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Citrus Research International, Nelspruit

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1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens (CRI)

1.1 SUMMARY

New EU phytosanitary regulations for FCM were published. CRI generated appropriate data and provided relevant inputs during the drafting of the new EU FCM regulations to support inclusion of least restrictive measures. The systems approach which CRI had promoted as the alternative to cold treatment, was included in the EU regulation. This was a lifesaving breakthrough for the industry, given the inability of the industry to apply cold treatment disinfestation. A massive impact on the industry's sustained viability was thereby averted. The regulation also recognised the non-host status of lemons for FCM, as recently demonstrated and published by CRI, thereby exempting lemons from the FCM requirements introduced to the EU phytosanitary regulations. An FCM Management System (FMS) was finalised on the basis of several years of preparatory research by CRI and this FMS provides a basis for ongoing compliance with the new EU FCM regulations. This was applied throughout the industry for the 2018 export season, with PhytClean as the implementation system. Improved future compliance with EU CBS regulations was pursued through amendments made to the CBS Risk Management System (RMS). These were based on investigations into the reasons for 2017 CBS interceptions. Sustained access to the Japan market was threatened by demands from Japan for additional risk mitigation data for *Bactrocera dorsalis*. CRI generated the required data, resulting in Japan indicating that citrus exports from South Africa could continue while they evaluate the data. All outstanding technical information required for enhanced access to the USA market was supplied to the USA, with the final regulatory response pending from USA. China accepted the FCM non-host status of lemons, but called for additional assurances that lemons are not a host for fruit flies. Such data were generated by CRI, published in a scientific journal and submitted to China in support of South Africa's request to exempt lemons from the cold treatment requirement. CRI research also demonstrated that the current cold treatment protocol for FCM, as currently required by several markets, is unnecessarily stringent and can be replaced with a far more fruit friendly alternative. The research results were published as a precursor to entering into negotiation with trading partners to revise the current export protocol conditions.

OPSOMMING

Nuwe EU-fitosanitiere regulasies vir VKM is gepubliseer. CRI het data gegenereer en relevante insette gelewer met die opstel van die nuwe EU VKM regulasies om die minste beperkende maatreëls te ondersteun. Die sisteem benadering wat deur CRI voorgestel is as 'n alternatief tot koue-behandeling, is in die EU-regulasie ingesluit. Dit was 'n lewensreddende deurbraak vir die bedryf, gegewe die onvermoë van die bedryf om koue-behandeling disinfestasië toe te pas. 'n Gewelddige impak op die bedryf se volgehoue lewensvatbaarheid is gevolglik afgeweer. Die regulasie het ook erkenning gegee aan die nie-gasheerstatus van suurlemoene vir VKM, soos onlangs deur CRI bewys en gepubliseer, en sodoende is suurlemoene van die VKM vereistes wat in EU-fitosanitiere regulasies vervat is, vrygestel. 'n VKM Bestuurstelsel ("FMS") is gefinaliseer op grond van verskeie jare se voorbereidende navorsing deur CRI en die FMS het die basis vir die voortgesette nakoming van die nuwe EU VKM regulasies neergelê. Dit is regdeur die bedryf vir die 2018-uitvoerseisoen toegepas, met PhytClean as die implementeringsstelsel. Verbeterde toekomstige nakoming van EU SSV regulasies is nagestreef deur wysigings aan die SSV Risikobestuurstelsel (RMS). Dit is op ondersoek na die redes vir 2017 SSV onderskeppings gebaseer. Voortgesette toegang tot die Japanse mark is deur nuwe vereistes van Japan vir *Bactrocera dorsalis* bedreig. CRI het die vereiste data gegenereer, wat daartoe gelei het dat Japan aangedui het dat sitrusuitvoere vanuit Suid-Afrika kan voortgaan terwyl hul die data evalueer. Alle uitstaande tegniese inligting wat benodig word vir verbeterde toegang na die VSA-mark is aan die VSA verskaf, met regulatoriese reaksie wat van die VSA uitstaande is. China het die VKM nie-gasheerstatus van suurlemoene aanvaar, maar het addisionele versekering dat suurlemoene nie 'n gasheer vir vrugtevlieë is nie, aangevra. Data is deur CRI gegenereer, gepubliseer in 'n wetenskaplike tydskrif, en aan China voorgelê ter ondersteuning van Suid-Afrika se versoek om suurlemoene van die vereiste koue-behandeling vry te stel. CRI se navorsing het ook bewys dat die koue behandelingsprotokol vir VKM wat tans deur verskeie markte vereis word, onnodig streng is en met 'n veel meer vrug-vriendelike alternatief

vervang kan word. Die navorsingsresultate is gepubliseer as 'n voorloper vir onderhandelinge met handelsvennote om die huidige uitvoerprotokolvoorwaardes te hersien.

1.2 EUROPE (EU)

FCM

In February 2017, through the WTO, the EU notified trading partners that the EU is amending Annexes I to V to Council Directive 2000/29/EC. The changes included new FCM regulations. In anticipation of the new FCM regulations, CRI engaged with affected parties and provided technical inputs during the drafting of the new EU FCM regulations. On 5 April 2017, CRI submitted a proposed communication to SA-DAFF which was submitted to the EU on 12 April 2017 in response to the EU's WTO notification of intent to implement new regulations. CRI issued interim FCM risk mitigation recommendations for the 2017 export season to prepare the industry for compliance with the anticipated new set of FCM regulations in 2018. These were communicated at the CRI Regional Packhouse Workshops, the March 2017 CMF meeting in Port Elizabeth and at the grower meeting in conjunction with the 2017 CGA Summit in Port Elizabeth. The relevant Variety Focus Groups were engaged for support. The recommendations were communicated in CRI Cutting Edge articles.

EU Directive 2017/1279 that amends Annexes I to V to Council Directive 2000/29/EC, with FCM specific measures was published on 14 July 2017. Non EU trading partners were required to communicate with the European Commission before 1 January 2018, notifying which compliance measures will be used. The regulation states that citrus (excluding lemons and some limes) from Africa, amongst other crops, must either be sourced from an FCM-free area, or must be subjected to an effective cold treatment, or another effective treatment to ensure freedom from FCM.

The systems approach which CRI had promoted as the alternative to cold treatment, was thereby included in the EU regulation. This was a lifesaving breakthrough for the industry, given the inability of the industry to apply cold treatment disinfestation as the FCM measure. A massive impact on the industry's sustained viability was accordingly averted. The regulation also recognised the non-host status of lemons for FCM, as recently demonstrated and published by CRI, thereby exempting lemons from the FCM requirements introduced to the EU phytosanitary regulations.

Over several years, in anticipation of such FCM regulations in the EU, CRI developed a science based FCM management system (FMS) which includes a systems approach for achieving compliance with FCM phytosanitary regulations. CRI formed an FMS Working Group (FMS WG), with representation from industry stakeholder groupings, including grower representatives from the variety focus groups. The FMS WG was engaged to provide inputs towards ensuring practical feasibility of the FMS within industry operations, prior to presentation to and discussion with SA-DAFF. SA-DAFF approved the release of a draft FMS in the interests of facilitating timely phytosanitary registration and starting implementation of the relevant good agricultural practices. This FMS was presented at CRI's IPM and Disease Management workshops during August and September 2017. The FMS was workshopped at the SA-DAFF planning meeting preceding SA-DAFF's annual citrus coordinating meeting for export to special markets, held in the Eastern Cape (11 October 2017). Here the FMS was presented as the official requirement for accessing the EU market for all citrus exports (excluding lemons and some limes) as of 01 January 2018. The FMS was also presented at SA-DAFF's regional follow up meetings. SA-DAFF adopted PhytClean as the official export registration and FMS implementation platform. Extensive industry workshopping took place to ensure IT systems are in place for 2018 exports. The pre-emptive development of the PhytClean system, a project driven by Paul Hardman (CGA), was co-funded in its start up by CRI and DST. The availability of PhytClean was critically important to effective implementation of the FMS.

CRI engaged with SA-DAFF to finalise wording of the communication to the EU, indicating the FCM measures that will be implemented to comply with EU Directive 2017/1279. SA-DAFF communicated this to the EU on 18

December 2017. The communication indicated that South African citrus will comply with option 16.6 (d) through the application of the citrus FCM systems approach. The FCM systems approach is incorporated into the FMS.

In January 2018 SA-DAFF confirmed that the FMS is to be used as the official system for ensuring compliance with the new EU FCM regulations. In January 2018 additional declarations for phytosanitary certification in order to comply with the FCM regulations were made available by SA-DAFF. A teleconference took place between SA-DAFF and the EU on 21 February 2018. On 21 February 2018 SA-DAFF requested more detail about the FMS from CRI to be provided to the EU. CRI compiled an abbreviated version of the citrus FMS and SA-DAFF provided the information to the EU on 19 March 2018.

Further meetings about the FMS and Phytclean were held with all role players to ensure that systems were in place for 2018 exports. The FMS and Phytclean were again presented at SA-DAFF and PPECB workshops in March and April 2018. An FMS Advisory Committee was established to guide operational implementation of the FMS in 2018. Further meetings were held with shipping lines to include regime codes for conventional shipping to the EU.

In April 2018 communications received from the EU indicated that not all limes species are recognised in the EU regulation as non-hosts for FCM. Accordingly, amended wording for phytosanitary certificate declarations were agreed with SA-DAFF and communicated to the industry. A revised citrus FMS, including clarification of details and improvements made during initial implementation, was submitted to SA-DAFF on 27 April 2018 for endorsement as the updated version (pending SA-DAFF response).

CBS

Twenty-three CBS interceptions on citrus from South Africa were notified by the EU in 2017. Most of the interceptions were on the Valencias that were exported in September and October 2017. A CBS-RMS working group meeting took place in November 2017 to discuss amendments to the CBS-RMS for the 2018 export season. The meeting agreed that the timing of orchard inspections for Valencias to be packed for export to EU in September and October needs to be amended and suitable changes to the CBS-RMS were agreed upon to give effect to this. CRI compiled a technical report on the potential reasons for the increase in CBS interceptions in 2017. CRI conducted a technical assessment of the information acquired during SA-DAFF investigations into each of the 2017 CBS interceptions as reported by SA-DAFF to the EU in December 2017. The CRI assessment included recommendations to improve compliance in 2018. Both CRI reports were submitted to SA-DAFF on 16 February 2018. CRI provided inputs to SA-DAFF on communications with the European Commission in January and February 2018 to assure the Commission that South Africa has adequately adjusted the CBS-RMS to ensure avoidance of a repeat of the 2017 CBS interceptions and that it is not necessary for the EU to impose additional CBS measures on South Africa to achieve compliance.

On 28 March 2018, SA-DAFF released an update of the CBS-RMS, including some changes that had not been agreed upon at the 2017 CBS-RMS Working Group meeting. These changes included validity periods for Valencia orchard inspection reports as of August, a requirement for repeated orchard inspections for lemons, and requirements for orchard inspections for degreened fruit. On 9 April 2018 SA-DAFF released a revised CBS-RMS. Objections were raised by members of the CBS-RMS Working Group. CRI convened a teleconference on 02 May 2018 to discuss the concerns. CRI compiled a report on the CBS risk associated with degreened fruit and presented this with recommendations on further CBS-RMS changes to SA-DAFF on 18 May 2018. SA-DAFF released an updated version of the CBS-RMS on 23 July 2018.

In April 2018 communication received from the EU indicated that not all lime species are recognised as non-hosts for CBS in the EU regulations. Accordingly, SA-DAFF released revised wording for phytosanitary certification declarations to comply with the EU CBS regulations for limes.

The changes to Annexes I to V to Council Directive 2000/29/EC, as referred to above, included relatively minor changes in relation to CBS. In the new rules provision was made for exporting country NPPOs to notify the Commission (before 1 January 2018) of the areas that are officially recognised by the exporting country NPPO as CBS free. On 5 April 2017, CRI submitted a proposed response to SA-DAFF which was submitted to the EU on 12 April 2017. The EU Directive 2017/1279 that amends Annexes I to V to Council Directive 2000/29/EC was published on 14 July 2017. For the 2018 export season the status quo in relation to CBS remains, since the emergency measures as set out in Commission implementing decision (EU) 2016/715 of 11 May 2016 remain in force until revoked. CRI provided SA-DAFF with proposed wording on measures that South Africa will implement to comply with EU Directive 2017/1279. Communication was sent to the EU on 18 December 2017. The communication included notification that South Africa is free from Citrus canker and *Pseudocercospora angolensis* and details of CBS pest free areas. CBS free areas were notified as: Western Cape, Northern Cape and Free State provinces and the magisterial districts of Christiana and Taung in the North West province. In January 2018 additional declarations for phytosanitary certification to comply with the EU regulations pertaining to CBS free areas were made available by SA-DAFF and in April 2018, SA-DAFF confirmed that all the magisterial districts in the Northern Cape will be treated as CBS free areas.

OTHER

The EU has published a new Plant Health Regime that will replace Council Directive 2000/29/EC (the framework plant health regulation). Implementation of the new Regime will come into force in late 2019. SA-DAFF is to convene a workshop to discuss the implications for trade.

1.3 JAPAN

In September 2016, a request was submitted to Japan-MAFF to amend the current export protocol to include all navel oranges cultivars as the protocol currently only specifies Washington and Cara Cara Navel cultivars. A subsequent meeting between Fruit SA and Japan-MAFF resulted in communication from the SA Agricultural attaché in Japan to Japan-MAFF, requesting confirmation that Navelates can be exported. A response from Japan-MAFF on 14 December 2017 indicated that Navelates are accepted. However, the official request made by SA-DAFF to allow all navel oranges remains pending since September 2016.

On 1 November 2017, SA-DAFF received a letter from Japan-MAFF in which it was stated “However, it would become impossible to continue the importation of the South African fresh fruits products with the current conditions on the protocols, if it is not possible to confirm that the current disinfestation conditions by cold treatments would be valid enough towards *Bactrocera invadens*”. CRI provided inputs to SA-DAFF on 12 November 2017. SA-DAFF provided feedback to Japan-MAFF on 22 December 2017. The feedback included detail about the distribution *B. dorsalis* in South Africa and indicated that the solution potentially lies in Japan accepting the revised time-temperature protocol for citrus that has been pending a response from Japan-MAFF for 3.5 years, since the temperature in such a revised protocol has been validated as effective for *B. dorsalis*.

In communication received on 1 February 2018, Japan-MAFF indicated that the request to amend the cold treatment to 1.4°C or lower for 16 days would not address their concerns about *B. dorsalis* and urged South Africa to prioritise their requested trial work on *B. dorsalis* cold tolerance, to avoid disruption to the SA citrus exports to Japan. On 14 February 2018 a meeting was held with SA-DAFF to discuss the situation. CRI provided a proposal to SA-DAFF on 21 February 2018 and SA-DAFF submitted a response to Japan-MAFF on 28 February 2018. The response included arguments as to why there would be no technical justification for disrupting citrus exports to Japan and an undertaking that the *B. dorsalis* trial work, demanded by Japan-MAFF, will be conducted, with an anticipated completion date of end March 2018. On 29 March 2018 SA-DAFF requested the SA Attaché in Japan to inform Japan-MAFF that SA is the process of finalising the report on the trials conducted. On 29 March 2018 CRI supplied SA-DAFF with two reports on the trials conducted to compare the cold tolerance of *B. dorsalis* and *C. capitata*. The results confirmed that Medfly (*C. capitata*) cold treatments can be used for disinfestation of *B. dorsalis*. SA-DAFF submitted the reports to Japan-MAFF on 4 April 2018. On 11 April 2018 SA-DAFF informed

CRI that Japan-MAFF acknowledged the receipt of the reports and that they are in the process of evaluating the data. Japan-MAFF also indicated that South Africa would not be listed as a country in which *B. dorsalis* is present and that exports of permitted fruit would not be disrupted, pending their assessment of the trial results as submitted by SA.

The two long outstanding issues pertaining to this market remain outstanding by the end of this reporting period. SA-DAFF submitted a request to Japan-MAFF in November 2009 to allow access for all mandarins (except Satsumas), under the current protocol for Clementines. Japan responded in 2013, calling for separate disinfestation treatment data for each of the 32 mandarin cultivars. SA-DAFF submitted a response to Japan-MAFF in September 2014. The request for inclusion of all mandarin cultivars remains outstanding, pending a technically justifiable response from Japan-MAFF. SA-DAFF and Industry have made several follow up queries over this time. The most recent Japan-MAFF response is that they will deal with one market access request at a time and it has been decided to prioritise an application for export of South African avocados to Japan.

In 2009, CRI completed the research and provided SA-DAFF with the requisite scientific reports to support a request from SA to revise the current cold treatment conditions for the export of all eligible citrus types to Japan. This package included Medfly efficacy data conducted in accordance with the Japan cold treatment trial guidelines. SA-DAFF submitted the request to include a treatment option of 1.4°C or lower for 16 consecutive days. In November 2009 Japan-MAFF requested the supporting scientific data of the Phase 2 trials. CRI supplied the data and SA-DAFF submitted it in July 2010. Japan-MAFF acknowledged receipt of the data, but also requested further information on the Phase 1 trials. In February 2011, SA-DAFF submitted a CRI report on the experimental work conducted on the Phase 1 trials of the revised cold treatment condition. In November 2011 Japan-MAFF informed SA-DAFF that problems were identified with the report on Phase 2 of the trials and advised on procedures to repeat phase 2 trials. CRI repeated the trials (repeat of phase 2) according to the Japan requirements and SA-DAFF submitted the report to Japan-MAFF in September 2014. The initial request to revise the temperature protocol was submitted in 2009 and the final revised data package to support this request was submitted in 2014, pending a decision from Japan-MAF.

1.4 USA

The outstanding issues for this market remain (1) the equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all SA citrus production areas), (2) expansion of CBS pest free areas to include the whole of the W Cape in the work plan, (3) adoption of CBS pest free places of production in the area of low pest prevalence (Far Northern Limpopo) and (4) revised work plan and pest list. In January 2017 President Trump gave instruction that all Federal regulations that have not been sent for publishing in the Federal Register are to be reviewed. SA-DAFF followed up with USDA-APHIS in May 2017 on all the outstanding issues and the USDA-APHIS informed SA-DAFF that they would communicate as soon as there are any new developments. SA-DAFF did follow up again with USDA-APHIS in August 2017 on all the outstanding matters and invited them for a bilateral meeting in October 2017 in South Africa. The USDA-APHIS again informed SA-DAFF that all the issues are receiving attention and that they will update them as soon as information becomes available. USDA-APHIS also indicated that they are not available to undertake a visit to South Africa but they suggested a Digital Video Conference (DVC) which took place on 20 November.

The USDA-APHIS again indicated during the DVC that they are evaluating the data for the 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 program and will communicate the outcome to SA-DAFF. They also indicated that the revised pest list will be provided as part of the updated workplan before the start of the next citrus export season.

The USDA-APHIS also requested further information on the production units and packhouses in the CBS affected areas that will take part in the program and also information on the ports that will be used to enable them to make

logistical arrangements. A workshop took place between CGA, CRI, SA-DAFF and PPECB on 14 February 2018. The implications and challenges for exports from the non CBS free areas were discussed. SA-DAFF sent a questionnaire to all producers and packhouses who had provisionally registered for this market to indicate whether they can comply with all the requirements pertaining to this market. SA-DAFF submitted the requested information about the production units and packhouses that will take part in the program and which ports are going to be used to USDA-APHIS on 26 March 2018.

In order to finalise all the outstanding issues, SA-DAFF sent an invitation to USDA-APHIS for a bilateral meeting to take place in April 2018 in South Africa, but the USDA-APHIS requested alternative dates in May 2018. By the end of this reporting period, a date for the bilateral meeting was still pending from USDA-APHIS.

1.5 CHINA

Two issues remain pending for this market by the end of this reporting period – a request submitted to AQSIQ to exempt lemons from the current cold treatment requirement (24-days) by recognising the non-host status of lemons for fruit flies and a request to accept bulk shipping of fresh citrus fruit from SA.

In August 2017, SA-DAFF received feedback from AQSIQ indicating that they had accepted that lemons are not a host of FCM, but requested further scientific evidence that lemons are not a host of fruit flies. They requested that experimental work be conducted in accordance with the guidelines of the relevant ISPM (ISPM 37 - Determination of host status of fruit to fruit flies (Tephritidae)). CRI consolidated results from relevant research that it had conducted into a scientific paper that was accepted for publication in a scientific journal. A draft response was submitted to SA-DAFF on 14 November 2017. The draft response included the scientific publication demonstrating that lemons are not a host for fruit flies. The research was conducted according to the IPPC guidelines as included in ISPM 37 and as requested by AQSIQ. An additional verification step was included in the study and therefore exceeds the requirements contained in ISPM 37. The draft response included a proposal to send trial shipments during the 2018 export season. In February 2018 further meetings took place between CRI and SA-DAFF to discuss the results of the experimental work done in the USA and South Africa pertaining to the host status of lemons for fruit flies. SA-DAFF submitted the information to AQSIQ on 28 February 2018.

On 13 April 2017, SA-DAFF submitted the requested information (prepared by PPECB and CRI) on the number of temperature sensors used in different cargo spaces with different cargo loading volume. In September 2017, SA-DAFF received feedback from AQSIQ, indicating that they remain concerned about the number of temperature sensors. A draft response by CRI and PPECB was submitted to SA-DAFF on 22 November 2017. In the response, a detailed explanation was given about the number of sensors per m³ within a vessel. The detail of a USDA approved vessel was used (South Africa only uses USDA approved vessels for bulk shipments of citrus fruit to trading partners requesting a cold treatment disinfestation for quarantine pests), a plan of the deck was included (detailing the number of fruit sensors per independent deck and/or common cooling space), together with a table indicating the minimum number of sensors required per deck or cooling space. The information indicated that the vessels to be used for shipments to China would be equipped with as many or more temperature sensors than the USDA specification (these specifications are used internationally as the benchmark for temperature monitoring in fresh produce shipping). SA-DAFF submitted the information to AQSIQ on 23 February 2018.

Regarding a request from China to export citrus fruit to South Africa, SA-DAFF received a response in February 2018 from AQSIQ on the list that was submitted to AQSIQ of pests present in China and their association with the fresh fruit pathway. CRI and SA-DAFF studied the information and held several meetings to evaluate the data. CRI provided a list of 67 pests to SA-DAFF in May 2018 for which further information is required from AQSIQ before a decision can be made on the import conditions for fresh Citrus fruit from China. The information was submitted to AQSIQ in June 2018.

1.6 INDIA

No report was received from the Indian Authorities on the trial pear shipment that was sent in February 2017 to demonstrate South Africa's ability to successfully apply an in transit cold treatment for fruit flies. In discussions held during a FRUIT SA visit to India in May 2017, it was indicated that trial shipments of citrus will also now be required. Several meetings were held with all role players to ensure that all understood the procedures to follow. The first citrus trial shipment consisting of five containers were shipped to India from Durban in June 2017 and were cleared on arrival in India. To date, SA-DAFF is still awaiting a report from India on the trial shipments. During a bilateral meeting in August 2017 in Cape Town, it was indicated that SA has to send two additional trial shipments per commodity (2 additional replicates of citrus trial shipments - they did not specify citrus types), to demonstrate our capability to conduct in-transit cold treatment for fruit flies. Due to unavailability of citrus fruit at the time, plans were made to send the two additional citrus trial shipments early in the 2018 export season.

1.7 VIETNAM

In September 2016 SA-DAFF submitted further information from CRI to the Plant Protection Department of the Ministry of Agriculture and Rural Development of Vietnam (PPD) in support of removing *Aspidiotus nerii* and *Pseudomonas syringae* pv *syringae* from the quarantine list. Concern about requirements for pre- and post-harvest management procedures in the first draft phytosanitary import requirements were also highlighted and the PPD was advised that South Africa cannot comply with some of the requirements. In the PPD's feedback of 12 April 2017 the PPD accepted the T107-e cold treatment (22 days) as the mitigation measure for FCM and the two fruit flies, but did not respond positively to the concerns raised by SA-DAFF about the pre and post-harvest management procedures in the draft protocol and the request to remove the two pests from the quarantine list. There was also no response to SA-DAFF's invitation to visit the South African citrus industry. In May 2017, SA-DAFF submitted a response with information supplied by CRI, again highlighting the reasons that SA cannot comply with some of the pre and post-harvest management procedures and the reasons that listing of the two pests cannot be scientifically justified. It was also indicated that new research results are available for an improved cold treatment for FCM. This information was re-sent to the PPD and the SA Embassy in Vietnam in September 2017, after the PPD indicated that they had not received the information that was sent in May 2017.

A response from the PPD was received on 10 January 2018. The PPD still insisted on the listing of the two pests (*A. nerii* and *P. syringae* pv *syringae*), the pre-harvest pest management procedures for *P. syringae* pv *syringae* and the sorting practices in the packhouse. They requested that the new research on FCM should be made available to the PPD for consideration. Meetings were held between CRI and SA-DAFF and feedback was submitted to the PPD on 14 March 2018. In the feedback SA-DAFF indicated that the listing of *A. nerii* as a quarantine pest with a low risk rating will be accepted, pending a scientific survey to provide further support that the pest is not present in commercial citrus orchards. The latest scientific articles by Moore *et al.* on an improved cold treatment for FCM and information on the sorting and inspection procedures in place for citrus exports to other trading partners were provided. Further scientific evidence to support the removal of *P. syringae* pv *syringae* as a quarantine pest was also included. No feedback was received from the PPD by the end of this reporting period. Although the Pest Information Packages for lemons, mandarins and grapefruit had been submitted in 2013, no response has been received from the PPD.

1.8 NEW MARKETS

1.8.1 The Philippines

The latest scientific information has been provided to the Philippine Authorities (the BPI) since 2014 to demonstrate that their list of quarantine pests needs to be revised and aligned with international standards as most of the listed pests do not follow the fresh fruit pathway or are not associated with *Citrus* spp. In April 2017, SA-DAFF sent a letter, extending an invitation to the technical experts from BPI to visit SA in order to finalise the import protocol for fresh citrus fruit from South Africa. However, in June 2017 the BPI responded that a follow up visit to South Africa is not needed to finalise the import protocol and that the five (5) remaining pests should remain on the quarantine

pest list. In September and October 2017 meetings between CRI and SA-DAFF were held to prepare feedback to the BPI. SA-DAFF submitted the feedback in December 2017. The South African Embassy was also included in the mailing list. Further information was provided to demonstrate that four (4) of the five (5) pests are not associated with citrus fruit and/or are not recorded pests of Citrus and /or Citrus in South Africa. Although the latest scientific information available indicated that one of the pests, *Aspidiotus nerii*, has never been found on citrus fruit in commercial South African citrus orchards (last record of the pest in South Africa was in 1997 on branches of lemon trees in a home garden), SA-DAFF indicated to the BPI that the listing of this pest as a quarantine pest with a low risk rating will be accepted, until such time as the results of a survey may provide further evidence for removal from the list. In feedback dated, 22 January 2018, the BPI indicated that they agreed to remove three of the five remaining pests from the quarantine list. The BPI accepted the listing of *A. nerii* as a low risk pest pending the result of the scientific survey to be conducted in commercial orchards. Despite all the scientific information provided, the BPI did not remove *Ceratitis quinaria* from the list. They justified their decision by referring to the fact the pest is currently listed by another trading partner as a quarantine pest. Meetings were held between CRI and SA-DAFF and on 26 February 2018, SA-DAFF submitted feedback to the BPI. In the feedback SA-DAFF indicated that the pest list of the other trading partner was finalised before the new information indicating that Citrus is not a host was available. This new information was only published in 2016. SA-DAFF informed the BPI that requests will be sent to other relevant trading partners to also update their pest lists regarding *C. quinaria*. No feedback was received from the BPI by the end of this reporting period.

1.8.2 Myanmar

SA-DAFF submitted a Pest Information Package (PIP) for all citrus types to Myanmar in March 2017. Last communication received from the Myanmar Authorities was in April 2017 when they indicated that South Africa can continue with exports while the PRA is in process.

1.9 FCM COLD TREATMENT CHANGES RELATED TO MULTIPLE MARKETS

A scientific paper validating the efficacy of improved cold treatments for FCM has been published. A process to amend the current protocols for FCM cold treatment conditions for the different fruit types and export markets was discussed at MAWG-meetings. CRI, HORGRO and SATI agreed on a communication to SA-DAFF, which included a dossier of data and a request that SA-DAFF engage with identified markets to adopt the improved treatment conditions. CRI submitted the dossier of data to SA-DAFF on 24 November 2017.

1.10 REGULATIONS

In this reporting period no new pest reports were sent to the IPPC on detections of *B. dorsalis* (previously *B. invadens*) incursions in South Africa. A status report on the presence of this fruit fly in South Africa was however posted on the IPPC's portal in February 2018. The status is as follows: The pest is considered to be present, actionable and under official control in the following provinces - Limpopo, Mpumalanga, Gauteng, North-West and some parts of KwaZulu-Natal. The pest is not present in the following provinces - Northern Cape, Western Cape, Eastern Cape and Free State.

Two African Greening monitoring surveys were conducted in 2017. One of the surveys had been conducted on the farm Wynkeldershoek in the Riebeeck Kasteel magisterial district of the Western Cape province and the other survey in the official greening free buffer zone in the Western Cape province including the magisterial districts of Knysna and Uniondale. Eight samples were drawn on the farm Wynkeldershoek and submitted to DAFF's Laboratory in Stellenbosch. All the samples tested negative for African Greening with conventional PCR methods. Ten duplicate samples were drawn in the buffer zone and were submitted to DAFF's Laboratory in Stellenbosch and CRI's Laboratory in Nelspruit. All ten samples tested negative in both laboratories with conventional PCR. However, the CRI laboratory also tested the samples with real-time PCR and found one sample positive for

African greening. The positive sample was collected from a home garden in Knysna. Meetings were held between CRI, DAFF and the owner of the tree. The positive tree has been removed by the owner. A delimiting survey will be conducted in this area in July 2018.

2 PORTFOLIO: INTEGRATED PEST MANAGEMENT

2.1 PORTFOLIO SUMMARY

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

For many years now, the research priorities within the IPM Portfolio have been determined by market access issues. This pertains particularly to retention and growth of existing markets. False codling moth continues to be the number one priority within the IPM Portfolio. However, its status was recently elevated to an unprecedented level, with the announcement by the European Union (EU) of impending regulation of FCM as a phytosanitary pest. This came into effect on 1 January 2018, affecting all exports of citrus from South Africa to the EU, except for lemons, which were excluded based on CRI's research demonstrating that lemons as exported are not a host. Nevertheless, approximately 40% of South Africa's citrus is exported to the EU, cementing FCM's position as the number one research priority. Consequently, with the IPM research portfolio, more funding and manpower was channelled towards FCM research than any other priority.

Other pests affecting market access that have attracted research focus and funding are fruit flies, particularly the Oriental fruit fly, which has been established in certain northern areas of the country for a few years now (and has been intercepted in the Western Cape), the recently identified Cape fly (a taxonomic separation from Natal fly), and mealybug. Apart from market access, biosecurity has become more important, particularly with the news that the Asian citrus psylla, *Diaphorina citri*, which is the vector of Asiatic greening disease (HLB), has been recorded in Tanzania and is moving southwards. Fortunately, HLB has not yet been recorded on the African continent outside of Ethiopia. However, this too appears to be spreading. In anticipation of this, a few projects have been conducted to develop and test management practices for potential use against *D. citri*.

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

During the past year, 21 projects were conducted within the FCM programme, five more than during the previous year. Fourteen of these projects entailed preharvest studies, six entailed postharvest studies and one covered a combination of the two. The preharvest studies covered the areas of microbial control, attractant related projects including mating disruption, the sterile insect technique, chemical control and ecological studies. Some highlights from the preharvest projects within the programme include the discovery of synergism between the FCM granulovirus and a yeast which was symbiotic with FCM, indications of selection for UV-resistant granulovirus, demonstration that bisexual releases of sterile moths within an SIT programme resulted in lower egg viability than male only releases, and completion of a study revealing several differences in FCM ecology in orchards of different ages. Highlights from the postharvest studies on FCM are that the efficacy of the full suite of shipping temperatures included in the FMS were determined, and further confirmation of promising efficacy of a CO₂ and cold combination treatment was recorded. Another highlight within the FCM programme is the completion and commercial implementation of the FCM risk management system (FMS), including the Systems Approach, which has been developed over several years.

The fruit fly research programme consisted of 11 projects. For the first time in a few years, two of the projects focussed on postharvest management of fruit fly pests. The most important finding from these projects was that Medfly is the most cold-tolerant fruit fly species and therefore cold treatment schedules which are effective against this species will be at least as effective against Natal fly and Oriental fruit fly. The main objectives of the preharvest management projects within the fruit fly programme during the past year were understanding the use of citrus by

fruit fly pests, understanding the population ecology of Oriental fruit fly, Natal fly and Cape fly, determining the efficacy of fruit fly monitoring tools and optimising pre-harvest control measures for fruit fly pests.

Within the mealybug and other phytosanitary pests programme, one projected focussed on mealybug, specifically the phytosanitary species, *Delotoccus aberiae*, one focussed on entomopathogenic fungi for foliar and fruit pests of citrus, including mealybugs, and the last one focussed on postharvest fumigation of both external and cryptic fruit pests. This last project demonstrated good efficacy with Vapormate against external pests and good potential with a combination of CO₂ and cold against internal pests, leading to an extension of this study under a new project within the FCM programme.

The final two programmes, which covered non-phytosanitary key pests and minor pests and mites, included five projects, a growth on last year. This was the result of an increased focus on the Asian citrus psylla, *Diaphorina citri*. Trials in Mauritius against *D. citri* and locally against aphids and green citrus leafhopper indicated that both systemically and foliar applied treatments hold potential for control of the pest, should it someday spread to South Africa.

During the past year, CRI research entomologists 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, DAFF Special Market workshops, PPECB workshops and several other smaller fora. This was extremely important, particularly in communicating critical messages, such as the FMS and the status and management of Oriental fruit fly in the country. Several Cutting Edges on important topics were also released to growers, as were articles in the SA Fruit Journal. Additionally, CRI research entomologists participated in certain international scientific meetings, such as International Organisation for Biological Control workshops, The International Symposium on Fruit Flies of Economic Importance, The Phytosanitary Measures Research Group Workshop and The Entomological Society of Southern Africa congress. Additionally, several key papers were published in scientific peer-reviewed journals, such as those demonstrating that lemons are not a host for fruit fly at the time of export and that improved cold treatments for FCM are effective.

PORTEFEULJE OPSOMMING

Vir baie jare nou is die navorsingsprioriteite binne die IPM Portefeulje deur marktoegangs faktore bepaal. Hierdie verwys veral na behouding en groei van huidige markte. Valskodlingmot bly die nommer een prioriteit binne die IPM Portefeulje, maar sy status is onlangs verder verhoog tot 'n ongekende vlak, met die aankondiging deur die Europese Unie (EU) van die dreigende regulasie van VKM as 'n fitosanitêre plaag. Hierdie is van 1 Januarie 2018 van toepassing en beïnvloed nou uitvoere van alle sitrus EU toe, behalwe suurlemoene, wat uitgesluit is as gevolg van CRI se navorsing, wat gewys het dat suurlemoene, soos wat hulle uitgevoer word, nie vir VKM 'n gasheer is nie. Nietemin word omtrent 40% van Suid-Afrika se sitrus EU toe uitgevoer, wat verseker dat VKM die nommer een navorsings prioriteit bly. As gevolg hiervan, binne die IPM Portefeulje is meer bevondsing en mannekrag na VKM navorsing gekanaliseer as enige van die ander prioriteite.

Ander plaë wat marktoegang affekteer en wat navorsings bevondsing gelok het is vrugtevlieë, veral die Oosterse vrugtevlieë, wat nou vir 'n paar jaar in sekere noordelike streke in die land gevestig is en selfs in die Wes-Kaap onderskep is, die onlangs geïdentifiseerde Kaapsevlieë (n taksonomiese onderskeiding van Natalsevlieë), en witluis. Afgesien van mark-toegang, het biosekuriteit ook meer belangrik geword, veral met die nuus dat die Asiatiese sitrus bladvloei, *Diaphorina citri*, die vektor van Asiatiese vergroenings siekte (HLB), in Tanzania aangeteken is en beweeg nou suidwaarts. Gelukkig is HLB nog nie in Afrika buite Ethiopia aangeteken nie, maar is ook besig om te versprei. In verwagting hiervoor is 'n paar projekte uitgevoer om bestuurspraktyke te ontwikkel en te toets vir hulle vermoë teen *D. citri*.

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

Gedurende die laaste jaar is 21 projekte binne die VKM program aangepak, wat vyf meer as die vorige jaar is. Veertien van hierdie projekte het vooroes studies behels, ses het na-oes studies behels en een het 'n kombinasie van die twee behels. Die vooroes studies het die volgende onderwerpe gedek: mikrobiële beheer, lokkingsverwante projekte, insluitend paringsontwrigting, die steriele insek tegniek, chemiese beheer en ekologiese studies. Sommige hoogtepunte vanuit die vooroes projekte binne die program sluit in die ontdekking van sinergisme tussen die VKM granulovirus en 'n gis wat simbioties met VKM is, aanduidings van seleksie van granulovirus met bestandheid teen UV, bewyse dat biseksuele loslatings binne 'n SIT program laer eier vatbaarheid veroorsaak as enkel-geslag loslatings, en voltooiing van 'n studie wat verskeie verskille in VKM ekologie tussen boorde van verskillende ouderdomme uitgewys het. Hoogtepunte van die na-oes studies op VKM sluit in voltooiing van studies of doeltreffendheid van die volle reeks verskepings temperature wat in die VKM risiko bestuur stelsel (FMS) gebruik word, en verdere bevestiging van die belowende werking van 'n CO₂ en koue kombinasie behandeling. Nog 'n hoogtepunt binne die VKM program is die voltooiing en kommersiële toepassing van die FMS, insluitend die Stelselsbenadering, wat oor verskeie jare ontwikkel is.

Die vrugtevlug navorsingsprogram het 11 projekte ingesluit. Vir die eerste keer in 'n paar jaar het twee van die projekte op na-oes bestuur van vrugtevlug plaë gefokus. Die belangrikste bevinding van hierdie projekte was dat Medvlug die mees koue tolerante vrugtevlug spesie is en daarom sal koue behandelings skedules wat doeltreffend teen Medvlug is, minstens ewe doeltreffend teen Natalsevlug en Oostersevrugtevlug wees. Die hoofdoele van die vrugtevlug program gedurende die laaste jaar is om die benutting van sitrus deur vrugtevlugplaë te verstaan, die populasie ekologie van die Oosterse vrugtevlug, Natalse vlieg en Kaapse vlieg te verstaan, effektiwiteit van vrugtevlug moniteringshulpmiddels te bepaal en om vóór-oes beheermaatreëls vir vrugtevlugplaë te optimaliseer.

Binne die witluis en ander fitosanitêre plaë program het een projek op witluis gefokus, spesifiek die fitosantêre spesie, *Delotoccus aberiae*, een het op entomopatogeniese swamme vir blaar- en vrugteplaë gefokus, insluitend witluis, en die laaste een het gefokus op na-oes beroking van albei eksterne en kriptiese plaë. Die laaste projek het goeie werking met Vapormate teen eksterne plaë gewys en met 'n kombinasie van CO₂ en koue teen interne plaë, wat gelei het tot 'n uitbreiding van die studie onder 'n nuwe projek binne die VKM program.

Die finale twee programme wat nie-fitosanitêre sleutel plaë en minder belangrike plaë en myte gedek het, het vyf projekte ingesluit, 'n groei van verlede jaar. Hierdie was as gevolg van 'n verhoogde fokus op Asiatiese sitrusbladvloei, *Diaphorina citri*. Proewe in Mauritius op *D. citri* en plaaslik op plantluise en groen sitrusbladspringer uitgevoer is het aangedui dat albei sistemies toegediende en gespuite behandelings belowend was vir beheer van die plaag, indien dit in die toekoms tot in Suid-Afrika versprei.

Gedurende die laaste jaar was CRI navorsings entomoloë ook aktief met voorligting aan produsente, in die vorm van IPM en Siektebeheer lente werksinkels, voor pakseisoen Na-oes werksinkels, DAFF Spesiale Mark werksinkels, PPECB werksinkels en verskeie ander kleiner forums. Hierdie was uiters belangrik gewees, veral met die kommunikasie van kritiese boodskappe soos die FMS en die status en bestuur van Oostersevrugtevlug in die land. Verskeie Snykante op belangrike onderwerpe is ook aan produsente vrygestel, sowel as SA Vrugtejoernaal artikels. Daarbenewens het CRI navorsings entomoloë aan sekere internasionale wetenskaplike vergaderings deelgeneem, soos die Internasionale Organisasie vir Biologiese Beheer werksinkels, die Internasionale Simposium op Vrugtevlug van Ekonomiese Belang, die Fitosanitêre Maatreëls Navorsings Groep werksinkel en die Entomologiese Vereniging van suidelike Afrika kongres. Daarbenewens is verskeie belangrike artikels in wetenskaplike eweknie-hersiende joernale gepubliseer, soos die wat gewys het dat suurlemoene soos uitgevoer nie vir VKM 'n gasheer is nie, en die wat die doeltreffendheid van verbeterde kouebehandelings vir VKM gerapporteer het.

2.2 **PROGRAMME: FALSE CODLING MOTH** Programme coordinator: Sean D Moore (CRI)

2.2.1 Programme summary

During the research cycle of this report, the landscape for FCM changed dramatically, with the implementation of regulation of FCM as a phytosanitary pest by the EU, effective of 1 January 2018. This was anticipated for several years and is reflected in the focus of the FCM research programme, particularly regarding the postharvest projects. Within the FCM programme, 21 different projects were conducted, of which 14 addressed pre-harvest issues, six postharvest projects and one addressed a combination of the two.

Of the pre-harvest management trials, five focussed on aspects of microbial control. One of these completed a seven-year investigation on the efficacy of locally isolated entomopathogenic fungi in controlling soil-dwelling life stages of FCM (2.2.3), demonstrating up to 80% efficacy with one of the isolates, with a residual efficacy covering the entire season with only one application in spring. Subsequent research focused on additional benefits of EPF application, including sublethal effects on eclosing FCM, efficacy against FCM eggs and neonates, and compatibility with commonly applied agrichemicals, particularly fungicides. The other four microbial control projects were dedicated to baculoviruses, either the *Cryptophlebia leucotreta* granulovirus (CrleGV) or the newly discovered *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). The two projects devoted to CrleGV, investigated UV resistance and protection (2.2.14) and synergism with yeasts (2.2.21). After the third passage of UV-exposed CrleGV through FCM larvae, bioassays against neonate larvae demonstrated reduced lethal concentrations, relative to initial exposure to UV, thus indicating the possible selection of UV-resistance virus particles. In the virus-yeast synergism project, one species of yeast was found to be a female moth-attractant or oviposition stimulant and the same yeast species, when combined with CrleGV, was recorded to increase larval mortality. The first CrpeNPV project successfully genetically and biologically characterised the virus and identified the existence of synergism between CrpeNPV and CrleGV in laboratory bioassays (2.2.8). The final microbial control project, investigated the efficacy of the novel NPV in field trials (2.2.15). Results were variable, due to the unprecedented level of fruit splitting and fruit drop in Navels in the Eastern Cape. However, under these conditions, efficacy of the NPV was similar to Cryptogran and superior to all chemical insecticides used.

A further four of the pre-harvest management projects focussed on pheromone or other attractant studies. The first compared three commercially available mating disruption products in a field trial, recording notable difference in trap shutdown (2.2.22). Unfortunately, FCM levels were too low for any infestation to be recorded. In another project, the compound 7-vinyl decyl acetate was used in combination with the FCM pheromone for mating disruption (2.2.11). This treatment caused greater trap shutdown than the FCM pheromone on its own and a commercial mating disruption product. Inexplicably, this did not translate into reduced fruit infestation. In the third project it was found that, FCM males from Citrusdal were significantly more attracted to FCM females from their same population, when presented with a choice between females from several populations (2.2.5). The study is being expanded to test for possible implications on technologies that make use of the FCM pheromone and female attraction, such as monitoring, mating disruption and the sterile insect technique (SIT). The final attractant project was a bit different in that it sought to identify and test possible female attractants for potentially greater monitoring accuracy (2.2.16). Although certain volatiles had appeared promising in the laboratory, none were able to attract meaningful numbers of released sterile females in the field.

The final five FCM pre-harvest management trials addressed aspects of SIT, conventional pest control, the ecology and movement of the pest and FCM management under nets. The SIT project aimed to improve the performance of the moth under cooler conditions, by augmenting the diet with the cryoprotectant trehalose (2.2.17). This did not prove successful in a large field trial. However, there were several confounding variables. Positively, it was demonstrated in laboratory trials that bisexual releases of sterile moths resulted in a significantly lower egg viability than male only releases. In the next project, a conventional spray trial was conducted with all registered FCM products in the Letsitele area (2.2.4). FCM levels were too low to obtain significant differences between treatments for the second year running. However, methoxyfenozide was the only product for which no infested fruit were recorded. The next pre-harvest management trial examined the effect of the multi-crop systems that occur in

much of the Western Cape on the movement and pest status of FCM and fruit fly on citrus (2.2.2). The close occurrence of other crops to citrus appeared to pose more of a fruit fly threat than an FCM threat to citrus. However, adjacent pomegranates did pose a carob moth risk to citrus. Penultimately, a three-year project that investigated the effect of orchard age on FCM levels came to an end (2.2.7). This study determined that fruit from juvenile orchards was more susceptible to FCM than fruit from mature orchards that FCM survival on fruit from juvenile orchards was higher, and that egg parasitism, virus infection and EPF occurrence in the soil were lower in juvenile orchards. The final preharvest management project examined FCM management under nets (2.2.19). FCM levels were not lower under nets. This was probably related to high levels of FCM in these orchards before the nets were erected. However, this may change over time, as sterile moth recaptures and ratios of sterile to wild moths were higher under nets.

Of the six FCM postharvest projects, three focussed on control of the pest and the other three on detection of the pest. The first control project sought to develop incomplete cold treatments that can be used as a final step in a systems approach (2.2.10). This was done for 3, 4 and 5°C for durations of 16 to 26 days. The next project investigated the use of CO₂ fumigation as a means to shorten the cold treatment required in order to achieve disinfestation (2.2.20). Difficulty was experienced with the penetrability of the artificial diet and thus solutions will be required to this problem. However, in Valencias, mean mortalities for 5th instars with gas only, cold only and gas plus cold were 39%, 12% and 67%, respectively. The final project was supposed to investigate the potential of vapour heat for disinfestation of fruit for FCM (2.2.12). However, no further progress could be made on this project due to other *ad hoc* priorities requiring attention.

The first postharvest detection trial researched the use of fruit-emitted volatiles as indicators of FCM infestation (2.2.13). Some exciting prospects were identified in the first season of research. However, due to the extreme physiological problems with fruit, especially Navel oranges, in the Eastern Cape last season, it was not possible to verify these results. However, the study continues. The other postharvest detection trial expanded on the already successful use of a sniffer dog for detection of FCM infested fruit (2.2.18). The objective is to develop a device for collection of volatiles from cartons or pallets of fruit and to expose the dog to these samples remotely. The equipment necessary for assembling such a device was obtained. The final postharvest detection project was an *ad hoc* trial in response to an urgent request from Satsuma growers (2.2.9). The assertion was that if FCM-infested Satsumas are degreened, 100% of infested fruit will be highlighted, particularly due to decay. However, this was unfortunately found not to be the case.

The goal of the last project within the FCM programme was to develop a systems approach for FCM management, as an alternative to a stand-alone cold treatment (2.2.6). This was successfully completed and the system is now being commercially implemented within the citrus industry for all exports to the EU.

Programopsomming

Gedurende die navorsings-siklus van hierdie verslag het die landskap vir VKM dramaties verander, met die regulasie van VKM as a fitosanitêre plaag deur die EU wat effektief van 1 Januarie 2018 geïmplementeer is. Hierdie is al baie jare gelede verwag en is dus in die fokus van die VKM navorsingsprogram weerspieël, veral wat die na-oes projekte betref. Binne die VKM program is 21 verskillende navorsings projekte aangepak, waarvan 14 vooroes projekte was, ses was na-oes projekte en een was 'n kombinasie van die twee.

Van die vooroes bestuur proewe het vyf op verskillende aspekte van mikrobiese beheer gefokus. Een van hierdie het 'n sewe-jaar ondersoek oor die effektiwiteit van plaaslik geïsoleerde entomopatogeniese swamme vir die beheer van grond-wonende lewensstadiums van VKM gerapporteer (2.2.3). Tot 80% beheer is met een van die isolate gewys, met 'n residuele nawerking wat die hele seisoen gedek het met net een toediening in die lente. Daaropvolgende navorsing het gefokus op verdere voordele van EPS toediening, insluitend subletale effekte op ontpoppende VKM, doeltreffendheid teen VKM eiers en pasuitgeborede larwes en verenigbaarheid met algemene plaagdoders, veral swamdoders. Die ander vier mikrobiese beheer projekte het gefokus op bakuloviruse, of die

Cryptophlebia leucotreta granulovirus (CrLeGV) of die nuut ontdekte Cryptophlebia peltastica nukleopolihedrovirus (CrpeNPV). Die twee projekte op CrLeGV, het onderskeidelik UV bestandheid en beskerming ondersoek (2.2.14) and sinergisme met giste (2.2.21). Na die derde deurloop van die UV-blootgestelde CrLeGV deur VKM larwes het biotoetse teen pasuitgebroeide larwes verlaagde noodlotige konsentrasies getoon in verglyking met die oorspronklike blootstelling aan UV. Hierdie het dus die moontlike seleksie van UV-bestande virus partikels gewys. In die virus-gis sinergisme projek is dit gevind dat een van die gis spesies of 'n wyfie lokmiddel of 'n eierleggins stimulant was. Wanneer dieselfde gis spesie met CrLeGV gekombineer was, is larwe mortaliteit verhoog. Die eerste CrpeNPV projek het suksesvol die virus geneties en biologies gekarakteriseer en het die voorkoms van sinergisme tussen CrpeNPV en CrLeGV in laboratorium biotoetse geïdentifiseer (2.2.8). Die finale mikrobiese beheer projek het die doeltreffendheid van die nuwe NPV in veldproewe ondersoek (2.2.15). Resulte was wisselvallig gewees as gevolg van die ongekende vlakke van vrugbars en vrugval in die Oos-Kaap. Onder hierdie omstandighede was die werking van die NPV en Cryptogran baie dieselfde en was beter as enige van die chemiese middels wat gebruik is.

'n Verdere vier van die vooroes bestuur projekte het gefokus op feromone en ander aantrekkings studies. Die eerste het drie kommersieel beskikbare paringsontwrigting produkte in veldproewe vergelyk en het opletbare verskille in lokvalsluiting aangeteken (2.2.22). Ongelukkig is VKM vlakke te laag gewees om enige besmetting aan te teken. In nog 'n projek is die samestelling, 7-viniel desiel asetaat in kombinasie met die VKM feromoon gebruik vir paringsontwrigting (2.2.11). Hierdie behandeling het 'n groter mate van lokvalsluiting veroorsaak as die VKM feromoon alleen en 'n komersieel beskikbare paringsontwrigting produk. Overklaarbaar het hierdie nie tot verminderde vrugbesmetting gelei nie. In die derde projek is dit gevind dat VKM mannetjie motte van Citrusdal betekenisvol meer aan VKM wyfies van hulle eie populasie aangetrek is wanneer hulle 'n keuse tussen wyfies van verskeie populasies gegee is (2.2.5). Die studie word uitgebrei om te toets vir moontlike implikasies vir tegnologië wat gebruik maak van VKM feromone en wyfie aantrekkingskrag, soos monitering, paringsontwrigting en die steriele inskek tegniek (SIT). Die finale aantrekkings projek het so bietjie verskil in die opsig dat moontlike wyfie aantrekkingsmiddels ondersoek is vir verbetering in akkuraatheid van monitering (2.2.16). Alhoewel sekere vlugtigestowwe in die laboratorium belowend voorgekom het, kon geen van hulle beduidende getalle van losgelate sterile wyfie motte in die veld lok nie.

Die finale vyf VKM vooroes bestuur proewe het aspekte van SIT, konvensionele plaagbestryding, ekologie en beweging van die plaag, en VKM bestuur onder nette aangespreek. Die doel van die SIT projek was om die optrede van die mot onder koeler omstandighede te verbeter, deur om die dieet met die kouebeskermer, trehalose, by te voeg (2.2.17). In 'n grootskaalse veldproef was hierdie nie suksesvol nie, maar daar was verskeie verwarrende veranderlikes. Aand die positiewe kant is dit in laboratorium proewe gewys dat biseksuele loslatings van steriele motte tot 'n betekenisvolle verlaagde eierlewensvatbaarheid gelei het as loslatings van net mannetjie motte. In die volgende projek is 'n konvensionele spuitproef op Letsitele uitgevoer met al die geregistreerde VKM produkte (2.2.4). Vir die tweede agtereenvolgende jaar is VKM vlakke is te laag om betekenisvolle verskille tussen die behandelings te kry. Nietemin is methoxyfenozide die enigste produk waarvoor daar geen besmette vrugte aangeteken is nie. Die volgende vooroes bestuur projek het die effek van die multigewas stelsels wat in die Wes-Kaap voorkom op die beweging en plaagstatus van VKM en vrugtevlieë ondersoek (2.2.2). Die nabye voorkoms van ander gewasse aan sitrus het blykbaar 'n groter dreiging vir vrugtevlieë as vir VKM vir die sitrus veroorsaak. Granate langsaan het egter 'n karobmot dreiging vir sitrus veroorsaak. Voorlaastens het 'n drie-jaar projek wat die effek van boord ouderdom ondersoek het tot einde gekom (2.2.7). Hierdie studie het bepaal dat vrugte van jong boorde meer vatbaar vir VKM was as vrugte van volwasse boorde, dat VKM oorlewing op vrugte van jong boorde hoër was, en dat eier parasitisme, virus besmetting en EPS voorkoms in die grond almal laer was in jong boorde. Die finale vooroes bestuur projek het VKM bestuur onder nette ondersoek (2.2.19). VKM vlakke onder nette is nie laer nie, mees waarskynlik as gevolg van hoë vlakke van VKM in hierdie boorde voor die nette opgerig is. Hierdie kan egter oor tyd verander want steriele motte hervangste en verhoudings van steriele tot wilde motte was hoër onder die nette.

Van die ses VKM na-oes projekte het drie op die beheer van die plaag gefokus en drie op die opsporing van die plaag. Die doel van die eerste beheer projek was om onvoldoende koue-behandelings te ontwikkel, wat as 'n finale stap in 'n stelselsbenadering gebruik kan word (2.2.10). Hierdie is vir 3, 4 en 5°C gedoen vir vir duurties van 16 tot 26 dae. Die volgende projek het die gebruik van CO₂ ondersoek om die benodigde koue behandeling vir ontsmetting te verkort (2.2.20). Probleme is ondervind met die penetreerbaarheid van die kunsmatige dieet en dus word oplossings benodig. Nietemin was gemiddelde mortaliteit van 5de instars in Valencias met gas alleen, koue alleen en 'n kombinasie van die twee onderskeidelik 39%, 12% and 67%. Die laaste projek moes die moontlike gebruik van damp hitte vir ontsmetting van vrugte vir VKM ondersoek het, maar geen verdering vordering was moontlik as gevolg van ander *ad hoc* prioriteite wat aandag benodig het (2.2.12).

Die eerste na-oes opsporings projek het die gebruik van vrug-uitgestraalde vlugtigestowwe as aanwysers van VKM besmetting ondersoek (2.2.13). Sekere opwindende moontlikhede is in die eerste jaar van navorsing geïdentifiseer, maar as gevolg van die buitengewone fisiologiese probleme met vrugte, veral Nawellemoene, in die Oos-Kaap verlede seisoen, was dit nie moontlik om hierdie resultate te verifieer nie. Die studie word egter nog voortgesit. Die tweede na-oes opsporings proef het uitgebrei op die alreeds suksesvolle gebruik van 'n snuffelhond vir opsporing van VKM besmette vrugte (2.2.18). Die doel is om 'n toestel te ontwikkel vir versameling van vlugtige stowwe van kartonne of palletes van vrugte dat die hond op een sentrale punt aan die monsters blootgestel kan word. Die nodige toerusting vir die samestelling van die toestel is gekry. Die finale na-oes opsporings projek was 'n *ad hoc* proef in reaksie op 'n dringende versoek van Satsuma produsente (2.2.9). Die bewering is gemaak dat as VKM-besmette Satsumas ontgroen word sal 100% van die besmette vrugte uitgelig word, veral as gevolg van bederf. Dit is gevind dat hierdie ongelukkig nie die geval was nie.

Die doel van die laaste projek binne die VKM program was om 'n stelselsbenadering te ontwikkel vir VKM bestuur, as 'n alternatief vir 'n alleenstaande koue behandeling (2.2.6). Hierdie is suksesvol voltooi en die stelsel word nou kommersieel geïmplementeer binne die sitrusbedryf vir alle uitvoere na die EU.

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

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

Summary

Weekly monitoring for fruit flies and false codling moth was carried out in the Stellenbosch, Porterville and Riebeeck Kasteel during the period 2013-2018. Monitoring took place in pome fruit, stone fruit, grape, pomegranate and citrus blocks. The broad range of fruit types grown on farms at Riebeeck Kasteel and Stellenbosch contributed to the high levels of fruit fly trapped late in the season at citrus harvesting time. Fruit fly remained in stone and pome fruit orchards, even after harvesting had taken place. Whether this is related to the presence of extrafloral nectaries is as yet unknown. Nevertheless, the presence of very high numbers of fruit flies in orchards close to citrus must be viewed as a real danger. Harvesting of soft citrus at the Stellenbosch and Riebeeck Kasteel farms (during April / May) took place despite high numbers of fruit flies being caught in traps. The climatic conditions in the Western Cape create a difficult situation for farmers. Temperatures in autumn are mild and humidity rises as citrus harvest time approaches. Rainfall in autumn / winter will also limit bait efficacy. At Porterville, where citrus and pomegranates are grown adjacent to each other, carob moth numbers were considerably higher than at the Stellenbosch and Riebeeck Kasteel farms where the latter crop is absent. Two different fruit fly lures were evaluated over a 3-year period in order to compare the results obtained from Biolure 3-component lure (catching mainly females) and Capilure (catching only males). Major peaks of fruit fly activity, as measured by the two lures, were closely correlated. Overall, Capilure was more sensitive, despite only catching males.

Opsomming

Weeklikse monitering vir vrugtevlieë en valskodlingmot in die Stellenbosch, Porterville en Riebeek Kasteel gebiede gedurende 2013-2018 is uitgevoer. Monitering het in kernvrugte, steenvrugte, druiwe, granaat en sitrus bloke plaasgevind. Die wye reeks van vrugtetipes wat op plase gekweek word by Riebeek Kasteel en Stellenbosch het tot die hoë vlak van vrugtevlieë wat laat in die seisoen naby sitrus oestyd gevang is bygedra. Vrugtevlieë en valskodlingmot het in steenvrug en kernvrug boorde gebly alhoewel oestyd reeds plaas gevind het. Of dié gedrag met die teenwoordigheid van “extrafloral nectaries” verbind is is nog nie bekend. Nogtans, die teenwoordigheid van hoë getalle vrugtevlieë en valskodlingmot in boorde naby sitrus moet as ’n werklike gevaar beskou word. Die oes van sagtesitrus by Stellenbosch en Riebeek Kasteel plase (gedurende April / Mei) teen spyte van hoë getalle vrugtevlieë wat in valletjies gevang is. Klimaats toestande in die Wes-Kaap maak die situasie vir boere moeilik. Temperature in herfs is matig en die humiditeit vlakke naby sagtesitrus oestyd klim. Reënval in herfs ook die doeltreffendheid van lokaas toedienings sal beperk. By Porterville, waar sitrus en granate lanks mekaar geplant is, was karobmot getalle aansienlik hoër as by die Stellenbosch en Riebeek Kasteel plase waar die laasgenoemde gewas nie gekweek word nie. Twee verskillende vrugtevlieg aanlok middels, Capilure (trimedlure) wat mannetjies vang en Biolure (3 komponente aanlokmiddel) wat hoofsaaklik wyfies vang, was oor a 3-jaar periode getoets. Die bevolkingspieke was nou koreleer. Capilure was algehele meer sensitief, alhoewel die lokmiddel net mannetjies vang.

Introduction

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), Cape fly *Ceratitis quilicii* (Diptera: Tephritidae) (De Meyer et al. 2016) and false codling moth, *Thaumatotibia leucotreta* (Meyrick), are polyphagous pests with phytosanitary implications (Moore et al. 2016). They attack many different cultivated fruit types as well as wild host plants in southern African fruit-growing regions (Myburgh 1964, Grout et al. 2011, Barnes and Venter 2006, Moore and Hattingh 2012). The presence of these pests in fruit orchards can have serious negative consequences for South African citrus exports. In the Western Cape, fruit production (including citrus) tends to occur in relatively distinct areas around towns, e.g. Riebeek Kasteel, Porterville, Stellenbosch, Citrusdal, Piketberg, etc. with the intervening agricultural land being utilized for cereals or grazing for livestock. These in-between environments are likely to be relatively unsuitable for pests such as fruit flies and false codling moth as there is little or no natural vegetation remaining. The relative importance of false codling moth and fruit flies may therefore vary considerably between the different “pockets” of fruit production depending upon microclimate, pest control practices and the presence or absence of different fruit types cultivated within a given area. Past studies have investigated the role of alternative wild host plants of *T. leucotreta* (Honiball 2004, Kirkman and Moore 2007, Stotter 2009). However, none have focussed on the role of other cultivated hosts, which may be grown nearby and any possible relationship to FCM populations on citrus. Regarding fruit flies, the relative abundance of fruit fly species infesting Western Cape deciduous fruit was studied by Manrakhan & Addison (2007, 2014) and Medfly was found to be dominant. On stone fruit, fruit flies are also known to be serious pests (Engelbrecht et al. 2004) and grapes, particularly the more modern cultivars, are suitable hosts for fruit flies to complete their life-cycle (Barnes 2006). This is in contrast to older grape cultivars where higher juice content causes significant mortality of immature fruit fly stages (Engelbrecht et al. 2004).

Stated objectives

The pest potential of polyphagous species can be increased when multiple, or a succession of, host plants (at suitable growth stages) are available in close proximity (Kennedy and Storer, 2000). The objective of this project was to improve understanding of when false codling moth, and fruit flies in the Western Cape utilize different cultivated fruit crops and the importance of the close proximity of various cultivated fruit crops in possibly assisting pest populations to persist over long periods.

Materials and methods

Monitoring using traps for fruit flies and FCM was carried out in a wide variety of different orchards, including deciduous fruit (apples and pears), citrus (oranges), grapes and pomegranates. Trapping was undertaken in different areas of fruit production and included Stellenbosch, Riebeek Kasteel and Porterville. There is considerable variation in the types of fruit grown in these different locations as a result of climatic differences. For example, pomegranates could only be monitored in the Porterville area. For monitoring fruit fly, use was mainly made of Biolure 3-component lure-baited bucket traps (Chempac, Paarl, Western Cape). However, at Riebeek Kasteel, a comparison was also made between the catches obtained from Biolure-baited bucket traps (mainly catching female flies) and Capilure-baited Sensus traps (catching almost exclusively male fruit flies). This was done in order to generate data regarding the relative activity patterns of male and female fruit flies in relation to the spray / harvest threshold of 4 flies per week in a Capilure trap as recommended by Citrus Research International to citrus growers.

Seasons 2013/14 and 2014/15

Biolure-baited yellow bucket traps were set out at two locations: at Welgevallen (the Stellenbosch University farm) and at Ebenhaezer Farm at Riebeek Kasteel. These locations were chosen because of the wide diversity of the fruit crops grown. At Welgevallen farm (Stellenbosch), nectarines, plums, apples, pears, and citrus (Satsumas) are grown in 2 ha blocks in close proximity. Two Biolure-baited traps were placed in each of the blocks. As there were 2 separate blocks of plums, a total of 4 traps was placed in blocks of this fruit type. A grand total of 12 traps was thus placed at this farm. At Ebenhaezer Farm (Riebeek Kasteel), blocks were larger in size from 4 ha upwards. Here, nectarines, plums, wine grapes, table grapes and two types of citrus (Midknights and Satsumas) are cultivated. At this farm a total of 14 traps were placed out. The two citrus cultivars as well as two different cultivars of table grapes were monitored separately. Two Biolure-baited bucket traps were placed in each of the blocks to be monitored. In addition, during the 2014/15 season a 3rd location was added to the project, this being Houdconstant Farm at Porterville. Here citrus and pomegranates are grown adjacent to each other, enabling data to be collected on an additional crop type and its relationship to nearby citrus.

Seasons 2015/16, 2016/17 and 2017/18

During these seasons, trapping continued at Ebenhaezer Farm, Riebeek Kasteel and also at Houdconstant Farm, Porterville. Trapping was discontinued at Welgevallen, Stellenbosch after having collected 2 years of results, due to other experiments being run by university students which would have interfered with trap catch results.

Results and discussion

Season 2013/14

At Riebeek Kasteel, Cape fly was a significant factor in nectarines and catches were high until mid-December, post-harvest. Thereafter this species practically disappeared and did not migrate into later-maturing fruit types such as grapes or plums. It was found in citrus orchards from May onwards.

Regarding Medfly, this species was of less significance than Cape fly on early nectarines but persisted to infest later maturing fruit types in this area. During December, most flies were caught in nectarine and plum blocks. During January, numbers were generally very low but shifted to vineyards. Regarding citrus (Midknights and Clementines), numbers of flies were significantly below those in the grape and nectarine blocks during February and March but increased considerably during April and May as fruit coloured. At Stellenbosch, Cape fly persisted from December to July, in terms of highest trap catches. During December, nectarines were the most favoured host. Later in the season during February and March, plums followed by apples and then citrus were the preferred hosts. Trap catches in pears were generally low and this seems not to be a preferred host for Cape fly. Regarding Medfly, activity was generally very low in December being mostly confined to nectarines. During January, apples were the most favoured host. During February a well-defined peak of activity occurred on pears with apples and

plums being the next favoured hosts. Fly catches on apples and plums increased considerably in March. During late March and early April, citrus began to attract large numbers of flies as Clementines coloured up.

At Stellenbosch, during December, the vast majority of FCM were caught in nectarines, followed by plums. Despite being harvested in November, FCM numbers in nectarines were highest in 12 out of 19 sampling weeks. When plums and nectarines were considered together, FCM numbers in these fruit types were highest in 14 out of the 19 sample weeks. FCM numbers trapped in pear and apple orchards were lowest and these fruit types are not considered to be hosts. Regarding citrus, FCM was absent during December and only very low numbers were caught during January and February. During March numbers increased and during early April were up to 9 per trap.

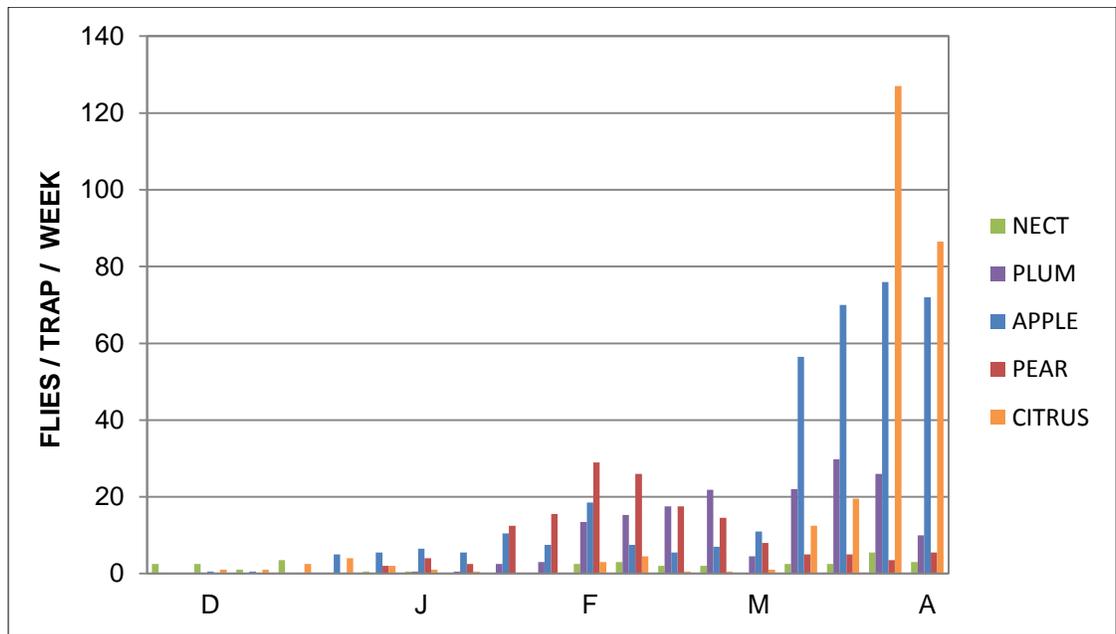


Figure 1. Numbers of Medfly caught in Biolure-baited bucket traps at Welgevallen Farm, Stellenbosch, in different fruit orchards, December 2013 – April 2014. Citrus was harvested in the first 2 weeks of April.

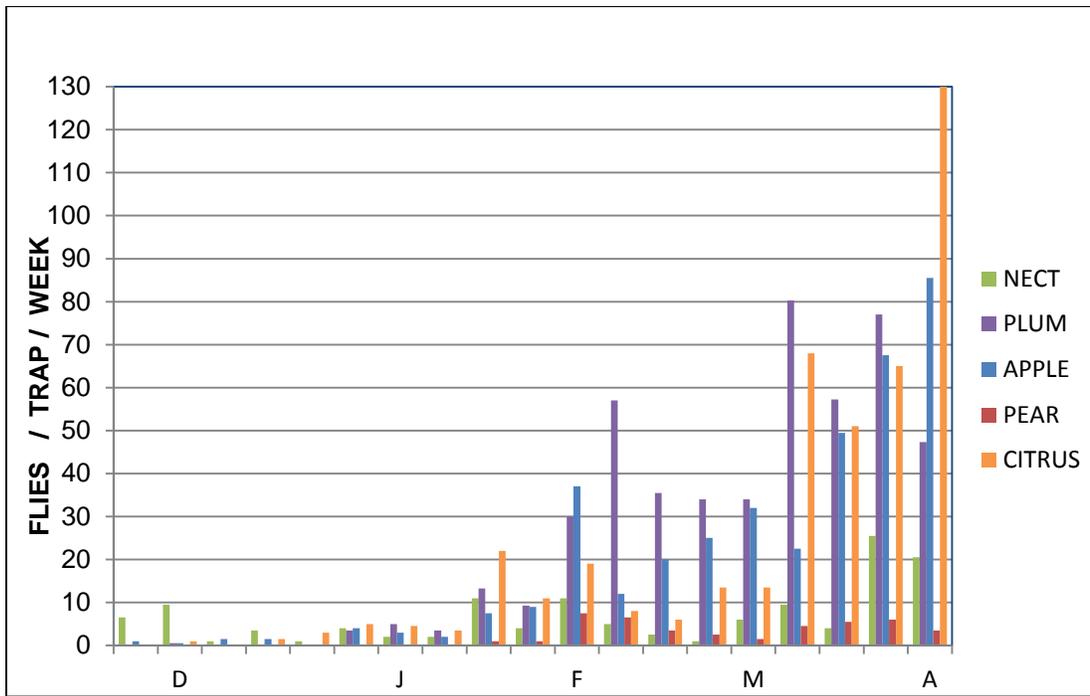


Figure 2. Numbers of Cape fly caught in Biolure-baited bucket traps at Welgevallen Farm, Stellenbosch, in different fruit orchards, December 2013 – April 2014. Citrus was harvested in the first 2 weeks of April.

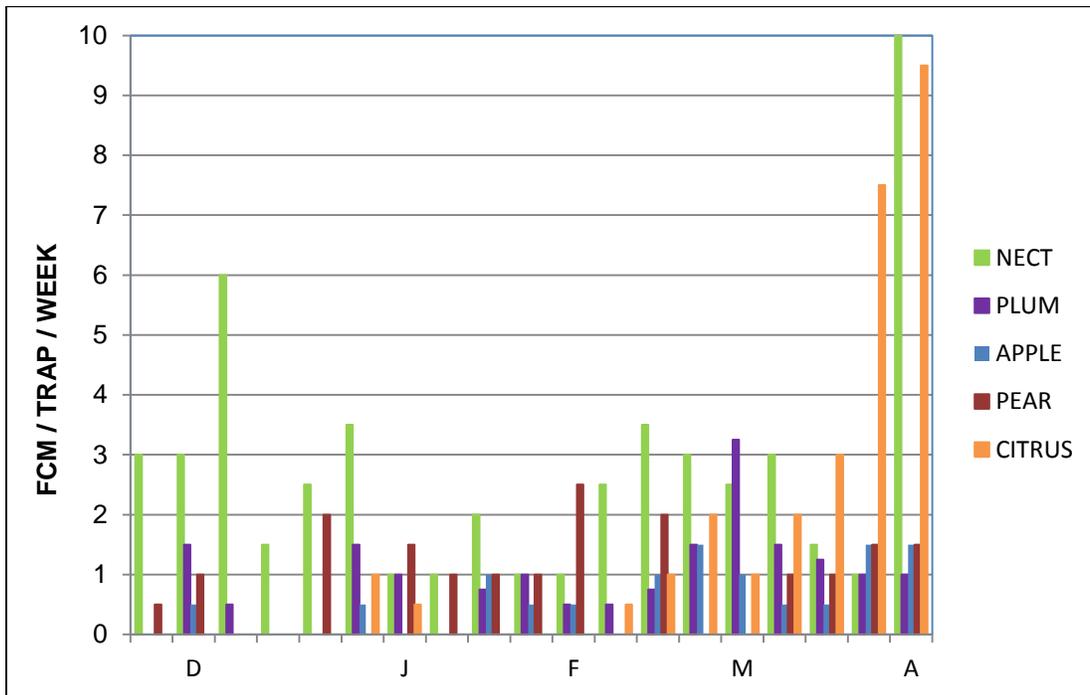


Figure 3. Numbers of FCM caught in yellow delta pheromone traps at Welgevallen Farm, Stellenbosch in different fruit orchards, December 2013 – April 2014. Citrus was harvested in the first 2 weeks of April.

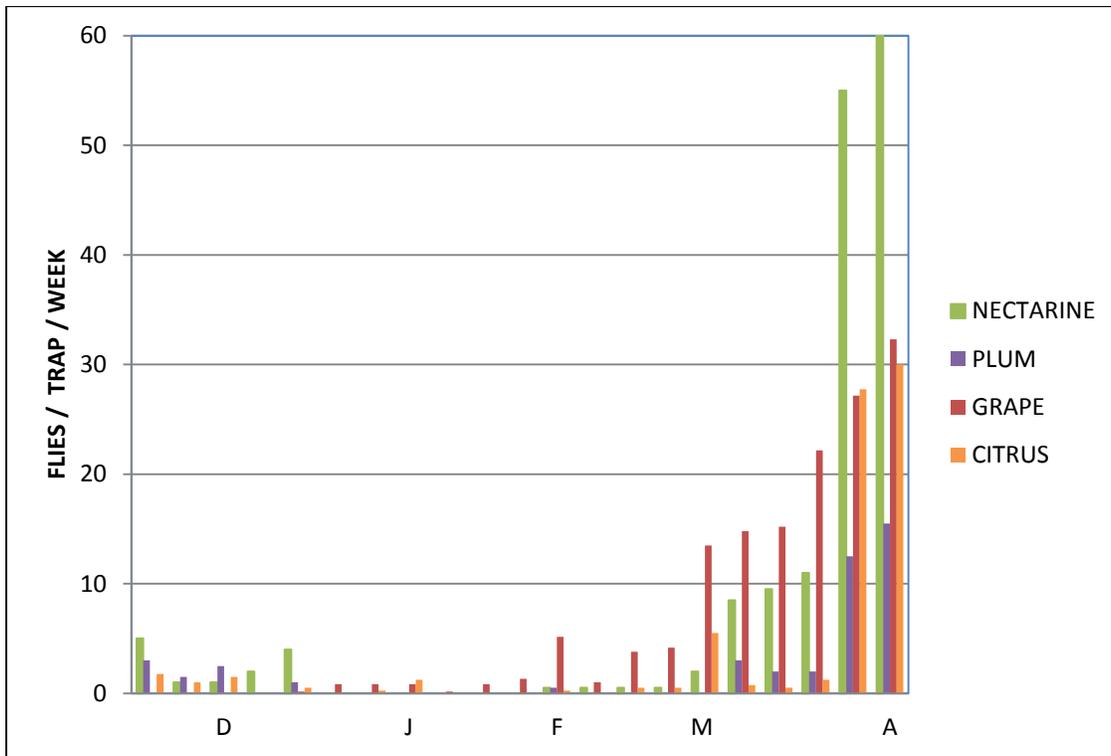


Figure 4. Numbers of Medfly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeek Kasteel, in different fruit orchards, December 2013 – April 2014

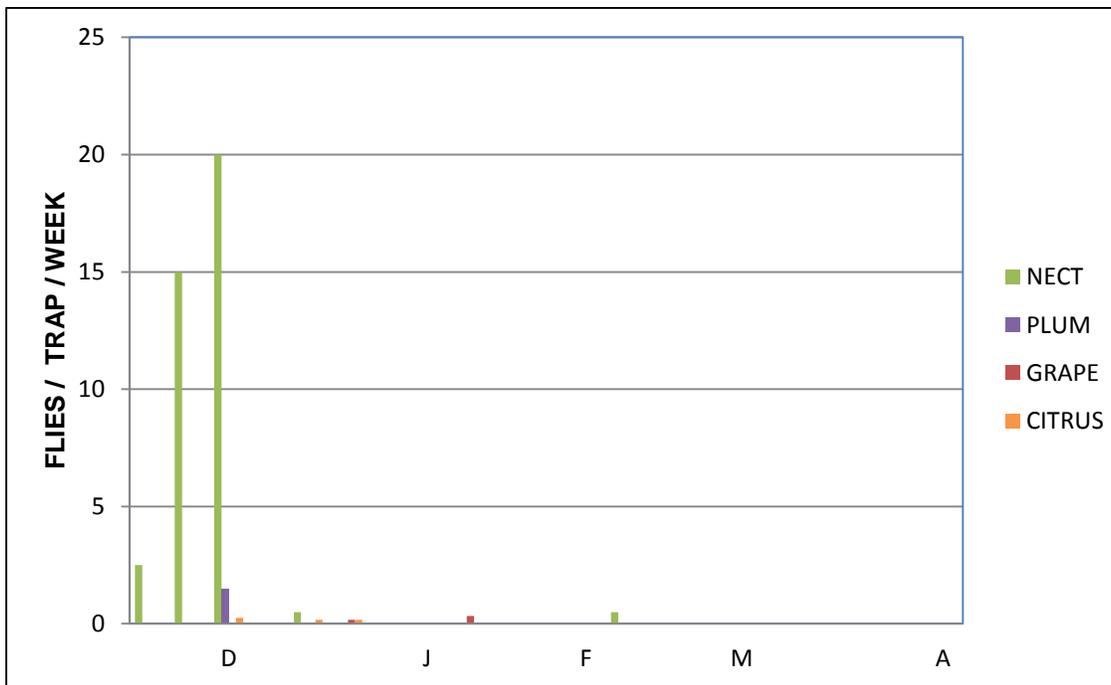


Figure 5. Numbers of Cape fly caught in Biolure-baited bucket traps at Ebenhaezer farm, Riebeek Kasteel, in different fruit orchards, December 2013 – April 2014.

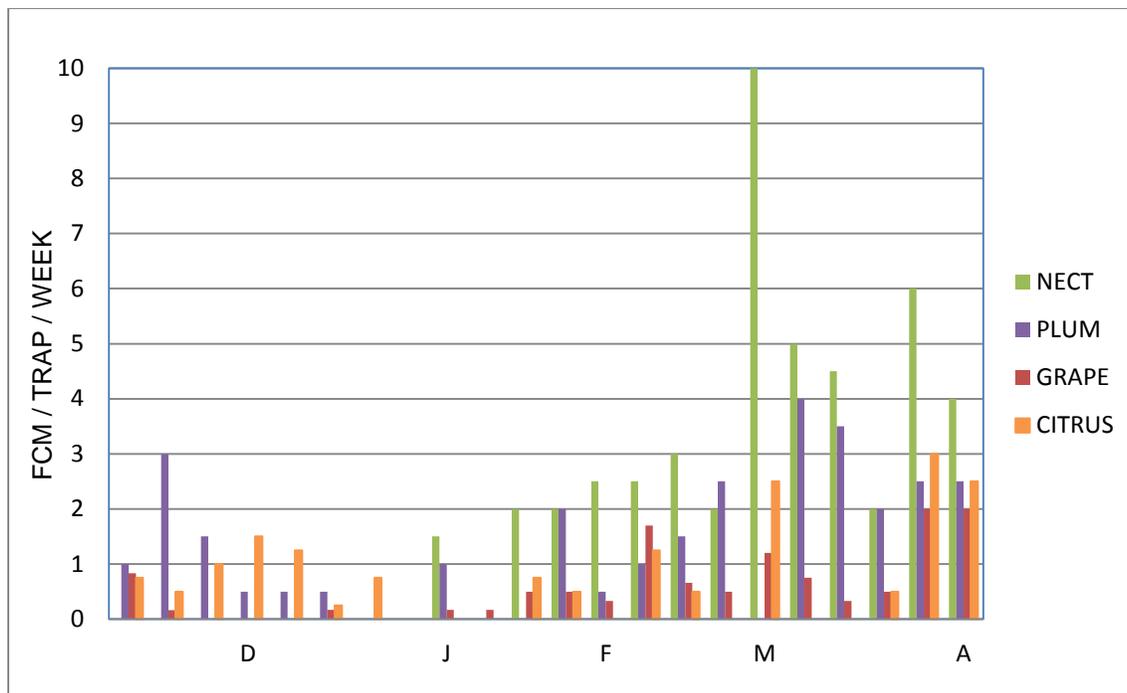


Figure 6. Numbers of FCM caught in yellow delta pheromone traps at Ebenhaezer Farm, Riebeek Kasteel in different fruit orchards, December 2013 – April 2014.

At Riebeek Kasteel, during late November and early December, FCM numbers peaked in a plum orchard prior to a peak in citrus in mid-December. Despite the early harvesting of nectarines, during November, FCM numbers in a block of this fruit type began to increase throughout January to March such that FCM numbers were highest in 9 out of 13 sample weeks. When FCM in plum orchards and nectarines were considered together, this total increased to 10 out of 13 weeks, despite both fruit types being harvested before the end of 2013. FCM numbers caught in vineyards were comparatively low. Regarding citrus, numbers were also very low compared to those caught in nectarine and plum orchards.

Season 2014/15

The 13 blocks from which results were obtained in 2013/14 continued to be monitored on a weekly basis in 2014/15. In addition, a farm where citrus and pomegranates are grown close together was included in the project for the purposes of monitoring FCM. There were marked differences in the patterns of activity between fruit fly and FCM. Regarding fruit-fly, peaks in trap counts were closely related to the ripening of each successive fruit type.

Regarding FCM, the peaks in male flight activity were not related to the ripening of fruit type. The results obtained from Riebeek Kasteel were particularly clear in that peaks of moth activity from October 2014 to April 2015 were very similar in their timing irrespective of which orchard type the traps were placed in. This would seem to indicate that FCM is far less restricted to a particular crop than fruit-fly and is able to disperse throughout a farm at any particular time. The nocturnal nature of FCM is obviously a factor in governing the pattern of its flight activity. These results indicate that other factors are governing flight activity rather than just host (fruit) maturity. This may have important consequences with regard to future pest management.

After 7 months of trapping, the cumulative totals of FCM trapped in citrus, plum and peach orchards at Riebeek Kasteel were very similar, ranging from 57 – 60 moths per trap. Nectarines showed the maximum with 66 moths per trap. Numbers in grapes were significantly lower at only 10.5 FCM per trap (cumulative total). This contrasts with fruitfly, where variation was far greater, with cumulative totals of Medfly on citrus, plum and peach of 43.3,

30.8 and 51.0 respectively. Nectarines showed the maximum number of Medfly at 122.5 per trap and grapes were at a cumulative total of 41.8 per trap.

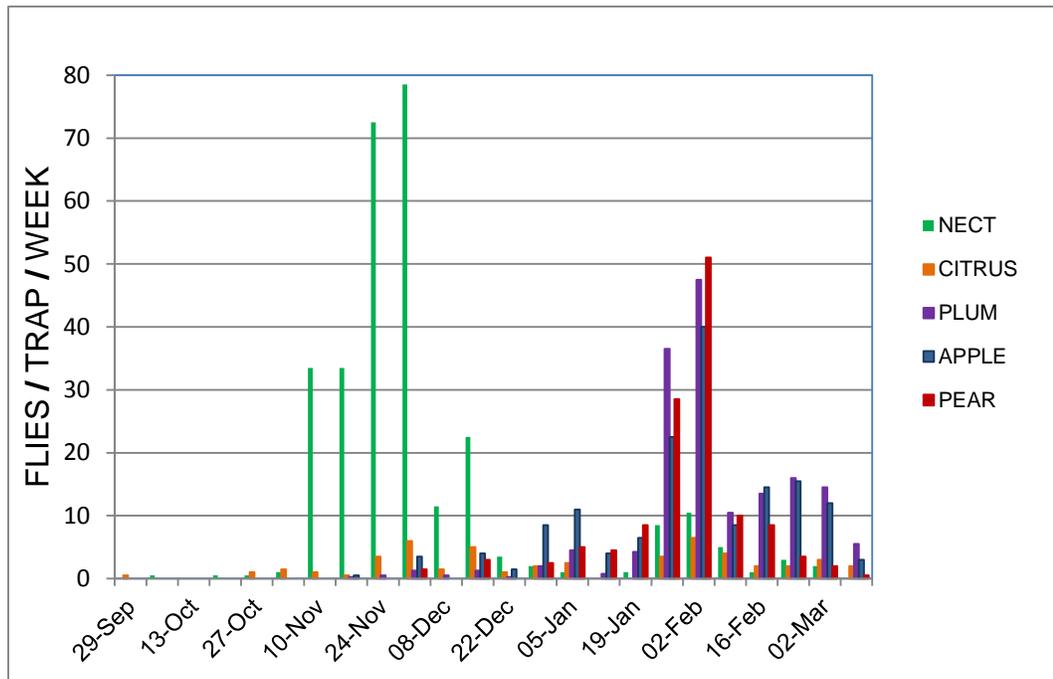


Figure 7. Numbers of Medfly caught in Biolure-baited bucket traps at Welgevallen Farm, Stellenbosch in different fruit orchards, September 2014 – March 2015.

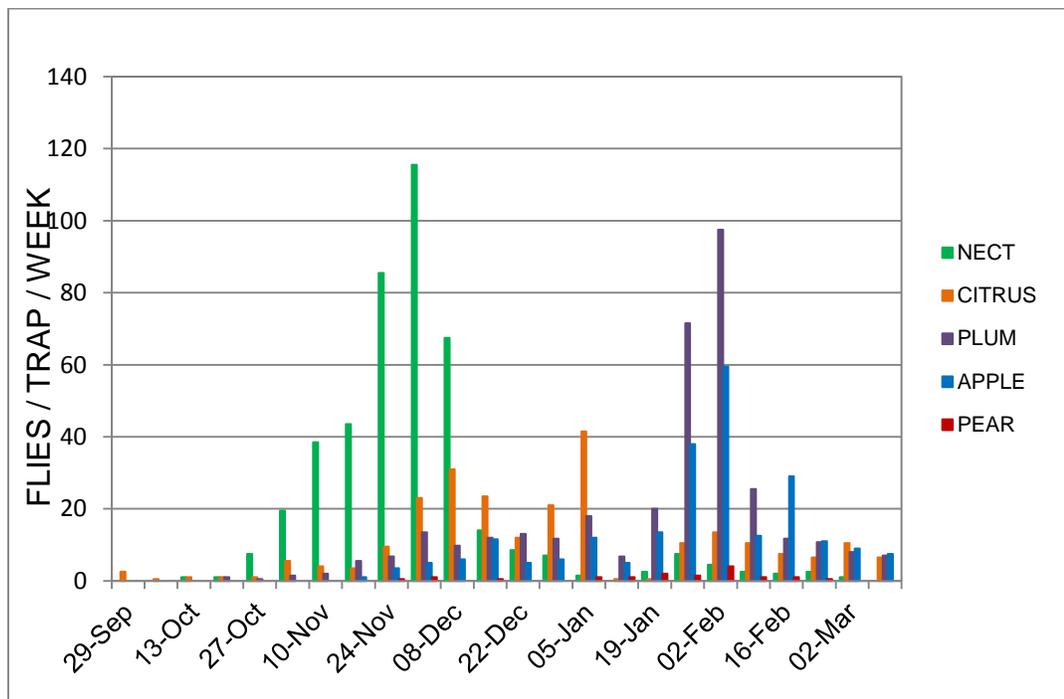


Figure 8. Numbers of Cape fly caught in Biolure-baited bucket traps at Welgevallen Farm, Stellenbosch in different fruit orchards, December 2013 – April 2014.

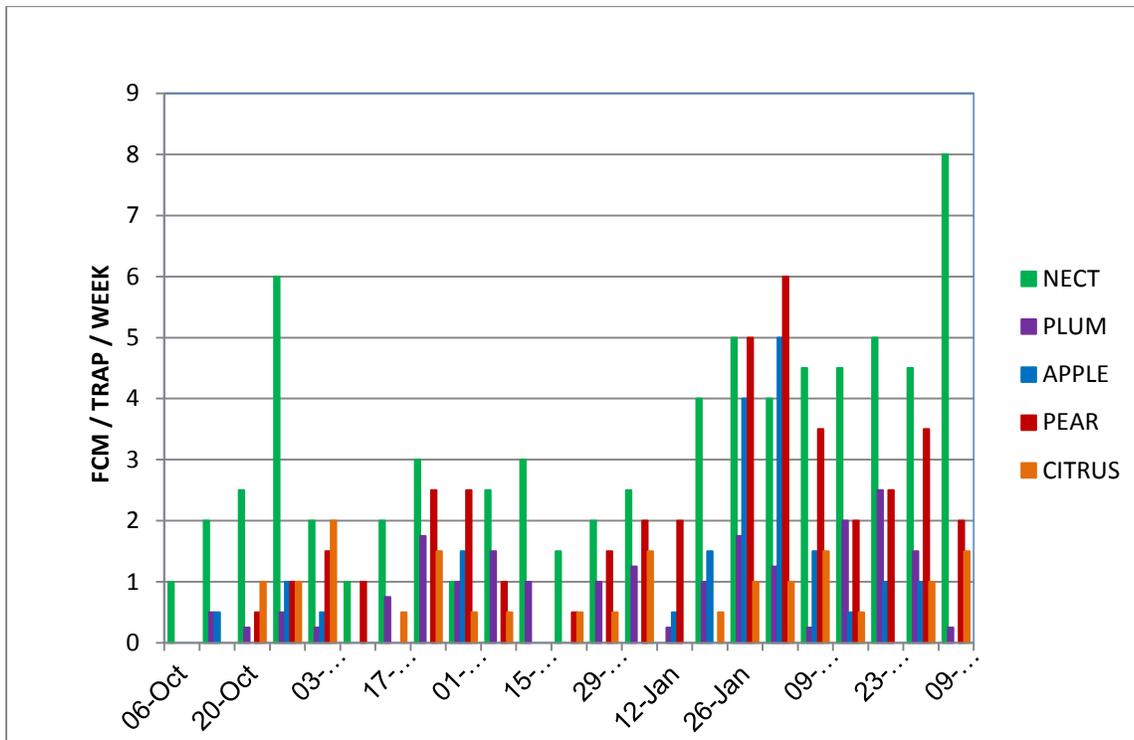


Figure 9. Numbers of FCM caught in yellow delta pheromone traps at Welgevallen Farm, Stellenbosch in different fruit orchards, October 2014 – March 2015.

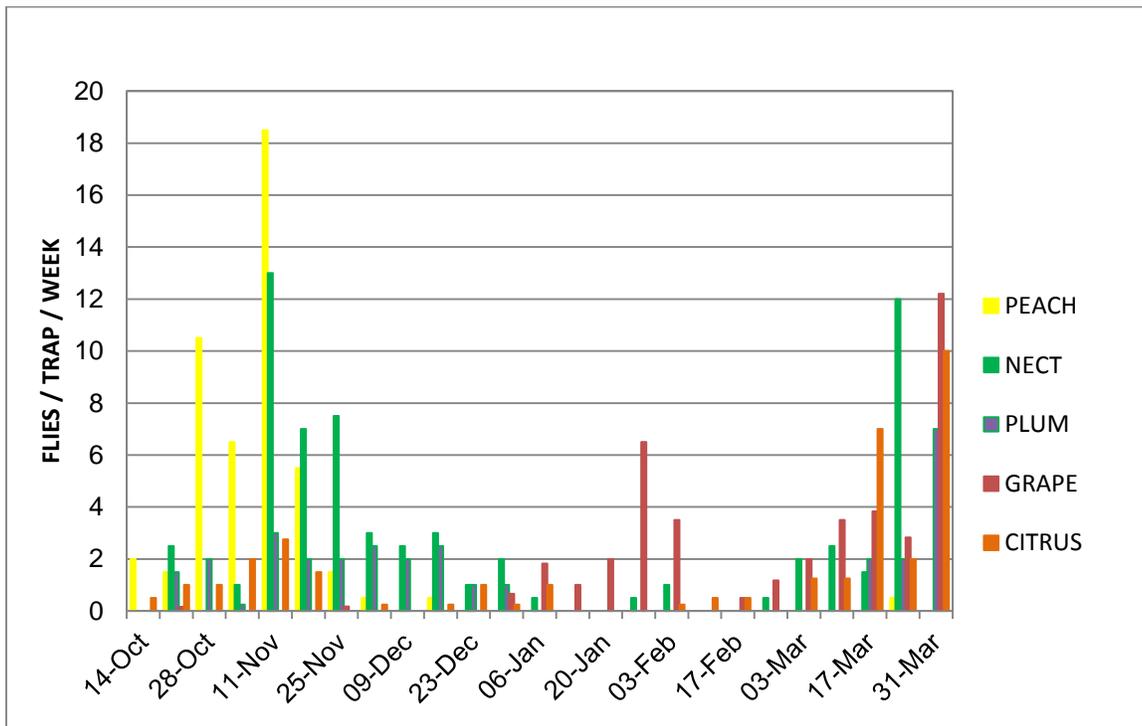


Figure 10. Numbers of Medfly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeeck Kasteel, in different fruit orchards, October 2014 – March 2015

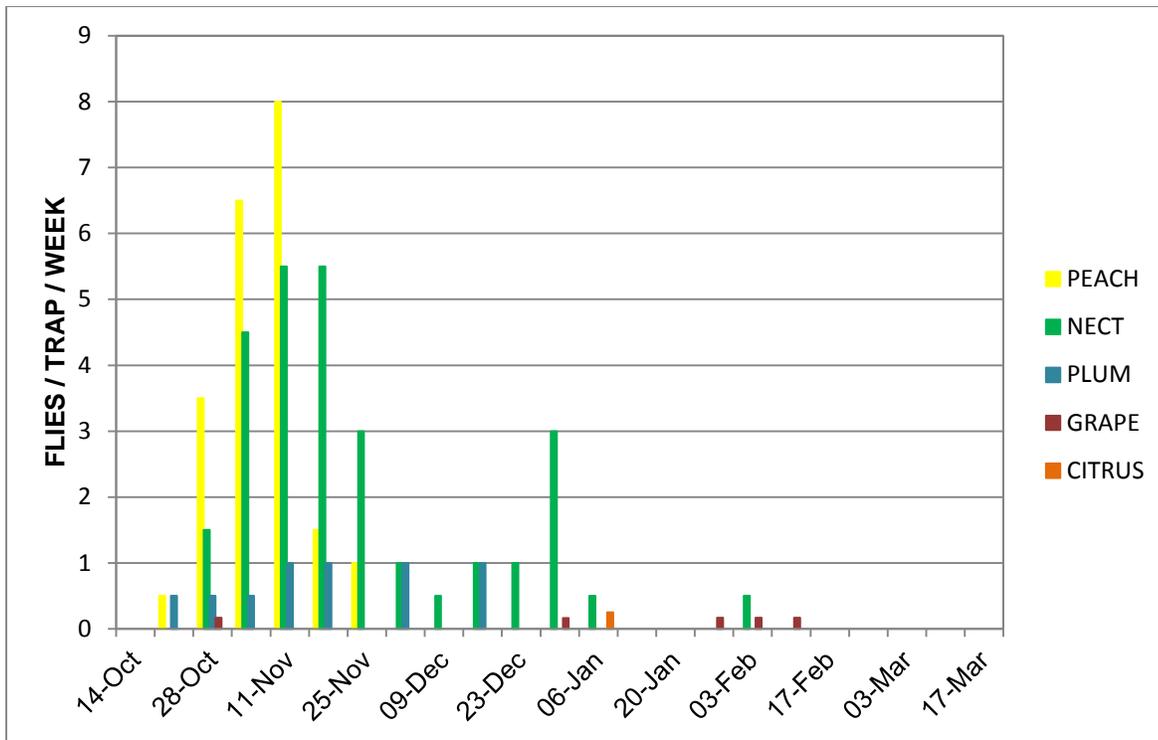


Figure 11. Numbers of Cape fly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeek Kasteel, in different fruit orchards, October 2014 – March 2015.

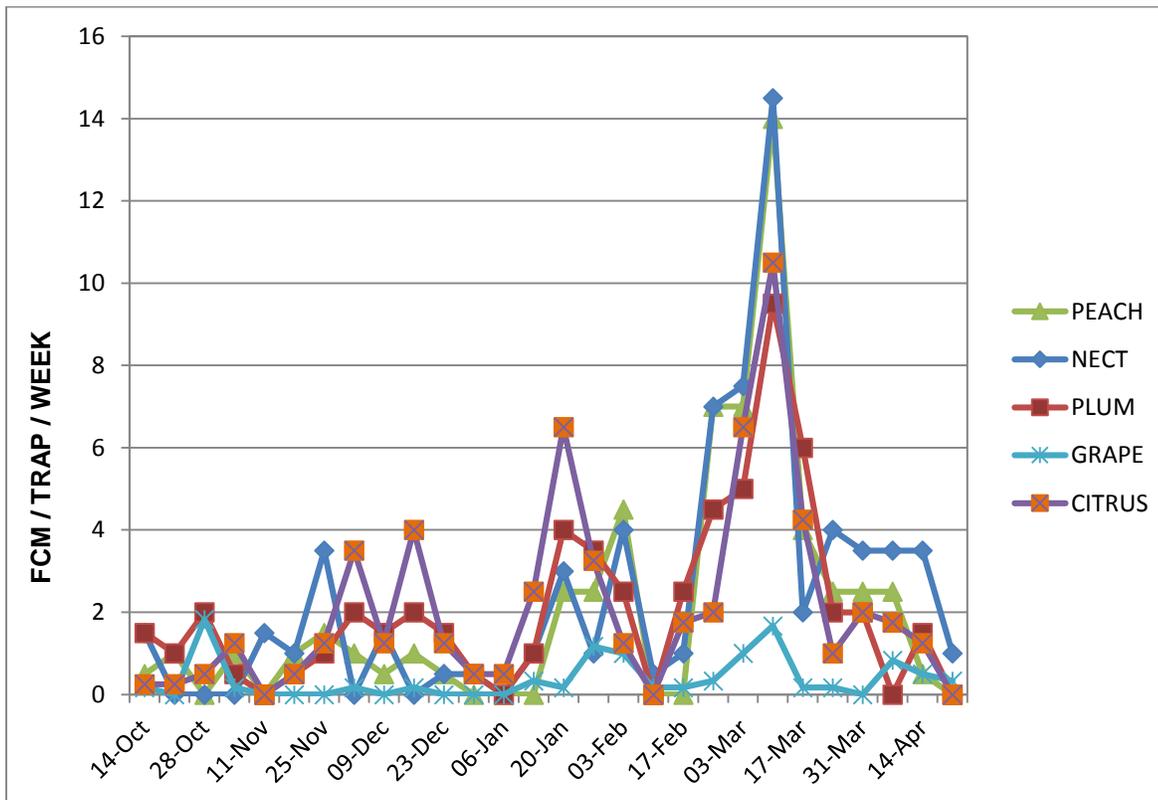


Figure 12. Numbers of FCM caught in yellow delta pheromone traps at Ebenhaezer Farm, Riebeek Kasteel in different fruit orchards, October 2014 – April 2015.

Season 2015/16

As in 2014/15, initial peaks of fruit fly activity in traps were correlated with the ripening of each fruit type. Trapping in autumn / winter revealed a similar pattern to 2014/15 in that, at the end of March / early April, in stone fruit orchards (nectarine, plum and peach) catches started to increase substantially. Numbers of flies trapped in nectarines were exceptionally high, peaking at 313 flies per week using Biolure traps in late April. The nectarine, plum and peach orchards had been harvested many months previously. The extremely high numbers in nectarines may possibly be related to the exudation of nectar by extrafloral nectaries which occurs on certain cultivars. Such high numbers of fruit flies pose a significant danger to nearby citrus blocks. At the very least, the ability to control fruit flies in nearby citrus would be placed under greater strain. High numbers persisted throughout May in all stone fruit and citrus orchards. Results of comparative fly catches between Biolure-baited traps and Capilure loaded Sensus traps are shown later in Fig. 18 after 3 years' data had been accumulated.

Regarding FCM, the peaks in male flight activity were once again not seen to be related to the ripening of each fruit type. The results obtained from Riebeek Kasteel were particularly clear in that peaks of moth activity from October 2015 to April 2016 were very similar in their timing, irrespective of which orchard type the traps were placed in. Peak flight activity of male FCM in specific fruit type blocks, as measured by presence in pheromone traps on multi-crop farms, is not always linked to fruit maturity. Flight activity can sometimes be extremely low / high in certain weeks in traps in all orchards (whether harvested or not) no matter what the fruit type. Flight activity can be high in stone fruit orchards that were harvested many months previously. Multi-crop growers should maintain a trap system in all orchards, no matter whether harvesting has occurred or not. The significance (risk) of high FCM counts in harvested orchards can only be estimated by fruit sampling / scouting in adjoining unharvested orchards.

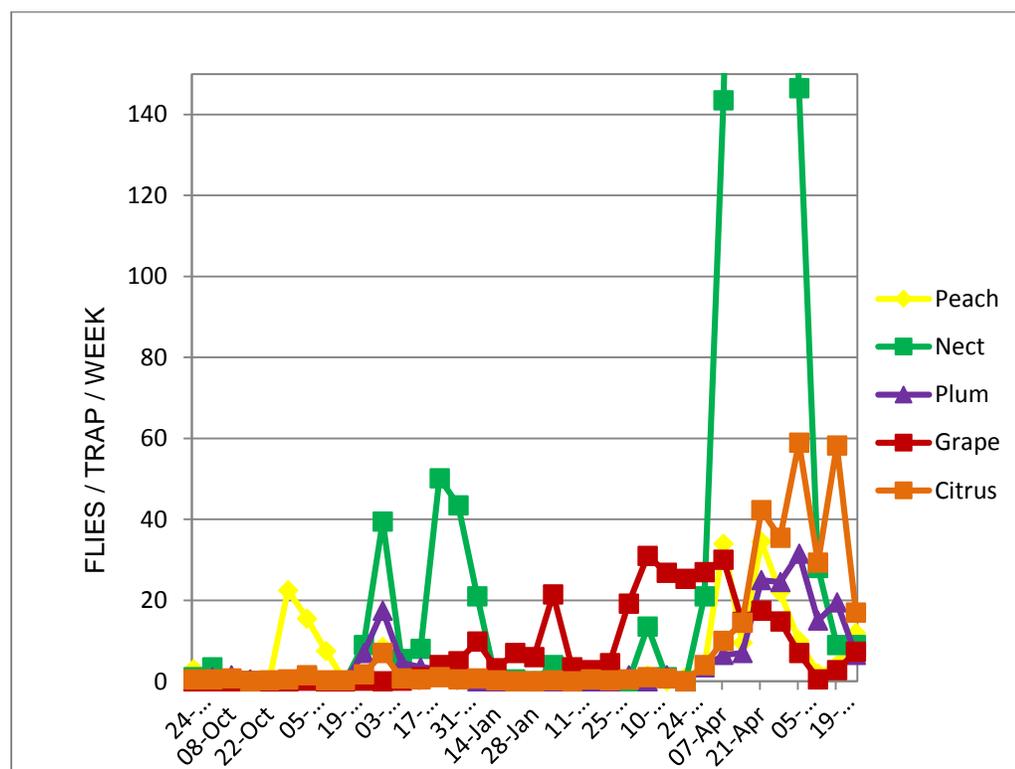


Figure 13. Numbers of Medfly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeek Kasteel, in different fruit orchards, Sep 2015 – May 2016

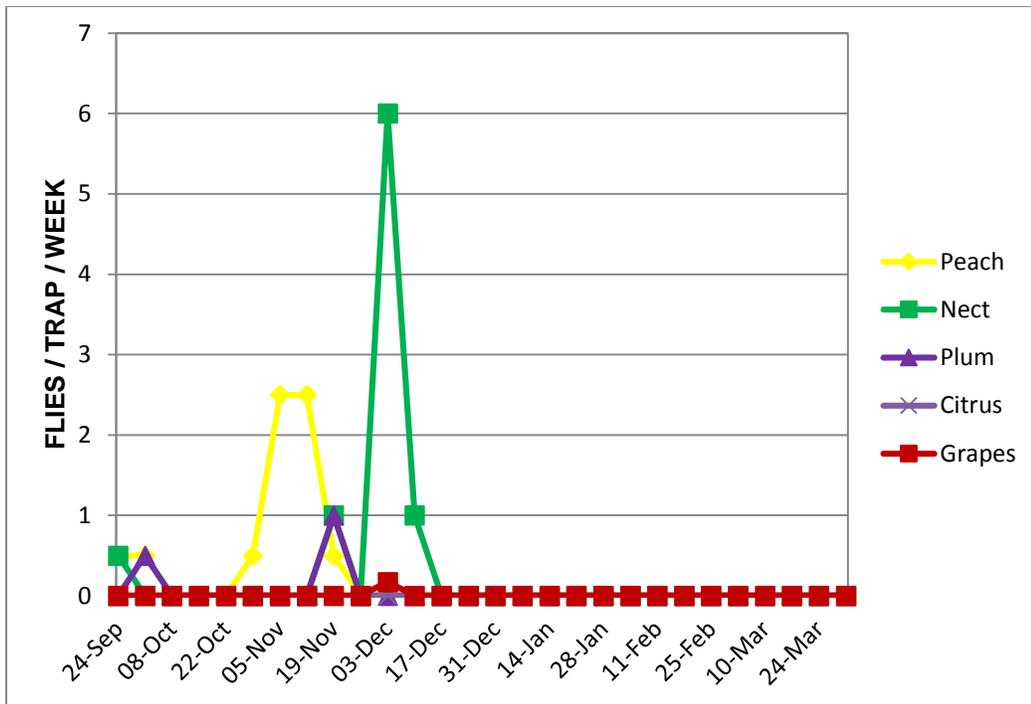


Figure 14. Numbers of Cape fly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeek Kasteel, in different fruit orchards, September 2015 – March 2016.

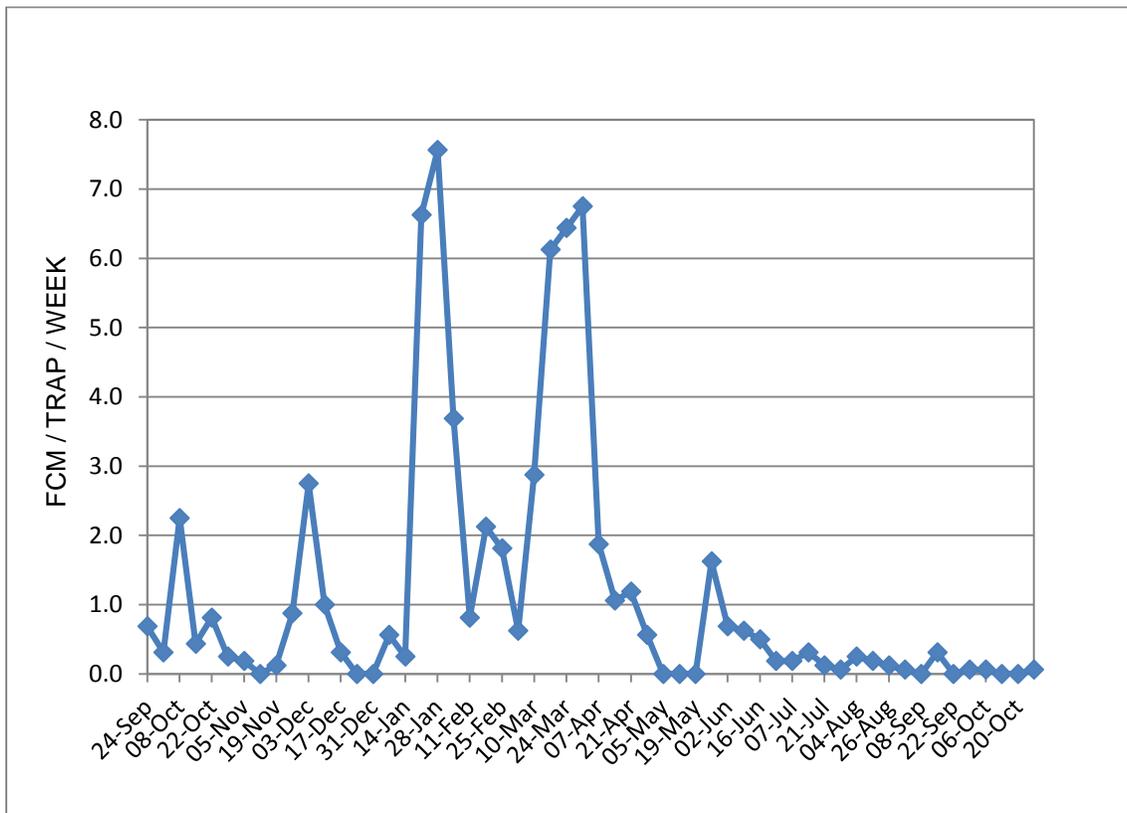


Figure 15. Numbers of FCM caught in yellow delta pheromone traps at Ebenhaezer farm, Riebeek Kasteel, all fruit crop types combined, September 2015 – October 2016.

At the Porterville farm, where fewer fruit types were cultivated (citrus and pomegranates only), Medfly numbers were considerably lower than at the more complex Riebeek Kasteel farm, not exceeding 12 flies per trap in any one week. Cape fly was very rarely caught, and not later than November. False codling moth numbers were also much lower, generally between 1 and 4 moths per trap without the pronounced peaks seen at Riebeek Kasteel. At Porterville, carob moth was also monitored using yellow delta traps with male attractant pheromone (Chempac, Paarl) because it is known as a pest of both pomegranates and citrus. Carob moth was regularly caught on both citrus and pomegranates at Porterville. This is in contrast to Riebeek Kasteel where a single indicator trap was maintained in a citrus block throughout the years of this experiment in which no carob moth at all was caught.

Season 2016/17

Weekly monitoring of FCM, fruit flies, and carob moth, continued during the 2016/17 season on two farms in the Western Cape. FCM and FF were monitored on nectarines, peaches, plums, table grapes, wine grapes and citrus at the first farm situated in Riebeek Kasteel. All three pest species (plus carob moth) were monitored in pomegranates and citrus at the second farm in Porterville.

The results of FCM trapping for the 2016/17 fruit-growing season are very different than those of the previous years studied. Late 2016 / early 2017 showed very low levels of FCM presence in all blocks at Riebeek Kasteel, with trap catches increasing the most in the citrus, but only to 2 per trap, in late April 2017. In general, in the Western Cape, much greater attention was focussed upon FCM incidence and control during 2016 / 17. This was as a result of increasingly strict export regulations on fruit crops regarding this pest. In October 2016, a Hortgro FCM seminar / information day was held in Stellenbosch to focus farmers' attention on this pest. The importance of ensuring "consignment freedom" from FCM on all fruit types was emphasised to the farmers that attended. Among other research presentations, Hortgro requested that a talk be given by the author to local farmers to help increase awareness of FCM population dynamics on stone fruit, grapes etc. on multi-crop farms and this was carried out. The low number of FCM caught during late 2016/ early 2017 may be attributed to improved control in the area following the FCM awareness campaign. To support this, Isomate supplies for the Western Cape were insufficient. Isomate could not be obtained for the continuation of project 1080. Fruit inspections were carried out on the tree for each fruit type for a number of weeks prior to harvest and showed no evidence of FCM infestation. The collection and monitoring of fruit collected after harvest and kept in the laboratory for approximately four weeks also produced no FCM adults or larvae. Fallen fruit from under five marked data trees, which was collected in each of the two citrus blocks, also indicated a very low level of fruit infestation.

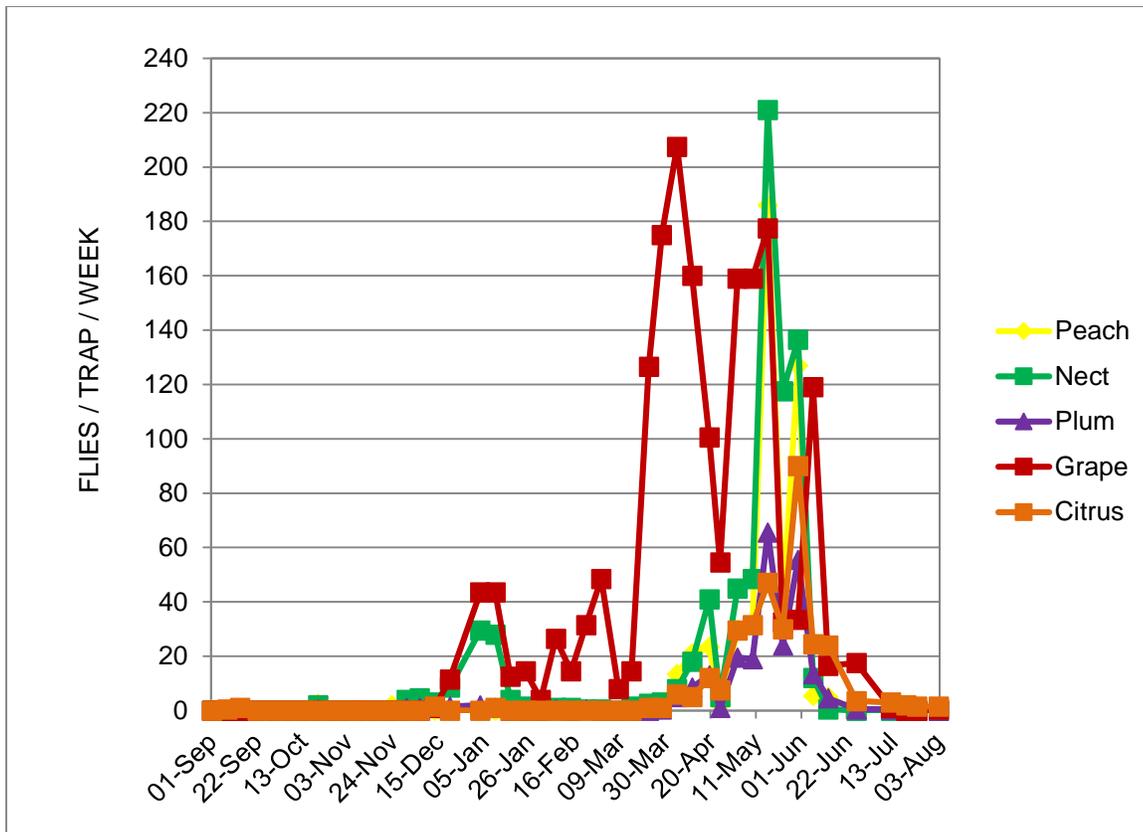


Figure 16. Numbers of Medfly caught in Biolure-baited bucket traps at Ebenhaezer farm, Riebeek Kasteel, in different fruit orchards, Sept 2016 – August 2017

Regarding fruit fly, numbers increased as fruits neared maturity and were harvested. However, fruit fly peaks were also recorded long after fruit harvest. The highest peak for fruit flies in citrus occurred in June 2016 with a mean of 102 flies/trap/week. Fruit fly traps placed in the nectarines recorded the highest average count/trap/week in 2016 with a mean of 545 flies. In 2017, table grapes recorded the highest numbers of fruit flies. Mediterranean fruit fly was the most common species recorded. Cape fly was present in very low numbers and only at cooler times of the year. The collection and analysis of fruit did not yield any fruit flies. At Porterville, an area where both citrus and pomegranates are grown, FCM counts remained below 4 per trap throughout 2016, with moth numbers generally being higher in the pomegranates than the citrus. Numbers increased in March and April 2017 with greater numbers in the pomegranates than in the citrus. The same trend was found for carob moth in both crops in 2016 being nearly all caught in the pomegranates. However, from January 2017 carob moth numbers increased substantially in the pomegranates with a peak mean of 13.5 moths / trap / week being recorded in this month and numbers remaining high throughout April and May. While peaks occurred in the citrus at this time, they were much lower than in the pomegranates. Nevertheless, citrus grown close to pomegranates could well be more vulnerable to false codling moth and carob moth attack than citrus grown without this crop in close proximity. This would have implications for different production areas in the Western Cape. Medfly numbers caught in pomegranate blocks were almost non-existent. Although Medfly is mentioned in a previously published list of pests recorded on pomegranates, the numbers trapped here indicate that this crop is not a preferred host for fruit flies. On the nearby citrus, a peak of 63 flies / trap / week was recorded in early June. Cape fly numbers were very low in both citrus and pomegranates. Between June and August 2016, pomegranates remaining on the tree after harvest were sampled and dissected to determine FCM and carob moth infestation. Ten randomly chosen fruit from each of two pomegranate orchards were collected weekly. An average of 9.1% of pomegranates sampled throughout the winter over 11 weeks were infested with FCM. This sampling was continued in 2017, with pre-harvest fruit being collected from February. In this case, five fruit from each tree were randomly sampled in order to limit losses for the grower, as it was difficult to detect infestation via fruit inspection on the tree.

Season 2017/18

In 2017 at Riebeek-Kasteel, FCM activity was low, but present in all the fruit type orchards during April to July. Peaks averages of 2 moths per trap per week were recorded in the citrus (May) and peaches (June). FCM activity began to increase towards the end of October 2017, with trap catches being highest in citrus from January to March 2018. In Porterville, FCM trap catches tended to occur more evenly throughout the season in both the citrus and pomegranate sites. Activity was generally higher in the pomegranate orchards, with a peak average of 12 FCM per trap per week being recorded in July in the pomegranates, compared to a peak average of five FCM per trap per week in the citrus. Peaks in trap catches tended to occur in both pomegranates and citrus at the same time, as also noted in late December 2017, when citrus peaked at an average of seven moths per trap per week and pomegranates at an average of 13 moths per trap per week. Carob moth was monitored at Riebeek-Kasteel throughout the season in the citrus, but no moths were caught. In contrast to Riebeek-Kasteel, carob moth activity was much higher at the Porterville site. Carob moths were trapped in both citrus and pomegranates during April in Porterville, but numbers were much higher in the pomegranates. Carob moth activity was low from mid-May to the end of September and began increasing again from October 2017, with slightly higher trap catches being recorded in the pomegranates. Carob moth activity was very high during 2016-17 season, but numbers decreased substantially during 2017-18.

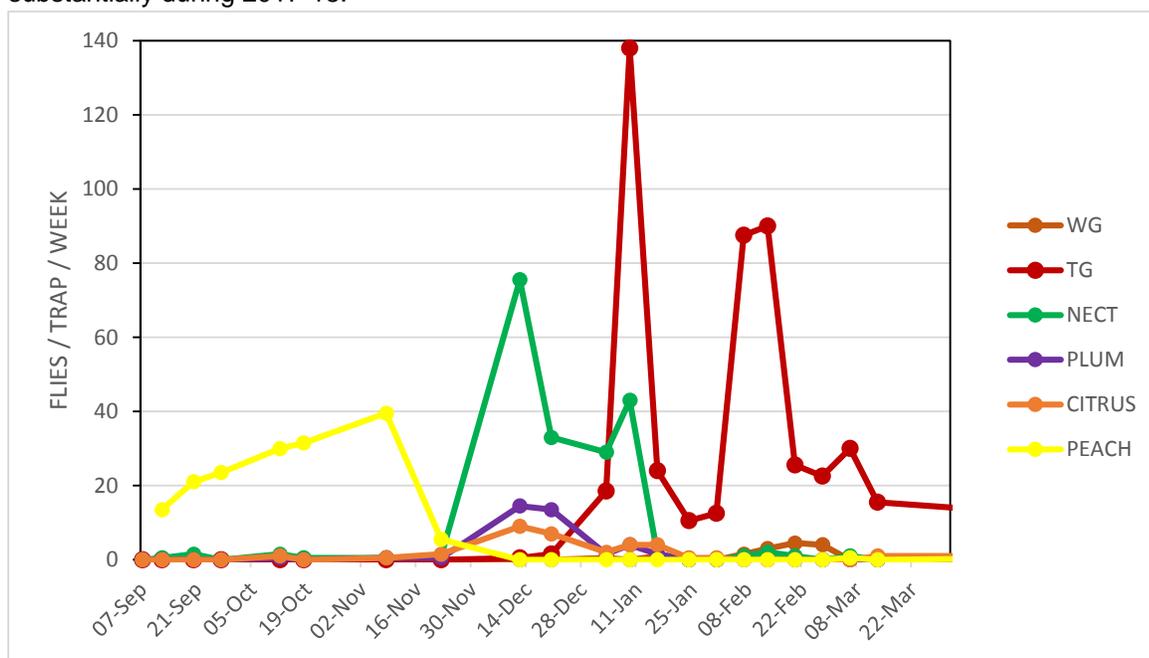


Figure 17. Numbers of Medfly caught in Biolure-baited bucket traps at Ebenhaezer Farm, Riebeek Kasteel, in different fruit orchards, September 2017 – March 2018

At Riebeek-Kasteel, Medfly peaks differed with different fruit crops' maturity. The highest counts were found for table grapes in April 2017 at a peak average of 207.5 flies per trap per week, with peaches, nectarines and citrus having peak fruit fly numbers of 186, 221 and 90 respectively in May. Fruit fly numbers decreased during July and remained very low until December 2017, increasing again in the nectarines, which were being harvested during this time. Table grape numbers began increasing from mid-December, reaching a peak average of 138 fruit fly per trap per week in mid-January 2018. Catches were low in the other crops, with an average of less than 15 flies per trap per week being caught in the peaches and citrus during January to March 2018. Cape fly numbers reached a peak average of 37 flies per trap per week in table grapes in April 2017, after which numbers dropped to almost zero for all fruit crops until December where trap catches increased the most in the nectarines, up to an average of 18.5 flies per trap per week. In Porterville, fruit fly activity in the citrus peaked with an average of 16 flies per trap per week in November 2017. Pomegranates are not a very suitable host for fruit flies and this was reflected

in the very low trap catch throughout the year as well. The same pattern applied to Cape flies, where more flies were trapped in the citrus than the pomegranates, but even these numbers were very low, with no more than 0.5 flies per trap per week being caught.

Comparison of Biolure and Capilure as attractants

Figure 18 depicts the results of Medfly trapping using Biolure-baited Yellow bucket traps and Capilure loaded Sensus traps from September 2015 to March 2018. It can be seen that population peaks revealed by the two different trapping methods are very closely correlated. This is despite the fact that Biolure tends to catch mostly females, and Capilure catches almost exclusively males. During the course of the experiment, a total of 3022 Medfly per Capilure trap were caught, of which 3010 (99.6%) were males and 12 (0.4%) were females. 938.5 Medfly were caught per Biolure trap, of which 373.5 (39.8%) and 565 (60.2%) were females.

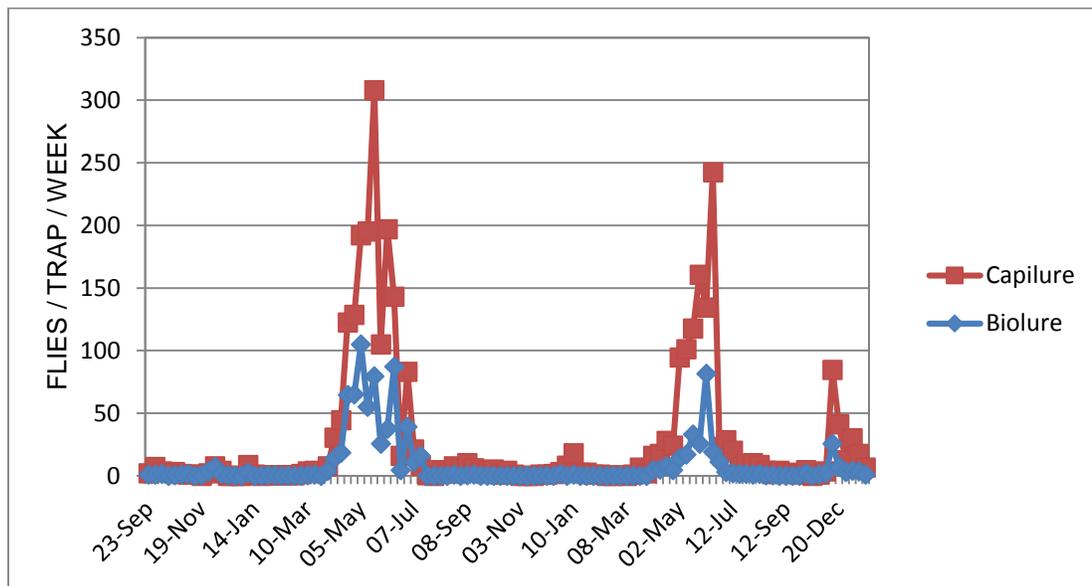


Figure 18. A comparison of the numbers of Medfly captured in Biolure-baited bucket traps versus Capilure loaded Sensus traps in a Satsuma orchard at Riebeek Kasteel, Sep 2015 – Jan 2018.

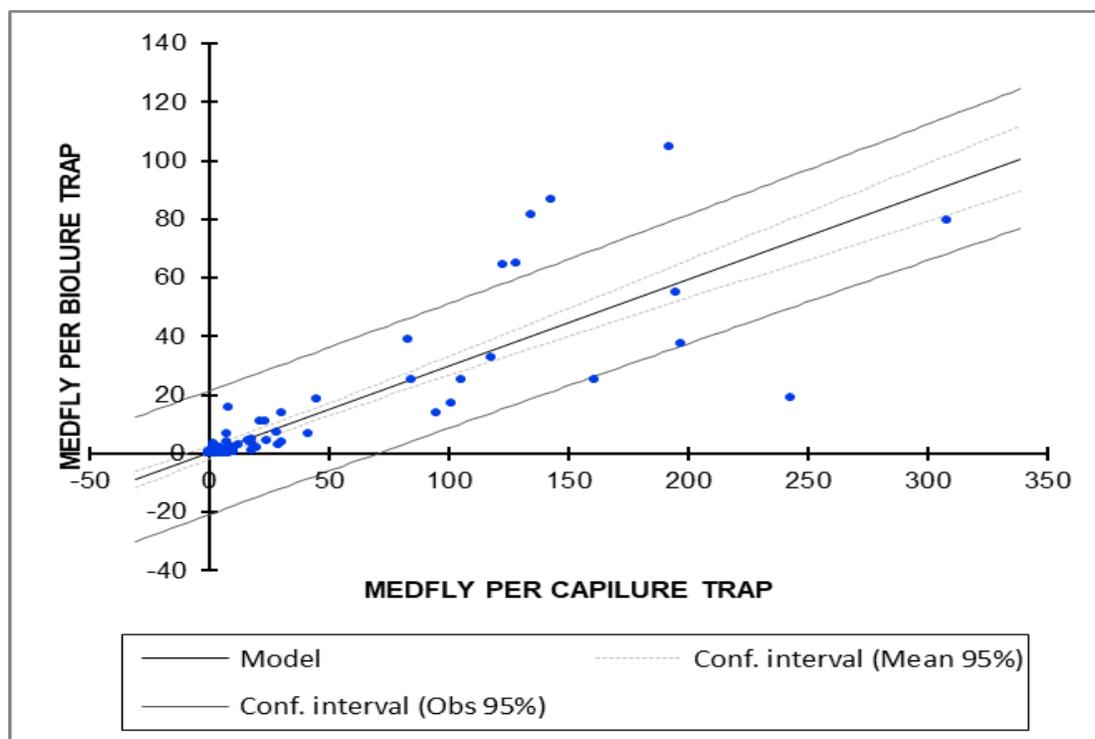


Figure 19. Linear regression of weekly numbers of male and female Medfly caught per trap in a) Biolure traps vs b) males in Capilure traps from September 2015 to March 2018, $R^2 = 0.71$, $P < 0.001$.

Linear regression was carried out, using XLSTAT 2018, on the observations to quantify any correlation between the numbers of the different sexes that were caught in the two types of traps. The strongest correlation (Fig. 19) was between numbers of males in Capilure traps and males plus females caught in Biolure traps with $R^2 = 0.71$ and $P < 0.001$. However, a regression of Capilure males vs Biolure females was almostly equally strong with $R^2 = 0.69$ and $P < 0.001$. A regression of Capilure males vs Biolure males yielded a poorer correlation with $R^2 = 0.62$, $P < 0.001$.

Overall, 5.3 male Medfly in a Capilure trap were caught for every 1 female Medfly in a Biolure trap. These results are a vindication of the threshold level for fruit fly control that is communicated to citrus growers. A number of years ago, the threshold was lowered from 7 male Medfly in a Capilure Sensus trap to 4 to introduce an additional level of security. Although traps that attract can never be used as an absolute measure of population density, the results show that at a level of 5 male Medfly in a Capilure-baited Sensus trap there is still female Medfly activity in the orchard that can be traced albeit with another type of lure.

Conclusion

Citrus, and fruit production in general, in the Western Cape is very different from that of other areas. Blocks of a particular fruit type tend to be small in comparison to those in other parts of South Africa. Many different types of fruit are grown on the same farm. Fruit flies attack all these different fruit: pome, stone, grape and citrus fruit types. False codling moth attacks stone fruit, grapes and citrus. The results of the project show, without adequate control and efficient post-harvest sanitation, how high numbers of these pests can persist in already picked orchards over long periods of time. Even where efficient post-harvest sanitation has been conducted properly, fruit flies can be found utilizing the cover afforded by foliage and probably feeding on honeydew from other insects (Israely et al. 1997). Only when the foliage was shed in winter in pome and stone orchards, and in vineyards, did fruit flies desert these niches.

Picking practices in pome, stone and grape blocks contribute to the likelihood of more fruit flies and false codling moth surviving to attack citrus later in the season. Grapes, particularly, are problematic as multiple picks are involved and so the process takes place over a long period. In any table grape block there are at least 3, and sometimes 4, separate picks: the 1st for well-shaped export grade fruit, the 2nd and perhaps 3rd for local market fruit and a 4th for the remainder to be sent for brandy distillation. Even for the final pick, sound grapes are required and so any bunches that are diseased or rotting are left in the vineyard. These tend to be left, either on the vine or on the ground creating ideal conditions for extended fruit fly and FCM survival. Cape fly was only seen to be a serious problem in the Stellenbosch area. Although this species was trapped at Riebeeek Kasteel and at Porterville it was only in very low numbers and in spring before temperatures became too hot.

FCM control in the areas monitored generally improved during the life of this project. A greater awareness of FCM was created by data that was generated by the project and by a Hortgro campaign to encourage farmers to control FCM on stone fruit in the Western Cape, which can only be good for citrus as well. By controlling FCM and fruit flies earlier in the season on other crops there should be less build-up of these pests in citrus orchards.

Future research

As mentioned in the results, the nectarine and plum cultivars involved in the monitoring experiment all possessed extrafloral nectaries. It is not yet known whether exudation of nectar from these nectaries contributes to the tendency of fruit flies and FCM to remain in orchards long after harvesting had taken place and should be further investigated.

The baiting (hanging of M3s) in vineyards and / or stone fruit orchards after harvest, creating a buffer zone, around citrus orchards particularly near to citrus harvesting time should be investigated and may help to reduce outside pest pressure on soft citrus. This is being addressed in project 1177.

Technology transfer

The following grower / scientific presentations utilized data generated by project 1081:

“Monitoring of FCM and fruit fly on multi-crop farms”: Talk presented at growers’ meeting at Robertson, Western Cape, October, 2015.

“Monitoring of fruit flies (Tephritidae) on multi-crop farms in the Western Cape Province of South Africa”: Poster presented at TEAM (Tephritid workers group) fruit fly symposium, Spier Estate, Stellenbosch, Western Cape, April 2016.

“Monitoring of FCM and fruit fly on multi-crop farms”: Talks presented at growers’ workshop at Ashton, Western Cape and Kakamas, Northern Cape, May 2016.

“Population fluctuations of Medfly & False Codling Moth on Western Cape multi-crop farms”: Talk presented at 9th Citrus Research Symposium, Drakensberg, Kwa-Zulu Natal, August 2016.

“The autecology of fruit flies (Tephritidae) & false codling moth (Tortricidae) on multi-crop farms in the Western Cape region of South Africa”: Talk presented at International Congress of Entomology, Orlando, Florida, USA, September 2016.

“Monitoring of False Codling Moth in orchards of stone & pome fruit, citrus and vineyards”: Talk presented at Hortgro FCM seminar, Stellenbosch, Western Cape, October 2016.

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2.2.3 FINAL REPORT: Entomopathogenic fungi for control of soil-borne life stages of FCM

Project 1024 (Mar 2011 – Mar 2018) by Sean Moore (CRI), Martin Hill (RU), Jo Dames (RU), Candice Coombes (RU), Wayne Kirkman (CRI), Martin Gilbert (CRI), Kim Stoltz (CRI), Claire Love (CRI), Mat Goddard (RU) and Sean Thackeray (RU/CRI)

Summary

The efficacy and persistence of three EPF isolates against FCM has been investigated under a variety of field conditions during the course of five field trials initiated over the 2013/2014 or 2014/2015 citrus growing season. Results have been exceptional thus far with an 80% reduction in FCM infestation recorded for isolate G Ar 17 B3 under micro-sprinkler irrigation, and 60% for isolate FCM Ar 23 B3 under drip irrigation. In addition, G Ar 17 B3 has shown to be an effective control measure when applied approximately two months before harvest, reducing

infestation in fruit by between 50 and 80%. In all trials, a reduction in fruit drop within all treatment blocks was also found. All isolates were found to persist for five months following application, with increases in fungal titre noted at some sites. Results suggest the better persistence of fungi in wetter soils than drier soils, an outcome of the irrigation system employed. In addition to field efficacy and persistence, the compatibility of these isolates with eight fungicides registered has been evaluated, indicating that only mancozeb might pose a minor problem for EPFs. Preliminary formulation assessments were also conducted. Candice Coombes completed her thesis and was awarded a PhD for this study. Research now focused on additional benefits of EPF application, including sublethal effects on eclosing FCM, efficacy of these isolates and FCM eggs and neonates as well as completing the compatibility assessment of these EPFs with commonly applied agrichemicals in citrus. Attempts were also made to determine a way to genetically identify these EPF strains after application in the field. However, the funded project has now come to an end.

Opsomming

Die doeltreffendheid en volharding van drie EPS-isolate teen VKM is ondersoek onder 'n verskeidenheid veldtoestande gedurende vyf veldproewe wat gedurende die 2013/2014- of 2014/2015-sitrusgroei-eisoen geïnisieer is. Die resultate was tot dusver uitsonderlik met 'n 80% -verlaging in VKM-besmetting wat vir isolaat G Ar 17 B3 aangeteken is onder mikrobeprosprinkelbesproeiing, en 60% vir isolaat FCM Ar 23 B3 onder drupbesproeiing. Daarbenewens het G Ar 17 B3 getoon dat dit 'n effektiewe beheermaatreël is wanneer dit ongeveer twee maande voor oes toegepas word, wat besmetting in vrugte tussen 50 en 80% verminder. In alle proewe is ook 'n afname in vrugval binne alle behandelingsblokke aangeteken. Alle isolate is gevind om te bly voortduur vir vyf maande na toediening, met toenames in swam titer wat op sommige plekke aangetoon word. Resultate dui op die beter nawerking van swamme in natter gronde as droër gronde, 'n uitkoms van die besproeiingsstelsel wat gebruik word. Benewens die doeltreffendheid en nawerking in die veld, is die verenigbaarheid van hierdie isolate met agt geregistreerde swamdoders geëvalueer, wat aangedui het dat slegs mancozeb 'n geringe probleem vir EPSe mag wees. Voorlopige formuleringsevaluasies is ook uitgevoer. Candice Coombes het haar tesis voltooi en 'n PhD vir hierdie studie ontvang. Navorsing is nou gefokus op addisionele voordele van EPS toediening, insluitend subletale effekte op ontpoppende VKM, die doeltreffendheid van hierdie isolate op VKM eiers en pasuitgeborede larwes asook die verenigbaarheid van hierdie EPSe met algemeen gebruikte chemiese plaagdoders in sitrus. Pogings is ook aan die gang om 'n metode te identifiseer om hierdie EPS isolate geneties te identifiseer na toediening in die veld. Die bevondse projek het egter tot einde gekom.

Introduction

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (1912) (Lepidoptera: Tortricidae) is considered one of the most economically damaging pest of citrus in South Africa (Grout & Moore 2015). FCM causes both pre- and post-harvest damage to fruit and in addition, is classified as a phytosanitary pest by lucrative export markets. As the bulk (74%) of citrus in South Africa is exported to these markets, the control of this pest is critical and is aimed at as close to zero as possible (CGA 2013; Moore 2012; Grout & Moore 2015). Due to stringent chemical restrictions placed on exported fruit, FCM control relies strongly on an integrated pest management (IPM) approach (Moore & Hattingh 2012). To date, only one commercial biological control product has been registered for use against the soil-dwelling life stages of FCM, whilst numerous products are registered against the above-ground life stages (Moore & Hattingh 2012; Moore 2016). In an attempt to provide greater control of the soil-dwelling life stage, entomopathogenic fungi (EPFs) have been under investigation since 2008/9 through collaboration between researchers at Citrus Research International (CRI) (Port Elizabeth, South Africa) and Rhodes University (RU) (Grahamstown, South Africa). Between 2013 and 2015, three fungal isolates, *M. anisopliae* FCM Ar 23 B3, *M. anisopliae* G 11 3 L6 and *B. bassiana* G Ar 17 B3, were shown to be capable of reducing FCM infestation within conventional citrus orchards by up to 80% (Coombes *et al.* 2016). Since then, the effects of these fungi on above-ground, targetable life stages (eggs and neonates) of FCM, the potential benefit of sub-lethal effects on eclosing FCM adults, and the compatibility of these isolates with commonly used insecticides, has been determined.

Objectives

Objectives 1 – 3 were undertaken during the Apr 2011 – Mar 2013 period

Objective 1: Complete dose-response and time-response laboratory bioassays with all of the isolates which showed good potential, against *T. leucotreta* 5th instar larvae, prepupae and pupae according to Goble *et al.* (2010)

Objective 2: Produce sufficient fungal spores of each of one or two of the most virulent strains for field trials

Objective 3: Determine efficacy and persistence in semi-field trials

Objectives 4 – 10 were completed during the Apr 2013 – Mar 2016 period.

Objective 4: To determine the most cost-effective means of mass producing each isolate

Objective 5: To identify a suitable formulation for field application

Objective 6: To determine an appropriate application mode for the fungal formulation

Objective 7: To evaluate the efficacy of each isolate in the field under semi-controlled conditions

Objective 8: To evaluate the efficacy of each isolate in the field following soil application to 1 ha treatment blocks

Objective 9: To determine the compatibility of each isolate with commonly applied agrichemicals

Objective 10: To further evaluate the field persistence of each isolate following soil application

Objectives 11 – 18 were undertaken during the Apr 2016 – Mar 2017 period.

Objective 11: Establish a mass production protocol

Objective 12: Identify a suitable formulation for field application

Objective 13: Investigate sub-lethal effects on FCM fecundity, longevity and developmental rate

Objective 14: Investigate fungal efficacy against FCM neonates

Objective 15: Investigate non-target effects (other biological control agents, beneficial insects etc.)

Objective 16: Investigate compatibility of fungal isolates with other commonly applied agrichemicals incl. lime and gypsum

Objective 17: Identify the most cost-effective application rate

Objective 18: Conduct sufficient field trials to enable registration, incl. trials in other regions

Objectives 19 – 20 were undertaken during the Apr 2017 – Mar 2018 period.

Objective 19: Complete/Initiate unachieved objectives listed for the period Apr 2016 – Mar 2017. This included objectives 12, 13, 14 and 16 detailed above

Objective 20: Genetic markers for strain identification

Materials and methods

Objectives 1 to 3 (for more detailed protocols see Coombes 2013 – MSc thesis)

Fungal cultures: Initially, all 12 fungal isolates identified by Goble *et al.* (2010) as capable of inducing greater than 80% mycosis in FCM soil-dwelling life stages were screened at a standard 1×10^7 conidia/ml concentration to assess whether attenuation (=reduced virulence) had occurred as a result of poor storage. Four isolates (*Beauveria bassiana* isolates: FF J&B R5, G Moss R10, G OL R 11 and FCM Rose R9) were excluded from subsequent FCM-EPF bioassays as a result. Thus, only eight isolates were investigated during FCM-EPF dose-response bioassays (*Metarhizium anisopliae* isolates: G OL R8, G 11 3 L6, G 14 2 B3 and FCM Ar 23 B3; *Beauveria bassiana* isolates: FCM 10 13 L1, G B Ar 23 B3, G Ar 17 B3 and G 14 2 B5), and only the three isolates regarded as most virulent were investigated during FCM-EPF time-response bioassays (G 11 3 L6, FCM Ar 23 B3 and G Ar 17 B3). All fungi were cultured on Sabouraud 4% dextrose agar supplemented with 50 mg/L rifampicin, 50 mg/L chloramphenicol and 1 ml/L iodine. Plates were always incubated at 26°C on a D12:L12 photoperiod for two to three weeks before use.

FCM-EPF dose-response bioassays: For each isolate investigated, four conidial concentrations were prepared. For each concentration, 5 ml was well mixed with 50 g autoclaved sieve sand to which 20 FCM fifth instars ready to pupate within 24 h, were added. A control was included (water only treatment). After one week, pupae were removed from their pupal casings and placed onto sterile soil housed in petri dishes over which eclosion chambers were placed. Any deceased individuals were surface sterilised and plated onto SDA plates and scored for mycosis after 3 days. The experiment was terminated 10 d after the first adult moth eclosed. The number of adult moths was recorded. Any pupae which failed to eclose were scored for mycosis as described above. Probit analysis, using the software PROBAN, was used to calculate the LC₅₀ and LC₉₀ for each tested isolate.

FCM-EPF exposure time-response bioassays: For each isolate, two conidial concentrations were tested. Bioassays were the same as described above, but FCM were exposed to each concentration for varying time periods. Logit analysis was used to determine the LT₅₀ and LT₉₀ for each isolate at each concentration.

Semi-field persistence trials: This trial was carried out in a citrus orchard at Mosslands. Briefly, 0.5 g of formulated product (fungal colonized rice grains) was mixed with 100 g of sterile sieved soil housed in net bags. These bags were buried approximately 5 cm below the soil surface underneath the tree canopies. Every month, for a period of six months, four bags were removed for each isolate (G 11 3 L6, FCM Ar 23 B3, G Ar 17 B3, control and two isolates used in commercial mycopesticides) and the number of colony forming units (CFUs) per gram of soil determined. Kruskal-Wallis analysis was used to statistically compare the CFUs/g monthly for each isolate. FCM fifth instars were also added to a sub-sample of each sample every month to determine % mycosis.

Application pot-trial: This trial replaced the previously suggested semi-field trial using mulch. Only two fungal isolates were used (G 11 3 L6 and G Ar 17 B3). A high and low concentration of two formulations – fungal colonized rice and conidial suspension – was applied to 400 g sterile soil either before the addition of ten FCM fifth instar larvae or 1 d thereafter. Tubs were house at 26° for four weeks and were monitored weekly for adult eclosion. Deceased individuals were scored for mycosis as previously described.

Objectives 4 to 10 (for more detailed protocols see Coombes 2016 – PhD thesis)

Semi-field cage trials: This trial was conducted in an organic 22-year-old Palmer Navel orange citrus orchard. Breathable mesh cages were buried within the upper soil layer, underneath the canopy of citrus trees and inoculated with the fungal isolates G 11 3 L6, FCM Ar 23 B3 and G Ar 17 B3 at three different concentrations – low, intermediate and high (0.5×10^{14} , 1×10^{14} and 2×10^{14} spores/ha, respectively) – and in the presence or absence of a mulch. A commercial mycopesticide, Broadband® (a.i. *B. bassiana* PPRI 5339) (BASF, South Africa) and untreated controls were included. Immediately after fungal application, 30 fifth instar larvae were allowed to drop into the cages to pupate. Following trial termination, the percentage eclosion was determined. Corrected FCM mortality within each treatment was calculated using Abbott's formula and analysed statistically via Kruskal-Wallis ($P < 0.05$) followed by a multiple comparison of mean ranks post-hoc test. Each treatment consisted of eight replicates.

Field trials: Five field trials were carried out over the 2013/2014 and 2014/2015 growing season in citrus orchards in the Sunday's River Valley area, Addo, Eastern Cape, South Africa. Three field trials (Atmar 1, Atmar 2 and Oranjelus) assessed the efficacy of the applied EPF isolates in reducing FCM infestation when applied early in the growing season before the onset of fruit drop (late October) whilst the remaining two field trials (Marwell and Stenhope) evaluated the efficacy of the applied isolates in reducing FCM infestation following application later in the growing season (mid-March), two or three months prior to harvest.

Application of fungi occurred as an aqueous suspension, water supplemented with the surfactant Break-thru®S240 (Evonik Industries, South Africa). Application occurred to the soil surface underneath the canopy of the citrus trees (navel oranges) in the late afternoon/early evening using a spray machine and hand-held spray guns. Treatment blocks were approximately 1 ha. At Atmar 1, isolates G 11 3 L6, FCM Ar 23 B3 and G Ar 17 B3 were applied at a

rate of 5×10^{13} spores/ha. At Atmar 2, only isolates FCM Ar 23 B3 and G Ar 17 B3 were applied at the same rate. At Oranjelus, isolates FCM Ar 23 B3 and G Ar 17 B3 were applied at three different rates $0.01 \times$, $0.1 \times$ and 5×10^{13} spores/ha. Only Bb1 was applied at Marwell and Stenhope. At Marwell application occurred at three different rates ($0.01 \times$, $0.1 \times$ and 5×10^{13} spores/ha); Stenhope, 5×10^{13} spores/ha (Here, Bb1 was applied via the sprinkler system).

For all field trials, the efficacy of the applied isolate was monitored via fruit drop surveys to determine the percentage FCM infestation within each treatment block in comparison to the control. Twelve centrally located data trees were used for each treatment.

Field persistence: During the course of field trials at Atmar 1, Atmar 2 and Oranjelus, to which fungi were applied at 5×10^{13} spores/ha, soil samples were collected at monthly intervals to determine the ability of the fungus to persist over a five month period, under a variety of conditions. A sample comprised of 20 sub-samples, which were collected using a soil corer (diameter = 7 cm; depth = 5 cm). The CFUs/g of soil was determined for each isolate. Fungal persistence was not recorded for trials conducted at Marwell and Stenhope, although the number of CFUs/g was recorded two weeks after trial initiation at Marwell and one week post-application and upon trial termination at Stenhope.

Physical suspension characteristics and toxicity of adjuvants: Dry aerial conidia of FCM Ar 23 B3 and G Ar 17 B3 were exposed to five adjuvants with different chemical properties and their ability to suspend, remain in suspension and resuspend after settling out was determined using a protocol adapted for that outline for chemical pesticide formulation testing in the CIPAC handbook Volume F. Fungal viability after 24 h exposure to the adjuvants was also determined by assessing percentage germination. The five adjuvants tested were: Breakthru® S240, Breakthru® S233, Breakthru® OE446, Breakthru® Advance and BP Medium Oil.

Fungus-fungicide compatibility (in vitro): The compatibility between isolates FCM Ar 23 B3 and G Ar 17 B3 and eight fungicides registered for use in citrus against CBS was determined. Vegetative growth, spore viability and sporulation was monitored on both fungicide-amended media and non-amended media (SDA only) after 1 h exposure to each fungicide. Fungicides were used at their highest recommended field rate. The fungicides investigated were: Benomyl (a.i. benomyl), Cabrio (a.i. pyraclostrobin), Copper oxychloride (a.i. copper oxychloride), Dithane M45 (a.i. mancozeb), Flint (a.i. trifloxystrobin), Fungaway (a.i. azoxystrobin), Pennfluid (a.i. mancozeb) and Sporekill (a.i. didecyldimethyl-ammonium chloride).

Objectives 11 to 18

Sublethal effects of fungi on FCM: FCM 5th instars were exposed to a range of fungal concentrations in sand-conidial bioassays. Eclosed adults were sexed and paired. The number of eggs oviposited and hatched was recorded. The longevity of the moths and time to eclosion was also recorded.

Fungal efficacy against FCM neonates: Field-collected oranges were surface sterilised in bleach, rinsed three times in distilled water and allowed to dry overnight. Initially, oranges were submerged for 30 s in a container housing a 1×10^7 and 2×10^6 conidia/ml concentration of the tested fungi (FCM Ar 23 B3, G Ar 17 B3 and Broadband). Two neonates (hatched within 24 h), were carefully placed onto each orange once dried. The trial was terminated 21 d thereafter and the number of FCM infested fruit recorded. Failing to achieve satisfactory results, a second attempt to assess efficacy was undertaken, with the exception that oranges were sprayed (using a handheld spray bottle) with 5 ml of the appropriate fungal suspension.

Fungal efficacy against FCM eggs: FCM were coupled and allowed to copulate overnight in plastic 20 ml pill vials. Adult females were then allowed to either oviposit on fungal-treated wax paper for 24 h. The number of eggs were counted, placed in petri dishes lined with moistened filter paper and incubated at 26°C. After 7 d, the percentage of eggs which hatched per treatment was determined. Similarly, FCM were allowed to oviposit on untreated wax paper for 24 h after which the number of eggs were counted and then treated (via spraying) with various

concentrations of fungal suspensions. Incubation occurred as described. Controls were included and 10 replicates were carried out per treatment. The entire experiment was repeated twice more. The fungal isolates tested were FCM Ar 23 B3, G Ar 17 B3 and the commercial mycopesticide, Broadband.

Fungus-chemical compatibility: The compatibility between isolates FCM Ar 23 B3 and G Ar 17 B3 and nine registered insecticides, was evaluated. The protocol followed that outlined for the fungus-fungicide experiments. Nine insecticides were tested: Agrimec (a.i. abamectin), Beretta (a.i. buprofezin), Dursban (a.i. chlorpyrifos), Hunter (a.i. chlorfenapyr), Klartan (a.i. tau-fluvalinate), Delegate (a.i. spinetoram), Mylomex 900 SP (a.i. methomyl), Scalex (a.i. pyriproxyfen) and Suprathion (a.i. methidathion). Insecticides were used at their highest recommended field rate.

Additional field trials:

Fungal isolates FCM Ar 23 B3 and G Ar 17 B3 were applied as before, but at more economically feasible application rates (5×10^{11} and 5×10^{12} conidia/ha) in an orchard of mature Palmer Navel orange trees on Hillside, Panzi Citrus Farms in the Eastern Cape over the 2015/16 growing season. Commercial mycoinsecticide treatment blocks (Eco-Bb and Real *Metarhizium*) were included. Commercial products were applied at 5×10^{12} conidia/ha. One FCM pheromone trap was hung in the centre of each treatment block and monitored each week from 4 November 2015 to 17 March 2016. Fruit infestation was evaluated weekly from 7 January to 17 March 2016 by analysing fruit drop underneath 12 trees in the centre of each treatment block.

A field trial was conducted in an orchard of mature Washington Navel orange trees on Crocodile Valley Estates in Mpumalanga, using the same rates as described for the Eastern Cape trial. However, there was no space to include any commercial products. Treatments were applied on 22 January 2016. FCM traps and infestation were monitored exactly as described above. Fruit infestation was evaluated from 16 February to 19 April 2016.

A similar trial was conducted in a mature orchard of Navelina Navel orange trees on Gelukwaarts Farm in Porterville, Western Cape, using Broadband as a commercial “standard”. However, it was applied to the soil in the same manner as the experimental EPFs, contrary to its registration as a foliar application. Treatments were applied on 27 January 2016 and fruit infestation was evaluated from 26 February to 20 April 2016 as described above.

Objectives 19 to 20

Genetic markers for strain identification: DNA was extracted from *B. bassiana* and *M. anisopliae* isolates using the modified salting-out-protocol. PCR and REN analysis (using three different restriction enzymes: EcoRI, HindIII and BamHI) was performed using established protocols. PCR samples were sent to Macrogen for sequencing of the ITS region.

Results and discussion

Obj. 1 - see Coombes (2013) or Coombes et al. (2015) for more detailed results and discussion

Dose-response bioassays identified the three most virulent fungal isolates (based on calculated LC50 values) against FCM fifth instars: *M. anisopliae* G 113 L6, *M. anisopliae* FCM Ar 23 B3 and *B. bassiana* G Ar 17 B3 (Table 1). In accordance with other similar literature, FCM mortality was dose-dependent.

Table 1. LC₅₀ and LC₉₀ values calculated using data generated from dose-response bioassays for each fungal isolate tested including two isolates used as the active ingredients of two commercial mycopesticides (Eco-Bb and Real *Metarhizium*). The three most virulent isolates are highlighted.

Fungal species	Isolate	LC ₅₀ (±SE)	LC ₉₀ (±SE)
<i>Metarhizium anisopliae</i>	G OL R8	4.58×10^6 (3.22)	3.09×10^8 (4.72)

	G 11 3 L6	6.26 × 10 ⁵ (1.93)	1.91 × 10 ⁷ (1.39)
	G 14 2 B3	5.64 × 10 ⁷ (10.0)	2.20 × 10 ¹⁰ (7.88)
	FCM Ar 23 B3	1.92 × 10 ⁶ (3.65)	1.67 × 10 ⁸ (2.23)
	ICIPE 69 (Real <i>Metarhizium</i>)	2.60 × 10 ⁷ (4.12)	2.08 × 10 ¹⁰ (7.14)
<i>Beauveria bassiana</i>	FCM 10 13 L1	2.88 × 10 ⁶ (3.65)	0.79 × 10 ¹⁰ (3.16)
	G B Ar 23 B3	0.36 × 10 ⁵ (1.95)	0.13 × 10 ⁸ (5.05)
	G Ar 17 B3	1.98 × 10 ⁵ (0.67)	1.02 × 10 ⁷ (0.90)
	G 14 2 B5	2.99 × 10 ⁵ (1.19)	2.45 × 10 ⁷ (2.78)
	Strain R444 (Eco-Bb)	2.16 × 10 ⁶ (2.40)	1.91 × 10 ¹⁰ (7.57)

Obj. 2

Not undertaken – Mass production of dry aerial conidia for use in field trials was outsourced.

Obj. 3 - see Coombes (2013) or Coombes et al. (2015) for detailed results and discussion

Laboratory application trials indicated that both tested isolates were capable of causing FCM mortality when applied as a spray or granules to the soil surface prior to the addition of fifth instars. When fifth instars were applied one day prior to fungal application, mortality did not exceed 20% suggesting that these fungi need to be applied before the presence of FCM in the soil.

Results of the semi-field persistence trial showed that although no correlation was found between laboratory undertaken bioassays (to measure % mycosis) and the number of CFUs/g of soil, fungal spores of all tested isolates were capable of persisting and initiating infection in FCM fifth instar larvae exposed to the soil for six months' post-trial initiation.

Obj. 4

Not undertaken – Mass production of dry aerial conidia for use in field trials was outsourced.

Obj. 5 - see Coombes (2016) for more detailed results and discussion

All tested adjuvants had similar physical emulsification characteristics, with the exception of BP Medium Oil. In terms of toxicity, none inhibited spore germination of G Ar 17 B3 (> 90% germination) at any temperature tested, whilst FCM Ar 23 B3 germination was reduced after 24 h exposure to some adjuvants at 25°C and 30°C. However, percentage germination was never below 70%. The main conclusion from this experiment was that the use of the surfactant, Breakthru® S240, during field trials, was highly unlikely to have hindered fungal efficacy in the field.

Obj. 6

During field trials application occurred via a spray machine and associated hand-held spray guns and on one occasion, via the micro-sprinkler irrigation system (at Stenhope). In all field plots to which fungi were applied, CFUs were recovered suggesting that either application approach is suitable. In addition, most field trials recorded a reduction in FCM infestation relative to a control in plots to which fungus had been applied, further supporting the suitability of either application approach.

Obj. 7 – see Coombes (2016) or Coombes et al. 2017 for more detailed results and discussion

Congruent with laboratory studies, efficacy was dose-dependent, with the tested isolates performing better than the commercially available product. Corrected mortality data suggests that all three isolates are capable of performing under field conditions, with isolates G Ar 17 B3 and FCM Ar 23 B3 perhaps better suited. To account for natural mortality, which was high (>50%), Abbott's formula was performed to determine the corrected mortality of FCM fifth instars within each cage (Abbott 1925). Median mortality percentage varied amongst treatments and was found to be significantly different (P < 0.05) (

Figure 1). Median mortality was calculated to be greatest in all treatments where fungus was applied at the highest rate; 90.00, 90.00 and 95.00% for isolate G 11 3 L6, FCM Ar 23 B3 and G Ar 17 B3, respectively. Although this level of control is impressive, the feasibility of applying these isolates at this rate to larger areas is improbable due to the cost of conidial production (Mulock & Chandler 2000). The effect of mulch on fungal efficacy remains unclear.

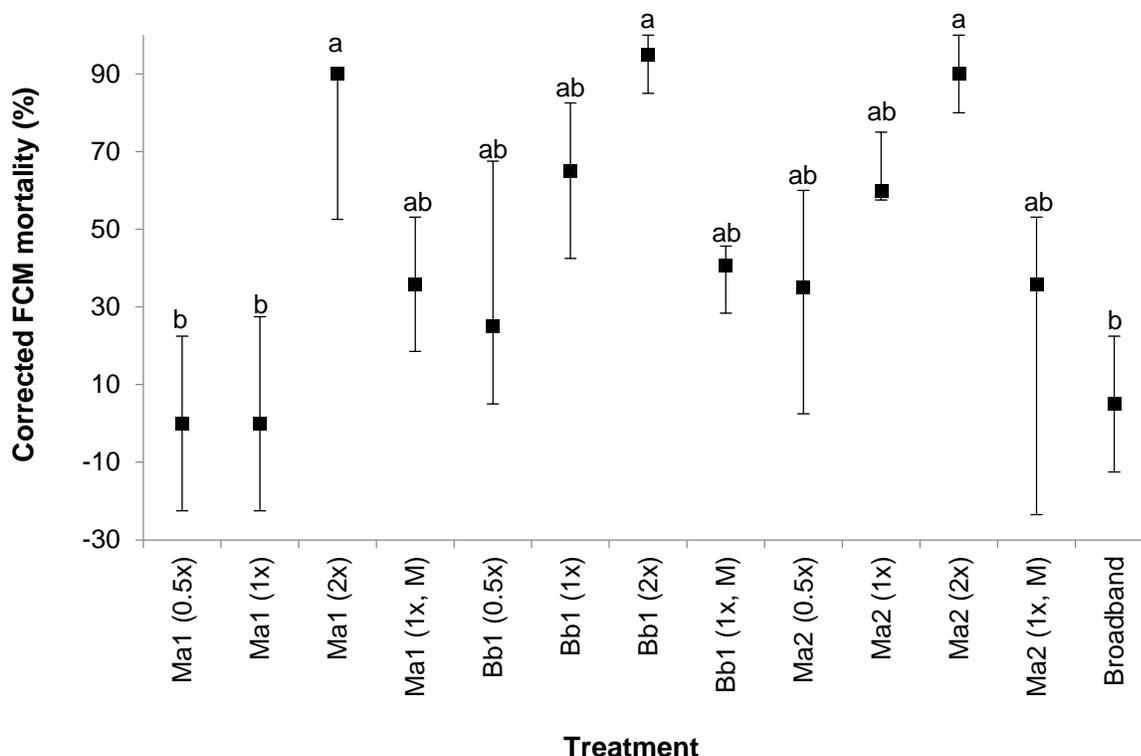


Figure 1. Median percentage, of eight replicates, of corrected (Abbott's formula) FCM mortality calculated for each treatment to which fungus was applied. Vertical bars denote the interquartile range. Different letters indicate significant differences between treatments. 0.5x, 1x and 2x refers to application rates 0.5×10^{14} , 1×10^{14} and 2×10^{14} spores/ha respectively; M, treatments to which mulch was applied

Obj. 8 – see Coombes (2016) or Coombes *et al.* 2016 for more detailed results and discussion

These field trials were the first report on the performance of these fungal isolates under field conditions against the soil-dwelling life stages of FCM.

A reduction in fruit drop and FCM infestation was recorded within all treatment blocks at all field sites and varied amongst isolates and field sites. For early application trials, Atmar 1 and Atmar 2, G Ar 17 B3 was found to perform better under micro-sprinkler than drip irrigation, whilst FCM Ar 23 B3 was more effective under drip irrigation (Figure 2). For late application trials (Marwell and Stenhope), G Ar 17 B3 reduced FCM infestation and fruit drop between 50 to 70% and 7 to 80%, respectively (Figure 3). At Marwell, the highest reduction in FCM infestation was recorded when G Ar 17 B3 was applied at 5×10^{12} conidia/ha, not the highest tested concentration, suggesting the potential to reduce application rate, whilst maintaining efficacy. No conclusive results could be obtained from Oranjelus due to low fruit drop and FCM infestation. Mean weekly fruit drop per treatment never exceeded 11, whilst the number of FCM infested fruit per treatment per week averaged less than 1.

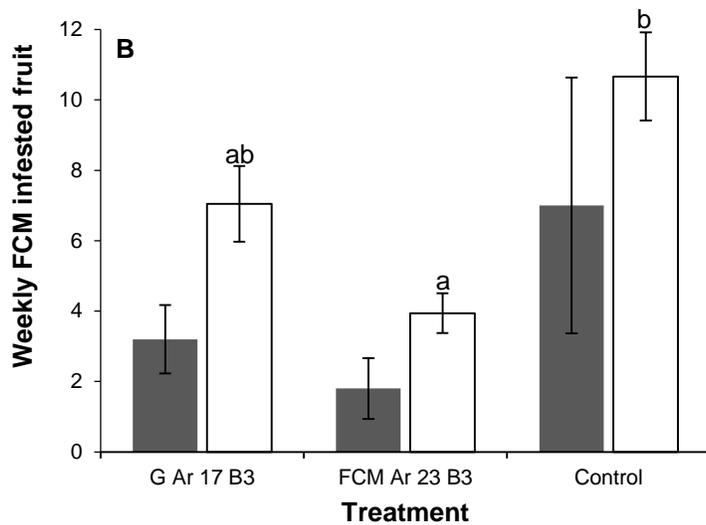
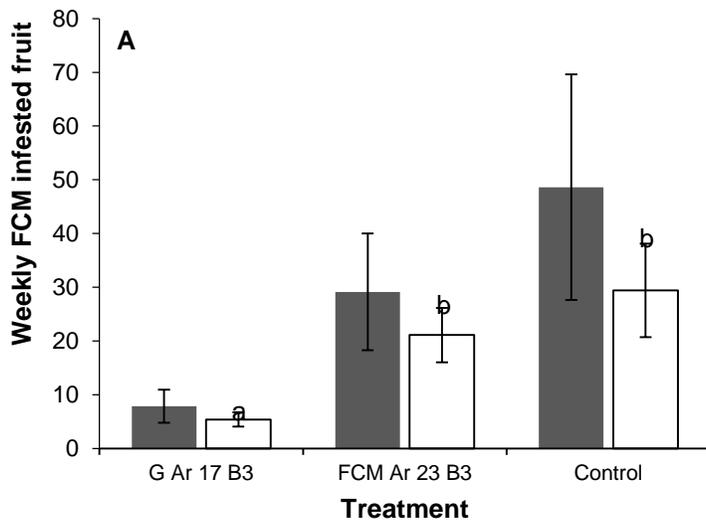


Figure 2. Mean (SE) weekly FCM infested fruit per 12 trees recorded 15 (grey) and 30 (white) weeks after fungal application, over a period of eight and 21 weeks respectively, for the control of FCM at Atmar 1 (micro-sprinkler irrigation) (A) and over a period of five and 18 weeks, respectively at Atmar 2 (drip irrigation) (B). Significant differences amongst treatments were found 30 weeks after treatment (Tukey's HSD test, $P < 0.05$), denoted by different letters.

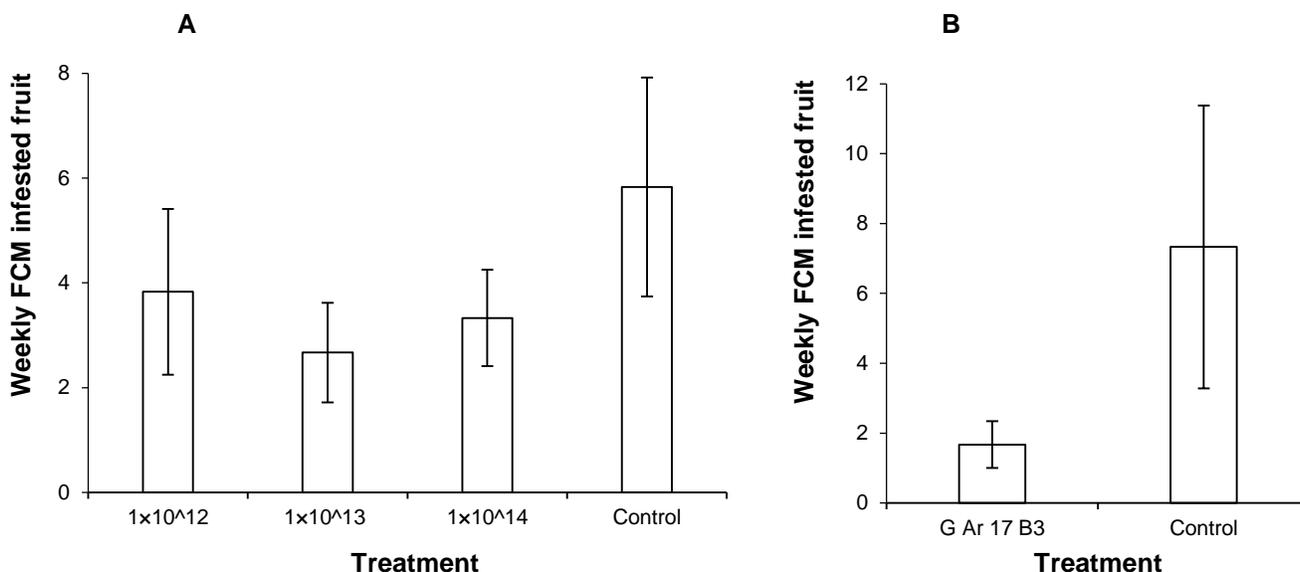


Figure 3. Mean weekly (\pm SE) FCM infested fruit per 12 trees recorded nine weeks after fungal application, over a period of six weeks, for the control of FCM at Marwell (A) and per ten trees recorded ten weeks after treatment, over a period of six weeks at Stenhope (B). At Marwell, all application rates of G Ar 17 B3 presented pertain to the quantity of conidia applied per hectare. G Ar 17 B3 was only applied at one rate at Stenhope, 5×10^{13} conidia/ha. Neither field trial produced significantly different results amongst treatments ($P > 0.05$).

Obj. 9 – see Coombes (2016) for more detailed results and discussion

The toxicity of the tested fungicides on the vegetative growth, spore viability and sporulation of isolates Ma2 and Bb1 was variable. Toxicity was more pronounced when experiments were conducted using amended media. Continual exposure to Dithane, Pennfluid, Benomyl and Cabrio prohibited the germination of both FCM Ar 23 B3 and G Ar 17 B3 conidia. With the exception of Sporekill, a reduction in the number of conidia produced for each isolate, in comparison to the control, was reduced between 60 and 99.9%. Minimal effect on vegetative growth, spore viability and sporulation was found when experiments were conducted on non-amended media following a 1 hour exposure period to each fungicide in aqueous suspension. G Ar 17 B3 appeared to be more sensitive to all fungicides, except Dithane which was highly toxic towards the spores of FCM Ar 23 B3.

Obj. 10 – see Coombes (2016) or Coombes *et al.* 2016 for more detailed results and discussion

At all field sites where persistence was monitored, fungi were isolated five months following application. Persistence varied subtly between isolates, but substantially amongst sites. Isolates G 11 3 L6, G Ar 17 B3 and FCM Ar 23 B3 persisted similarly at Atmar 1. Following an initial decline in fungal density post-application, density began to increase after approximately two months. With the exception of G 11 3 L6, this increase was apparent until trial termination and was found to not be significantly different to the fungal density recorded one week after application. It is suggested that this increase in fungal density is a result of host infection and as such, fungal recycling (Rath *et al.* 1995; Kessler *et al.* 2004). However, host density alone cannot fully explain the ability of these isolates to persist. At Atmar 2, where trap catches suggested higher FCM densities than Atmar 1, persistence was poorer for both isolates (Figure 4). Soil moisture differed substantially between these two sites, a result of the irrigation system employed. Soil moisture within the upper 10 cm was drier at Atmar 2 (< 15% soil moisture) than Atmar 1 (20% to 50% soil moisture). This may have impacted spore germination and thus infection as well as fungal sporulation and thus fungal recycling. The impact of soil moisture on the spores of FCM Ar 23 B3 and G Ar 17 B3 remains to be determined. At Oranjelus, trap catch data would suggest host density similar to Atmar 1, however sterile moths were more prevalent at Oranjelus which may have reduced actual host density in the soil. In addition, soil moisture ranged between 35% and 65%. These factors may have contributed to the increase in fungal density following application, but due to low host density, could not be maintained. The impact of other soil

factors which have been reported to influence persistence, including soil temperature, soil type and soil pH, could not be determined as these were highly similar across all sites.

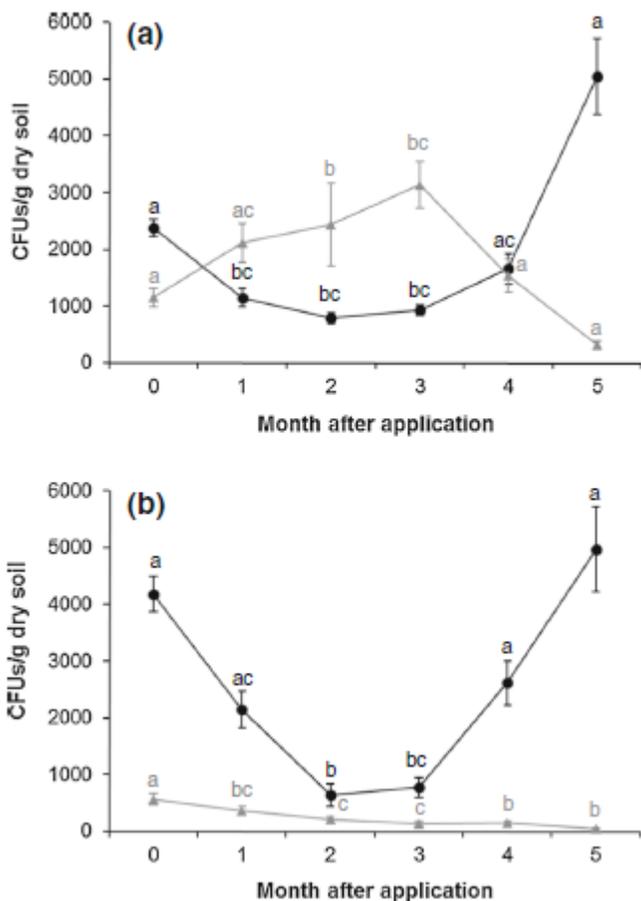


Figure 4. Mean (\pm SE) concentration of entomopathogenic fungal isolates *B. bassiana* G Ar 17 B3 (a) and *M. anisopliae* FCM Ar 23 B3 (b) as measured one-week post-application (0) and every month thereafter (1–5) for five months, in the soil at Atmar 1 under micro-sprinkler irrigation (filled circle) and Atmar 2 under drip irrigation (filled triangle). Different letters denote significantly different results (multiple comparison of mean ranks, $P < 0.05$) within each field site.

Obj. 11

Not undertaken – Mass production of dry aerial conidia for use in field trials was outsourced.

Obj. 12

See objective 5 above. No other formulation experiments were undertaken.

Obj. 13

The sublethal effect of eclosing adults following FCM fifth instar larvae exposure to the LC_{50} concentration of each fungal isolate (*B. bassiana* G Ar 17 B3 and *M. anisopliae* FCM Ar 23 B3) was considered to be negligible, relative to the control. No differences were found amongst treatments with respect to pupal weight, pupal length and adult longevity. In all cases, males were found to have a slightly longer pupation length and adult lifespan. Average fecundity, based on the number of eggs oviposited per female per day, was not statistically different amongst treatments ($F_{2, 157} = 1.21, p = 0.302$) (Figure 5).

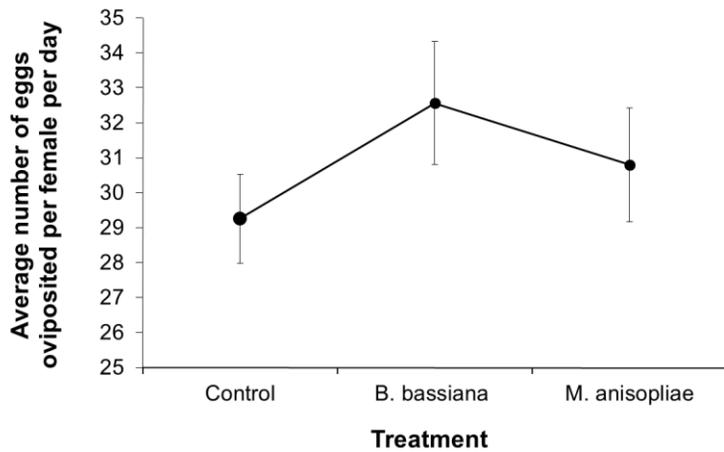


Figure 5. Average number of eggs oviposited per female per day. Vertical bars denote standard errors. No significant differences amongst treatments were found ($F_{2, 158} = 1.14$, $p = 0.32$)

Obj. 14

Eggs: Only *M. anisopliae* FCM Ar 23 B3 isolate had a significant effect reducing egg hatch by approximately 90% at both concentrations whether applied before or after oviposition occurred (Figure 6). Further dose-response bioassays using FCM Ar 23 B3 only were conducted and used to determine the LC_{50} and LC_{90} , calculated to be 1.43×10^5 and 1.93×10^6 conidia/ml, respectively.

Neonates: One replicate experiment suggested that no fungi, except Broadband®, was capable of preventing FCM infestation within fruit. However, it is thought that the oil formulation of Broadband® acted as a physical barrier preventing neonates from entering the fruit rather than the fungal active ingredient killing the insect. This may have been a result of the method used. Subsequent experiments to determine whether the formulation of Broadband® was responsible for lack of FCM infestation was unsuccessful as in all treatments initiated, control included, no oranges became infested with FCM.

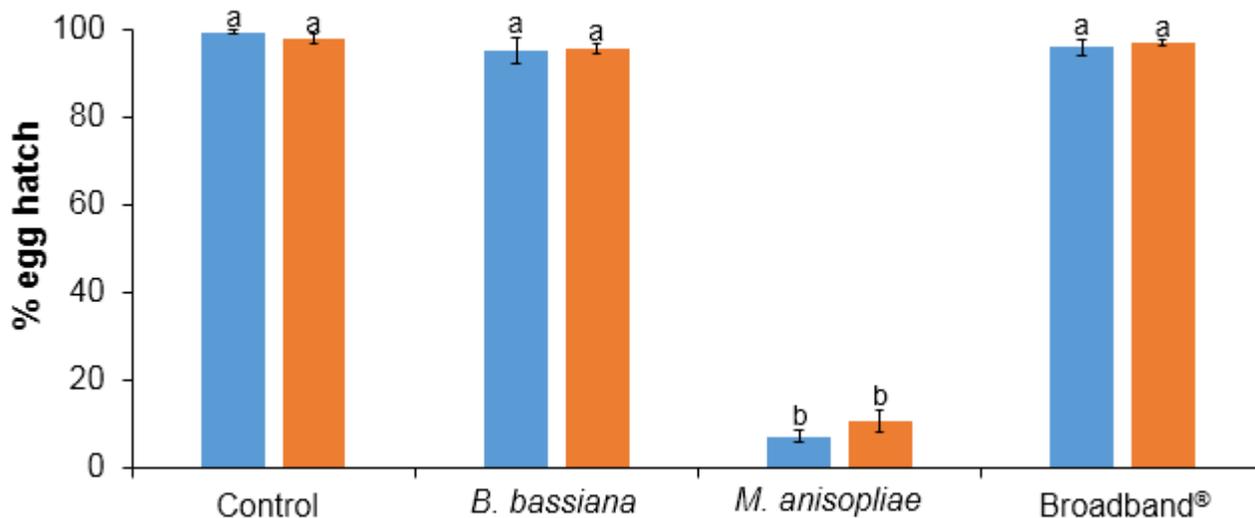


Figure 6. Average (\pm SE) percentage egg hatch for both tested fungi and the commercial mycopesticide, Broadband® when applied before (orange) and after (blue) eggs were oviposited at a concentration of 2×10^6 conidia/ml.

Obj. 15

Not undertaken – Isolate G Ar 17 B3 begun to show signs of attenuation (reduced virulence), thus a series of bioassays were conducted in place of this objective, in an attempt to solve this problem. To date, attempts have not been successful.

Obj. 16

Isolate *M. anisopliae* FCM Ar 23 B3 was compatible with all nine tested insecticides tested (Table 2), whilst the vegetative growth and sporulation of isolate *B. bassiana* G Ar 17 B3 was affected by five of the tested insecticides. Viability, with the exception of exposure to Beretta, remained relatively unaffected (Table 3).

Table 2. Viability, vegetative growth and sporulation of *M. anisopliae* FCM Ar 23 B3 when grown on insecticide-amended media. The means (\pm standard errors) and percentage expression (in bold) of each trait relative to the control is presented. Percentages range between 0% and 100%, with 100% representing a response identical to or greater than the control. Different letters denote statistically significant results within each grouping (Tukey's HSD test, $P < 0.05$).

Insecticide	Viability ($\times 10^7$ CFUs/ml)	Vegetative growth (area in cm^2)	Sporulation ($\times 10^7$ spores/ml)
Control	3.61 (0.236) ab ¹ -	44.43 (0.48) a -	4.25 (0.41) ab -
Agrimec	4.09 (0.188) ac 100%	38.40 (0.68) ce 86.44%	4.40 (0.40) ab 100%
Beretta	2.72 (0.342) b 75.37%	34.36 (0.78) b 77.33%	3.99 (0.34) ab 93.9%
Delegate	3.44 (0.144) abc 95.19%	35.27 (0.42) bc 79.38%	4.85 (0.57) ab 100%
Dursban	3.75 (0.140) a 100%	28.05 (0.41) d 63.14%	3.72 (0.49) ab 87.5%
Hunter	3.35 (0.115) ab 92.67%	40.06 (0.63) ef 90.18%	3.69 (0.42) ab 86.8%
Klartan	3.20 (0.227) ab 88.63%	43.37 (1.49) af 97.62%	5.43 (0.5.43) a 100%
Mylomex	3.75 (0.166) a 100%	37.54 (0.78) bce 84.50%	3.23 (0.2.98) b 75.9%
Scalex	3.43 (0.193) ab 94.83%	25.96 (1.06) d 58.42%	3.48 (0.46) ab 81.8%
Suprathion	3.49 (0.170) ab 96.46%	8.59 (0.50) g 19.35%	3.10 (0.59) b 72.9%

Table 3. Viability, vegetative growth and sporulation of *B. bassiana* G Ar 17 B3 when grown on insecticide-amended media. The means (\pm SE) and the percentage expression (in bold) of each trait relative to the control is presented. Percentages range between 0% and 100%, with 100% representing a response identical to or greater than the control. Different letters denote statistically significant results within each grouping (Tukey's HSD test, $P < 0.05$).

Insecticide	Viability ($\times 10^7$ CFUs/ml)	Vegetative growth (area in cm^2)	Sporulation ($\times 10^7$ spores/ml)
Control	4.37 (0.34) abcd ¹ -	20.97 (0.45) a -	17.4 (2.51) a -
Agrimec	5.19 (0.20) abcd 100%	14.24 (0.48) b 67.89%	16.6 (1.99) ab 66.89%
Beretta	3.27 (0.47) b 69.16%	13.50 (0.20) bc 64.37%	8.68 (1.99) bc 49.96%
Delegate	4.06 (0.36) abcd 85.94%	9.23 (0.66) d 44.03%	10.5 (2.10) acd 60.22%
Dursban	5.70 (0.5.9) cd 100%	14.12 (0.48) b 67.34%	4.04 (0.56) bde 23.22%
Hunter	4.87 (0.35) abcd 100%	15.60 (1.09) b 74.39%	14.7 (1.83) ac 84.82%
Klartan	5.41 (0.73) abcd 100%	18.32 (0.36) e 87.34%	13.5 (2.28) ac 77.55%
Mylomex	5.59 (0.42) acd 100%	11.09 (0.69) cd 52.86%	10.4 (1.43) acef 59.71%
Scalex	5.01 (0.49) abcd 100%	4.97 (0.36) f 23.69%	13.5 (1.54) ac 77.91%
Suprathion	4.17 (0.66) abcd 88.30%	1.10 (0.123) g 5.24%	3.92 (0.67) bdf 22.57%

Obj. 17

Rates lower than 5×10^{13} conidia/ha are possible. For *M.anisopliae* FCM Ar 23 B3, this appears to be 5×10^{12} conidia/ha. Isolate *B. bassiana* G Ar 17 B3 appears to be less dependent on the application rate in reducing FCM infestation with reduction possible at 5×10^{11} conidia/ha (see figure 7 below).

Obj. 18

Results of one additional field trial are provided in Figure 7. Isolates FCM Ar 23 B3 and G Ar 17 B3 were more effective in reducing FCM infestation than two commercially available mycopesticides and the control. Evaluation was based on a once-off survey of infested fruit within the tree canopy shortly before harvest. Similar trials were also conducted in the W Cape and Mpumalanga, but FCM levels were so low that meaningful results were not obtained.

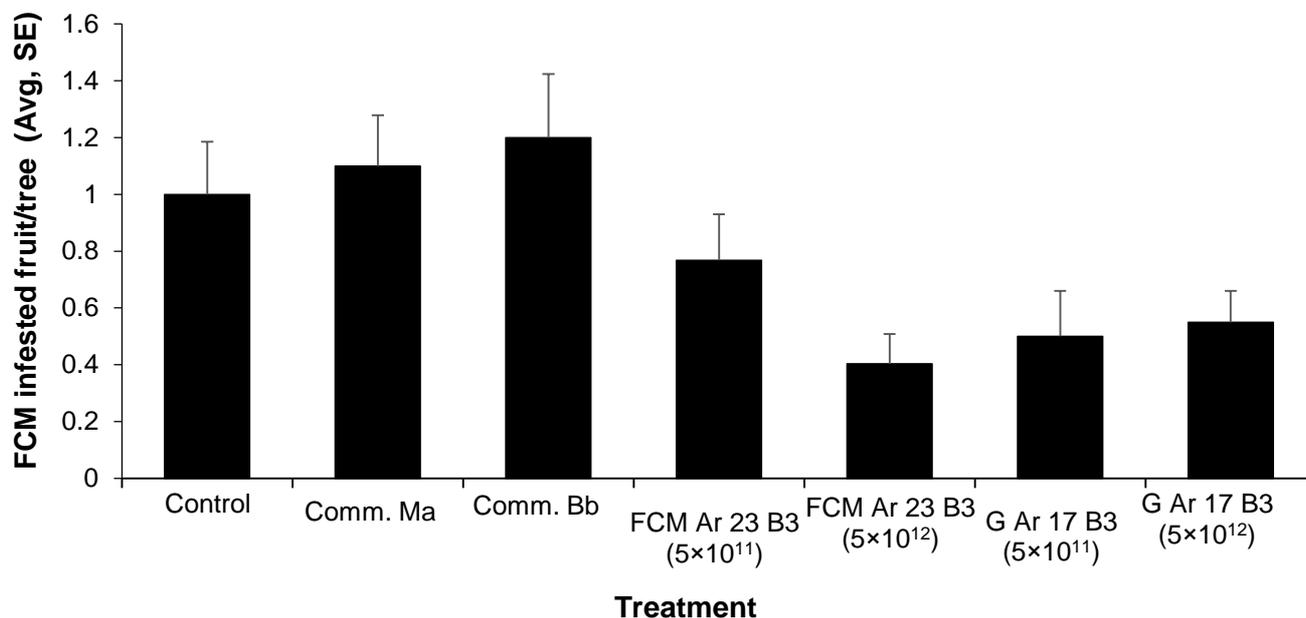


Figure 7. Results of the EPF application trial at Panzi citrus orchard across the 2015/16 growing season. Results were based off a once-off evaluation shortly prior to fruit harvest.

Obj. 19

Addressed above (obj. 12, 13, 14 and 16)

Obj. 20

REN analysis, using the restriction enzymes EcoRI, BamHI and HindIII, to identify a genetic fingerprint was unsuccessful. REN analysis using extracted fungal genomic DNA was capable of distinguishing between certain *Beauveria* isolates tested, but not all the tested *B. bassiana* isolates nor, did the restriction enzymes used, cut the genomic DNA of the *M. anisopliae* isolates.

However, PCR samples were sent for sequencing of the ITS region using the primers ITS1 and ITS4. This was done to determine whether samples of the three isolates, previously determined to be the most virulent, held at Rhodes University, River Bioscience and PPRI were genetically the same (Table 4). This was in attempt to understand why *Beauveria bassiana* G Ar 17 B3 was performing poorly in more recent laboratory bioassays. Results were interesting. The following groups were considered genetically the same based on phylogenetic analyses (neighbor-joining tree) (Figure 8).

- K1, Bb PPRI, Bb PPRI SC and a submission deposited in GenBank by Veronique Chartier-Fitzgerald during her MSc research on mealybug and thrips
- K2, K3, Bb New and Bb ORI
- Bb Old

- K4, K6, FCM Old, FCM PPRI and G 11 Old
- K5, K7 and FCM ORI
- G 11 ORI

Table 4. Description of the fungal samples sent for ITS region sequencing

Fungus	Description
K1	<i>B. bassiana</i> G Ar 17 B3, from Sylvan (RB sample)
K2	<i>B. bassiana</i> G Ar 17 B3, received Jan 2017 (RB sample)
K3	<i>B. bassiana</i> G Ar 17 B3, received 2015/2016 (RB sample)
Bb ORI	<i>B. bassiana</i> G Ar 17 B3, obtained from Vee and stored in glycerol 14.10.2016
Bb NEW	<i>B. bassiana</i> G Ar 17 B3, received Jan 2017 (CRI sample)
Bb OLD	<i>B. bassiana</i> G Ar 17 B3, first batch from Sylvan (CRI sample)
Bb PPRI	<i>B. bassiana</i> G Ar 17 B3, received 04.09.2017 (PPRI sample); original isolate isolated by T. Goble
Bb PPRI SC	<i>B. bassiana</i> G Ar 17 B3, 1st subculture of PPRI sample
K4	<i>M. anisopliae</i> FCM Ar 23 B3, 4 kg bag (RB sample)
K5	<i>M. anisopliae</i> FCM Ar 23 B3, received Jan 2017 (RB sample)
K6	<i>M. anisopliae</i> FCM Ar 23 B3, received 2015/2016 (RB sample)
FCM ORI	<i>M. anisopliae</i> FCM Ar 23 B3, obtained from Vee and stored in glycerol 29.09.2016
FCM OLD	<i>M. anisopliae</i> FCM Ar 23 B3, first batch from Sylvan (CRI sample)
FCM PPRI	<i>M. anisopliae</i> FCM Ar 23 B3, received 02.11.2017 (PPRI sample); original isolate isolated by T. Goble
K7	<i>M. anisopliae</i> G 11 3 L6, (RB sample)
G 11 ORI	<i>M. anisopliae</i> G 11 3 L6, obtained from Vee and stored in glycerol 29.09.2017
G 11 OLD	<i>M. anisopliae</i> G 11 3 L6, first batch from Sylvan (CRI sample)

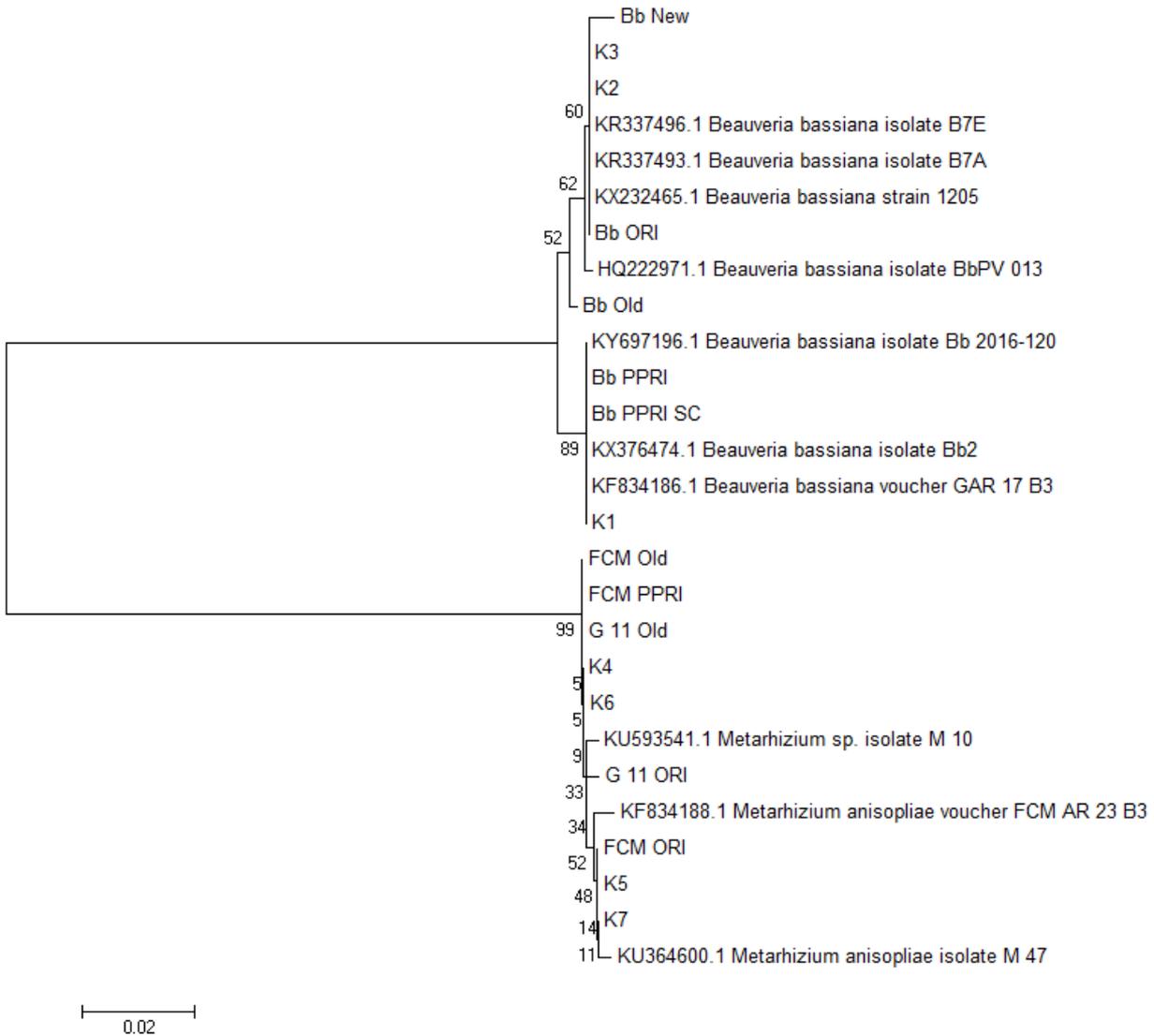


Figure 8. A neighbour joining tree showing the relationship between the fungal samples. Closest matches on GenBank to the sequenced isolates are included.

Conclusion

From this project, two fungal isolates, *B. bassiana* G Ar 17 B3 and *M. anisopliae* FCM Ar 23 B3 have been identified as suitable candidates for the control of false codling moth when applied to the soil surface in conventional citrus orchards.

Technology Transfer

- Coombes, CA., Hill, MP., Moore, SD., Dames, JF. 2017. Potential of entomopathogenic fungal isolates for control of the soil-dwelling life stages of *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in citrus. *African Entomology* 25(1): 235-238.
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2.2.4 **FINAL REPORT: The efficacy of registered treatments for FCM control in Limpopo**

Project 1112 (Apr 2015 – July 2017) by Sean Moore, Wayne Kirkman, Rooikie Beck (CRI), Sean Thackeray, Mellissa Peyper (CRI/RU), Francois Joubert (RU), Jacolene Meyer, Marili Mouton (QMS Laboratories) and Hannah Otto (Insectec).

Summary

In this project, we proposed to test and compare the efficacy of control measures that are registered for use against FCM on citrus. This is because it cannot be assumed that results generated from trials elsewhere in the country will be identical for Letsitele or any other region for that matter; and because anecdotal information indicates differences in efficacy of certain products between certain regions. During the 2015/6 season, two trials were conducted: one involving mating disruption (MD) and attract and kill (A&K) products and one involving products registered as sprays. Monitoring of traps and fruit infestation was conducted weekly until the end of March 2016, and was terminated at this time, as FCM infestation was too low to justify continuation thereof. FCM infestation was too low to establish any significant differences between spray treatments and the untreated control. Although FCM infestation of fruit in the MD and A&K trial was similarly low, there were large differences in trap catches, a good indication of treatment efficacy. Trap catches were significantly lower in the Isomate treatment, followed by Checkmate and Last Call, all of which were lower than the untreated control. The spray trial was repeated in April 2017, but once again, the FCM levels were too low to show significant differences between treatments. Runner was the only treatment to deliver no infested fruit.

Opsomming

In hierdie projek beplan ons om die werking van produkte wat op sitrus teen VKM geregistreer is te toets en met mekaar te vergelyk. Hierdie is omdat dit nie sommer aanvaar moet word nie dat resultate van proewe elders in die land identies sal wees vir Letsitele of enige ander streek. Boonop, anekdotiese inligting dui aan

dat daar verskille in die werking van sekere produkte in sekere streke mag wees. Gedurende dit 2015/6 seisoen is twee proewe uitgevoer: een het paringsontwrigting (PO) en lok-en-vrek (L&V) produkte vergelyk en die ander het geregistreerde spuit-produkte vergelyk. Monitoring van lokvalle en vrugbesmetting is tot Maart 2016 weekliks uitgevoer en is toe gestop omdat VKM besmetting te laag was om verdere monitoring te regverdig. VKM besmetting was te laag om enige betekenisvolle verskille tussen spuit behandelings en die onbehandelde kontrol te kry. Al was VKM besmetting in die PO en L&V proef ewe laag, was daar groot verskille in lokval vangste, 'n goeie aanduiding van doeltreffendheid van die behandelings. Lokval vangste is laagste in die Isomate behandeling, gevolg deur Checkmate en Last Call, en al die behandelings is laer as die onbehandelde kontrole. Die spuitproef is in April 2017 herhaal, maar weereens was die vlakke van VKM te laag om beduidende verskille tussen die behandelings uit te wys, hoewel Runner die enigste behandeling was wat geen besmette vrugte gelewer het.

Introduction

Although extensive comparative trials have been conducted over a number of years with products registered for the control of FCM on citrus (Moore & Hattingh, 2012), most of these trials have been conducted in the Eastern Cape. Some trials have been conducted in the Western Cape and Mpumalanga, but very few have been conducted in Limpopo. The comparative efficacy of products tested extensively (particularly in the Eastern Cape) is clear and conclusive. However, anecdotal reports from Limpopo contradict some of these findings. Therefore, it cannot be assumed that the products which are the most effective in one region will be the same products that are the most effective in another region. Timm et al. (2010) found distinct genetic differences between FCM populations in different regions and even sub-regions; Opoku-Debrah et al. (2014) found significant biological differences between certain populations; and it is concluded that different FCM populations even have different sex pheromone compositions (Angelini et al, 1981; Hall et al., 1984; Attygalle et al., 1986). There is therefore good reason to suspect that susceptibility to the various control options may differ between regional populations of FCM. Additionally, different management programmes are employed in different regions, exposing FCM populations to different selection pressures and more specifically, differences in resistance development likelihood to various pesticides. Due to the increasing phytosanitary pest status of FCM, we cannot afford any lack of clarity on the relative effectiveness of the various pesticides for FCM and this must be investigated, particularly in Limpopo – due to the size and importance of the production region and the uncertainty regarding pesticide efficacy.

Objectives

To compare the efficacy of all registered control options for FCM on citrus (sprayable pesticides, mating disruption and attract and kill products) in field trials in Limpopo Province.

Materials and methods

2015/6

Mating disruption/attract and kill

In September 2015, two orchards of Turkey Valencia oranges with a history of conspicuous FCM infestation were selected on Letaba Estates in the Letsitele region of Limpopo Province for a mating disruption/attract and kill trial. One replicate of each treatment was applied to 1.5 ha in each orchard, thus replicated twice. The first treatments were applied on 19 October 2015, comparing Isomate, Checkmate and Last Call FCM. Each product was applied according to registration, except that the area of application was smaller than prescribed. Previous trials conducted in the Eastern Cape have demonstrated that treatment blocks of this size are adequate to obtain a reliable result, if all monitoring is conducted in the dead centre of each treatment block (in order to avoid any edge effect). One FCM pheromone trap was hung in the centre of each treatment block and monitored weekly from 23 October 2015 to 29 March 2016. Checkmate and Last Call were reapplied on 19 November 2015, 14 December 2015, 11 January 2016, 08 February 2016 and 07 March 2016. The second application of Isomate was applied on 11 January 2016. Ten data trees were marked in the centre of each

treatment block. Fruit drop was collected and dissected on a weekly basis from 15 December 2015 to 29 March 2016 (including those from an untreated block) and cause of drop determined. Fruit drop was evaluated by Du Roi QMS. .

Spray trial

Another orchard of Turkey Valencia oranges on the same farm was selected for a spray trial. The trial was laid out in a single tree randomised block format, replicated 10 times. On 16 December 2015, Cryptogran, Cryptex, Meothrin, Cypermethrin, Delegate, Coragen, Runner, Alsystin and Broadband were applied at registered rates, using a hand gun applicator, 2mm nozzles and 20 bar pressure, at an average rate of 17.56 L/tree. Weekly, from three weeks after spraying, fruit drop under all trees (including untreated control trees) was collected and dissected and the cause of fruit drop (especially if by FCM) determined. Fruit drop was evaluated by Du Roi QMS.

2017

Spray trial

An orchard of Midnight Valencia oranges with a history of conspicuous FCM infestation were selected on Letaba Estates in the Letsitele region of Limpopo Province. The trial was laid out in single tree randomised block format, replicated 10 times. On 11 and 12 April 2017, Cryptogran, Cryptex, NPV, Meothrin, Cypermethrin, Delegate, Coragen, Runner, and Broadband were applied at registered rates, using a hand gun applicator, 2mm nozzles and 20 bar pressure, at an average rate of 24.2 L/tree. Weekly, from three weeks after spraying, fruit drop under all trees (including untreated control trees) was collected and dissected and the cause of fruit drop (especially if by FCM) determined. Fruit drop was evaluated by Hannah Otto (Insectec).

Results and discussion

2015/6

Mating disruption/attract and kill

Although FCM infestation of fruit in the MD and A&K trial was very low, there were large differences in trap catches, a good indication of treatment efficacy. The total trap catches per treatment, monitored weekly from 23 October 2015 to 29 March 2016, were untreated control 112, Isomate 3, Checkmate 34 and Last Call 69. This equated to an average weekly moth catch per trap of 2.3, 0.06, 0.71 and 1.44 per treatment respectively (Table 1), with the Isomate catches significantly lower than the untreated control. Fruit infestation was too low to pick up any differences between treatments (Table 1), and control infestation was zero.

Table 1. FCM trap captures and infested fruit per treatment, monitored from 23 October 2015 to 29 March 2016 at Letaba Estates.

Treatment	Total moths per treatment	Moths per trap per week	Total FCM infested fruit	FCM infested fruit/tree/week
Untreated control	112	2.1a	0	0
Isomate	3	0.06b	0	0
Checkmate	34	0.71 ab	4	0.013
Last Call	69	1.44a	2	0.006

*Different letters in column denote significant differences, Non Parametric: Kruskal-Wallis test: $p < 0.05$.

Spray trial

FCM infestation was too low to establish any differences between spray treatments, and the untreated control infestation was zero (Table 2).

Table 2. FCM infested fruit per treatment, monitored from 04 January 2016 to 29 March 2016 at Letaba Estates.

Treatment	Rate per 100L (ml or g)	Total infested fruit	Infested fruit/tree/week
Untreated control		0	0
Cryptogran + molasses + BreakThru	10 + 250 + 5	1	0.008
Cryptex	3.3	0	0
Meothrin	30	1	0.008
Cypermethrin	25	1	0.008
Delegate	20	0	0
Coragen	17.5	2	0.015
Runner	60	0	0
Alsystin	20	0	0
Broadband	50	1	0.008

2017

Spray trial

Unfortunately, once again FCM infestation was low, and none of the treatments significantly reduced FCM infestation (Table 3). Runner was the only treatment that resulted in no infested fruit over the 10 weeks of evaluation.

Table 3. FCM infested fruit per treatment, monitored from 01 May 2017 to 03 July 2017 at Letaba Estates.

Treatment	Rate per 100L (ml or g)	Total infested fruit	Infested fruit/tree/week
Untreated control		5	0.05a*
Cryptogran + molasses + BreakThru	10 + 250 + 5	3	0.03a
Cryptex	3.3	5	0.05a
NPV + molasses +BreakThru	10 + 250 + 5	8	0.08a
Meothrin	30	2	0.02a
Cypermethrin	25	3	0.03a
Delegate	20	5	0.05a
Coragen	17.5	1	0.01a
Runner	60	0	0.0a
Broadband	50	5	0.05a

*Different letters in column denote significant differences, Non Parametric: Kruskal-Wallis test: p =0.1974

Conclusions

FCM infestation of fruit in the mating disruption/attract and kill trial was very low, but there were large differences in trap catches, a good indication of treatment efficacy. This would indicate that Isomate was the most effective treatment, followed by Checkmate and Last Call. Unfortunately the infestation in the two spray trials was too low to show significant differences, but in the second trial Runner was the only treatment to result in no infested fruit.

Technology Transfer

None yet.

Further objectives and workplan

None

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2.2.5 FINAL REPORT: Assessment of pheromone specificity in FCM populations with focus on pest monitoring and regional rollout of SIT

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

Summary

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is considered the most important indigenous pest of citrus in southern Africa. It is recognized by several markets as a phytosanitary organism and the efficient control of this pest is now more important than ever. The pheromone communication between the male and female moths has been exploited in order to control FCM through the sterile insect technique (SIT). The sterilized males used for all SIT programmes across South Africa come from a culture that originates from wild material collected from the Citrusdal area of the Western Cape Province. The aim of this study was to determine if any differences in attractiveness of females to males exist between different geographical populations of FCM and if so what impact this would have on the male's ability to locate females from other populations via the volatile sex pheromone released by the female. Laboratory trials with Y-tube olfactometers and flight tunnels tested the attraction of male moths to virgin females, but did not yield any consistent results. Field experiments were conducted with sterile male Citrusdal moths released and recaptured in yellow delta traps in two separate trials. For one trial, the traps were baited with live virgin females from five different geographical populations including Addo, Nelspruit, Marble Hall, Citrusdal and the Old colony, which is a mixture of several populations. For the other trial traps were baited with various artificial pheromone blends including three regional blends which included South Africa, Ivory Coast and Malawi and three commercial blends including Pherolure, Isomate and Checkmate. For the virgin female trial the Citrusdal males showed a significant preference for females from their own population. There was also a significant difference in the recaptures from the different artificial pheromones. The South African blend was the most attractive of all the regional and commercial blends. A cross-mating trial was also conducted under laboratory conditions in petri dishes with five different FCM populations including Citrusdal, Addo, Marble Hall, Nelspruit and Old (mixed origin). Females produced more eggs when mated with males from the same population for the Addo, Marble Hall, Nelspruit and Old (mixed origin) populations. The only case in which this was statistically significant was for the Marble Hall population. All the crosses produced viable eggs and the origin of the male or female did not influence egg hatch. The results from this study may lead to improvements in both the control and monitoring of FCM populations. The control methods include mating disruption, attract-and-kill and SIT.

Tailoring these methods for a specific growing area with a pheromone blend originating from the area or releasing sterile moths from a culture that originates from the area may optimize the available monitoring and control options. This study was completed as an MSc thesis.

Opsomming

Valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) word beskou as die belangrikste inheemse plaag van sitrus in suidelike Afrika. Dit word deur verskeie markte geken as 'n fitosanitêre organisme en die doeltreffende beheer van hierdie plaag is nou selfs meer belangrik as ooit te vore. Die feromoon kommunikasie tussen die mannetjie en wyfie motte is uitgebuit om VKM deur middel van die steriele insek tegniek (SIT) te beheer. Die steriele mannetjies wat gebruik word vir alle SIT programme deur Suid-Afrika is van 'n kultuur afkomstig wat van wilde insekte gevestig is wat van die Citrusdal area in die Wes-Kaap Provinsie versamel is. Die doel van hierdie studie was om te bepaal of daar enige verskille was in die aanloklikheid van wyfies vir mannetjies van verskillende streeks bevolkings van VKM en as dit die geval was, watter impak dit sou hê op die mannetjie se vermoë om wyfies van verskillende populasies te vind deur middel van die vlugtige seksferomoon wat deur die wyfies vrygestel is. Laboratorium proewe met Y-buis olfaktometers en vlugtonnels het die aantrekkingskrag van ongepaarde wyfies vir mannetjie motte getoets maar het nie konsekwente resultate gelewer nie. Veldproewe is met steriele mannetjie Citrusdal motte uitgevoer, waar motte losgelaat en in geel delta lokvalle hervang is in twee aparte proewe. Vir een van die proewe is lewendige ongepaarde wyfies van vyf geografies verskillende populasies in die lokvalle gebruik. Motte was van Addo, Nelspruit, Marble Hall en Citrusdal afkomstig en van 'n ou kolonie, wat 'n mengsel van verskeie populasies was. Vir die ander proef is verskeie verskillende sintetiese feromoon mengsels as lokmiddels in die lokvalle gebruik, insluitend drie streeks spesifieke mengsels, wat Suid-Afrika, Ivoorkus en Malawi ingesluit het, en drie kommersiele mengsels, insluitend Pherolure, Isomate en Checkmate. Vir die ongepaarde wyfie proef het die Citrusdal mannetjies 'n betekenisvolle voorkeur vir wyfies van hulle eie populasie gewys. Daar was ook 'n betekenisvolle verskil in die hervangste van die verskillende sintetiese feromone. Die Suid-Afrikaanse mengsel was die mees aantreklik van al die streeks en kommersiele mengsels. 'n Kruisparings proef is ook uitgevoer in petribakke in die laboratorium met vyf verskillende VKM populasies, insluitend Citrusdal, Addo, Marble Hall, Nelspruit en Ou (gemengde oorsprong). Wyfies het meer eiers gelê wanneer hulle met mannetjies van dieselfde populasie gepaar is vir die Addo, Marble Hall, Nelspruit en Ou populasies. Die enigste geval waar hierdie statisties betekenisvol was, was in die geval van die Marble Hall populasie. Al die kruisings het lewensvatbare eiers geproduseer en die oorsprong van die mannetjie of wyfie het nie eier-uitbroeiing beïnvloed nie. Die resultate van hierdie studie kan tot verbeteringe in albei beheer en moniterings praktyke vir VKM populasies lei. Die bestrydings maatreels sluit in paringsontwrigting, lok-en-vrek en SIT. Aanpassing van hierdie metodes vir 'n spesifieke produksie streek met 'n feromoon mengsel wat van die streek afkomstig is of loslating van steriele motte van 'n kultuur wat van die spesifieke streek afkomstig is kan dalk die monitering en bestrydings opsies optimaliseer. Hierdie studie is as 'n MSc tesis voltooi.

Introduction

South Africa is a major exporter of citrus fruit and as of 2014 was the second largest exporter globally (CGA 2015). Most citrus fruit is adversely affected by a large number of insect pests. In South Africa there are 23 important arthropod and molluscan pests of citrus (Moore *et al.* 2008). False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is one of the pests and is considered to be the most important pest of citrus in South Africa (Moore 2012). The expectation by international markets for high quality fruit and strict phytosanitary conditions, force farmers to prioritize the control of FCM (Moore & Kirkman 2009). FCM numbers need to be kept to a minimum and this means optimizing all the available control methods. One of the non-chemical based methods that is used is the sterile insect technique (SIT). It was first implemented in South Africa during 2007 by Xsit (Pty) Ltd. The method that is used utilizes the release of sterilized male moths into the wild to compete with wild males for females. This method has been shown to be effective in the Citrusdal area of the Western Cape, but is also being implemented in other parts of the country. Xsit only use males from their culture, originated from field collections in Citrusdal, for all their SIT programmes across South Africa. However, this raises questions around regional differences and compatibility. These questions pertain not only to SIT but also to monitoring and pheromone lure suitability. The aim of this project

was to determine if there were any pheromone variations between different FCM populations across South Africa. These possibilities were tested in laboratory based and field based experiments, to determine if female location by males could be affected and if attraction to different isomer blends differed regionally. This study was completed as an MSc study. For more detailed information, the thesis can be consulted.

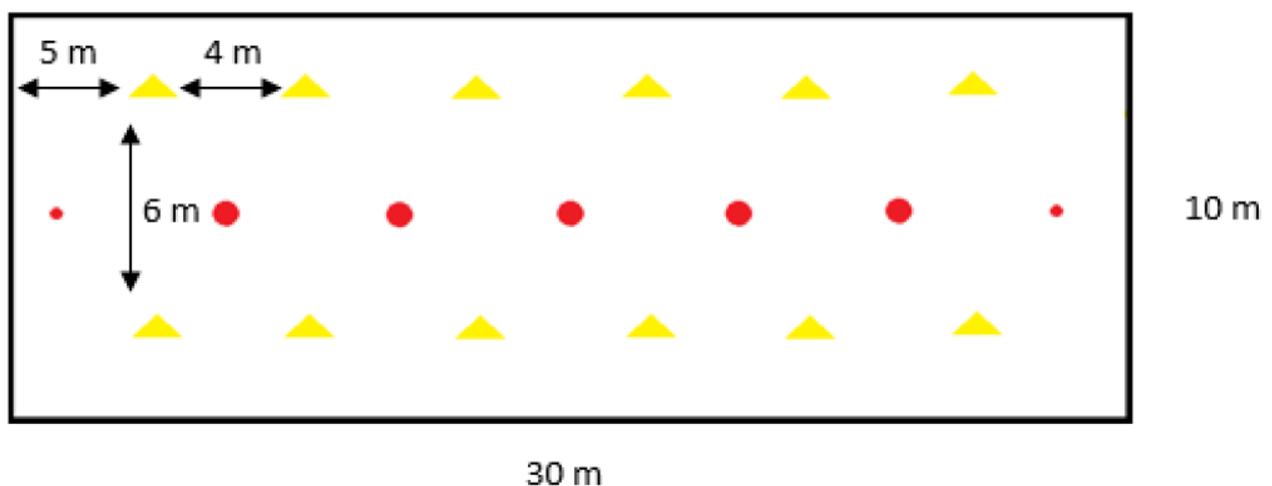
Objectives

- A laboratory trial was conducted with a Y-tube olfactometer and flight tunnel. This study attempted to reproduce the attraction of males to virgin FCM females under laboratory conditions. This would help to test the attraction of males to females from different geographical populations.
- To test the attraction of male moths to different artificial pheromone blends and virgin females from different geographical populations under field conditions. This would shed light on the impacts on SIT and FCM monitoring.
- To test possible postmating isolation that may take place when a male and female FCM mate and reproduce. This objective was investigated with a cross-mating trial.

Materials and methods

A detailed report of the methodologies followed can be found in the MSc thesis.

The attractiveness of virgin females from the Addo, Nelspruit, Marble Hall, Citrusdal and Old cultures to sterile male FCM (Xsit Citrusdal culture) was tested in field trials (three replicates) (Fig. 1). For the trial, 6 treatments were tested, each represented by two virgin females per cage per delta trap replicated 6 times each. The 6th treatment was a control with no females present.



- ▲ Delta trap position
- Release point for sterile male moths = 200 moths
- Release point for sterile male moths = half quantity 100 moths

Figure 1. Placement of traps and the release points of sterile male moths in a polyethylene tunnel for testing the response of the male moths to traps baited with females from different regional populations.

The six treatments used for attracting release male moths in the tunnel trials were (Fig. 2):

Commercial Blends

- FCM Pherolure (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: trans-7-dodecenyl acetate) (40%:10%:50%)

- Checkmate (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate)
(78%:22%)
- Isomate (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: trans-7/8-dodecenol)
(69.5%:29.5%:1%)

Regional blends

- Ivory Coast (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate)
(69%:23%:8%)
- Malawi (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate)
(32%:52%:16%)
- South Africa (trans-8-dodecenyl acetate: cis-8-dodecenyl acetate: dodecyl acetate)
(76%:10%:14%)

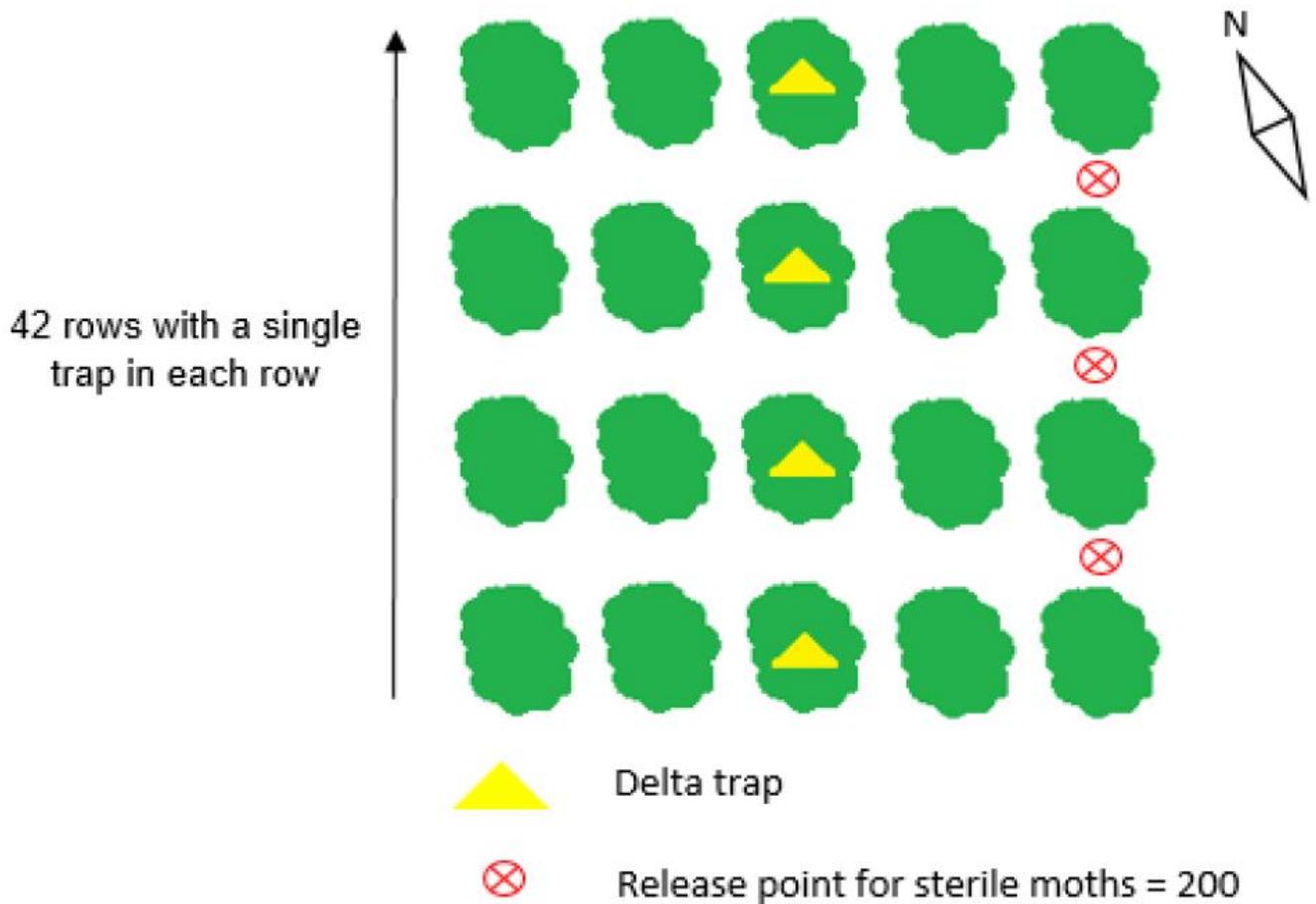


Figure 2. Trap placement and sterile moth release points used for field trials for testing the response of the male moths to traps baited with different blends of synthetic pheromones.

Results and discussion

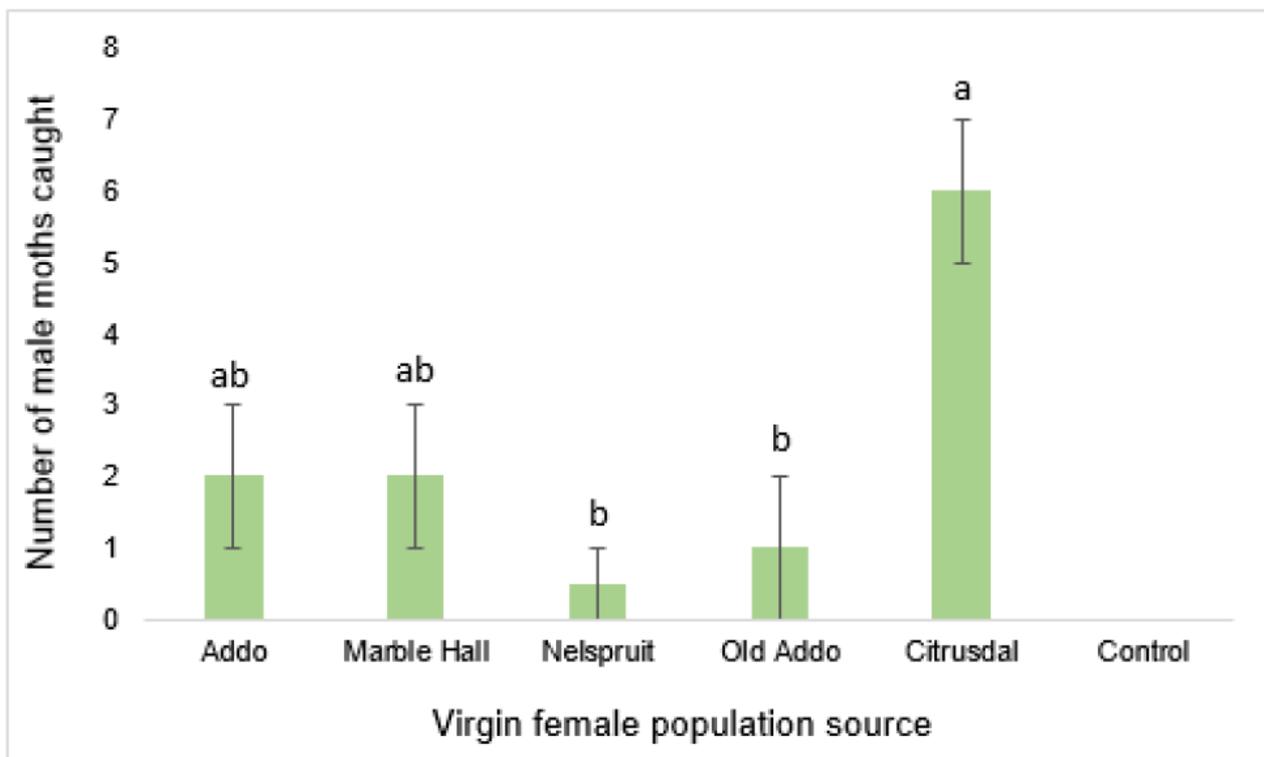


Figure 3. Mean (\pm SE) number of sterile FCM males caught in delta traps using live virgin females from different populations (Replicate 2). Results for Replicate 3 were very similar; catches in Replicate 1 were too low.

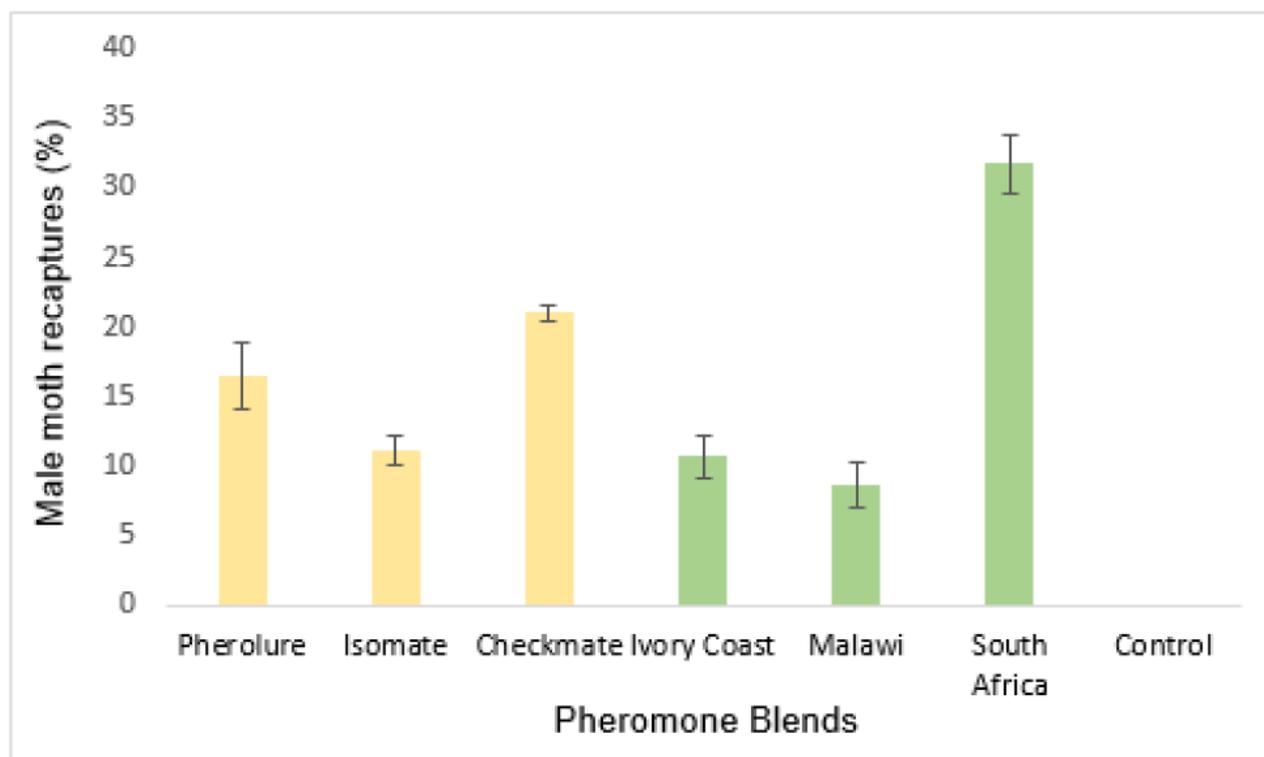


Figure 4. Mean (\pm SE) recaptures per synthetic pheromone blend as a percentage of the total recaptures for three replicates combined.

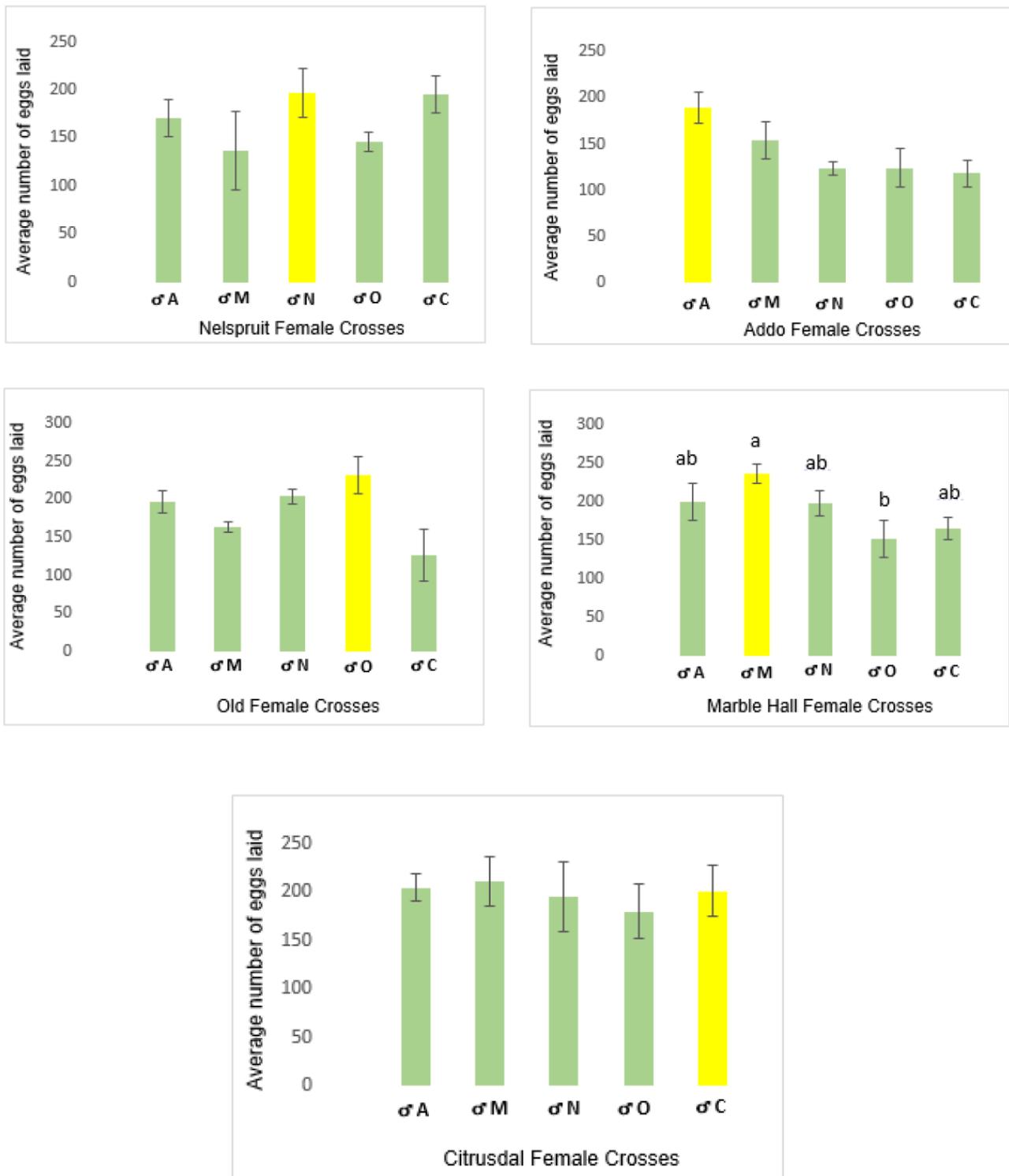


Figure 5. Number of eggs laid by FCM females mated with males from different geographical populations across South Africa (the acronyms used indicate the culture: Old (O), Addo (A), Nelspruit (N), Marble Hall (M) and Citrusdal (C). Bars with different letters are significantly different ($P < 0.05$).

Conclusions

Citrusdal males showed a significant preference for females from their own population. There was also a significant difference in the recaptures from the different artificial pheromones. The South African blend was the most attractive of all the regional and commercial blends. In a cross-mating trial, females produced more eggs when mated with males from the same population for the Addo, Marble Hall, Nelspruit and Old (mixed origin) populations. However, this was only statistically significant for the Marble Hall population. All the crosses

produced viable eggs and the origin of the male or female did not influence egg hatch. The results from this study may lead to improvements in both the control and monitoring of FCM populations.

Technology transfer

- Presentation at the ESSA meeting 2015: Assessment of pheromone specificity in *Thaumatotibia leucotreta* (Meyrick) populations with focus on pest monitoring and regional rollout of the sterile insect technique in citrus
- Poster at 9th CRI conference 2016: Assessment of pheromone specificity in *Thaumatotibia leucotreta* (Meyrick) populations with focus on pest monitoring and regional rollout of the sterile insect technique in citrus

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2.2.6 FINAL REPORT: Verification of proposed inspections standards within an FCM systems approach

Project 1085 (April 2014 – March 2018) by Sean Moore, Wayne Kirkman (CRI), Mathew Goddard (RU), Sean Thackeray (River Bioscience), Mellissa Peyper (RU), Paul Cronje (CRI) Gary Sharp (NMU), Ken Pringle (SU) and Vaughan Hattingh (CRI)

Summary

A systems approach was previously developed for phytosanitary risk mitigation associated with *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in citrus fruit exported from South Africa, as an alternative to a stand-alone cold disinfestation treatment. This study was conducted to test and improve the system by applying it to 10 Nova mandarin orchards for the full duration of the 2015/16 citrus production season. Once the systems approach equation for calculating the level of phytosanitary risk mitigation was improved, it was shown to be effective in measuring compliance with specified pre- and post-harvest infestation thresholds in all orchards. Improvements made to the systems approach included the use of weekly average infestation thresholds for orchard inspections, rather than four-weekly rolling averages, and increasing packinghouse delivery inspection samples to at least 600 fruit. Additionally, the size of this sample has a marked effect on the risk mitigation of the systems approach and thus larval mortality required from shipping temperature. Values for the proportion of infested fruit detectable on delivery to packinghouse and the grading efficacy in the packinghouse were revised according to results reported in this study. Furthermore, the equation for calculating the level of phytosanitary assurance provided by the systems approach was improved to provide a 95% assurance that the maximum potential fruit infested with live FCM after application of the system is no greater than that infested with live FCM after a Probit 9 level efficacy post-harvest disinfestation treatment has been applied to fruit with a pre-treatment infestation level of no more than 2%.

Opsomming

'n Stelselsbenadering is voorheen ontwikkel vir fitosanitêre risiko versagting gekoppel aan valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in sitrusvrugte uitgevoer van Suid-Afrika, as 'n alternatief vir 'n alleenstaande koue ontsmettings behandeling. Hierdie studie is uitgevoer om die stelsel te toets en te verbeter deur om dit in 10 Nova mandaryn boorde vir die volle tydperk van die 2015/16 sitrus

produksie seisoen uit te voer. Nadat die stelselsbenadering wiskundige vergelyking vir berekening van die vlak van fitosanitêre risiko vermindering verbeter is, is dit gewys dat dit doeltreffend was om die mate van nakoming met spesifieke voor- en na-oes besmettings drempelwaardes in alle boorde te meet. Verbeterings aan die stelselsbenadering sluit in weeklikse gemiddelde besmettings drempelwaardes vir boord inspeksies, eerder as vier-weeklikse rollende gemiddeldes, en die verhoging van pakhuisafleweringsmonsters vir inspeksies tot minstens 600 vrugte. Bonop het die grote van hierdie monster 'n beduidende effek op die risiko versagting van die stelselsbenadering en dus die nodige larwe mortaliteit van die verskepingstemperatuur gehad. Waardes vir die proporsie besmette vrugte opspoorbaar op aflewering by die pakhuis en doeltreffendheid van gradering in die pakhuis is volgens die resultate wat in hierdie studie gerapporteer is hersien. Verder, die wiskundige vergelyking om die vlak van fitosanitêre versekering te bereken wat deur die stelselsbenadering voorsien is, is verbeter om 'n 95% versekering te voorsien dat die maksimum moontlike besmetting met lewendige VKM na toepassing van die stelsel, nie hoër is as besmetting na toepassing van 'n Probit 9 vlak doeltreffendheid na-oes ontsmettings behandeling van vrugte wat nie meer as 'n 2% besmettings vlak gehad het voor die behandeling toegepas is.

Introduction

The South African citrus industry is dependent on export of fresh fruit to many markets around the world, with approximately 70% of South Africa's citrus crop being exported (CGA 2013, 2015). Some of these export markets, such as USA, Peoples Republic of China and South Korea require a disinfestation cold treatment for citrus fruit from South Africa, as a phytosanitary risk mitigation measure for false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (SA-DAFF 2015). This is due to its endemism to sub-Saharan Africa (Moore 2002) and its pest status on some types of citrus fruit in southern Africa (Newton 1998; Grout and Moore 2015). The cold treatment developed for FCM by Myburgh (1965) entails maintenance of temperatures below 0°C for 22 d. Moore et al. (2017) subsequently determined that the cold treatment could be improved with the following treatments still achieving Probit 9 level efficacy: 19 days at temperatures $\leq 1.2^{\circ}\text{C}$ or 16 days at temperatures $\leq -0.1^{\circ}\text{C}$. However, certain citrus types and cultivars are susceptible to chilling injury (Lafuente et al. 2003; Cronjé 2007) and thus not suitable for export under such cold treatment protocols.

Recently the European Union (EU) also regulated FCM as a phytosanitary pest [Commission Directive 2017/1279] (EU, 2017). The regulation states that citrus from Africa, amongst other crops, must either be sourced from a FCM-free area or place of production, or must be subjected to an effective cold treatment or another effective treatment to ensure freedom from FCM.

An estimated 35-45% of South Africa's citrus exports go to Europe each year (CGA 2013, 2015). Although it is possible to subject the relatively small volumes of fruit that are exported to niche markets, such as USA and South Korea, to a stand-alone cold treatment as a phytosanitary risk mitigation for FCM, this is not possible for the large volumes of fruit that are exported to Europe. However, alternative options, such as systems approaches (Follett and Neven 2006), are increasingly being accepted and implemented (e.g. USDA, APHIS and BAPHIQ 2008; Jang et al., 2015; Anonymous 2017a & b; NWHC, 2017). A systems approach is defined as "the integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests" (FAO 2002, 2007). Aluja and Mangan (2008) define a systems approach as "the integration of pre and postharvest practices, from the production of a commodity to its distribution and end use, that cumulatively meet predetermined requirements for quarantine security".

Consequently, and in anticipation of the EU regulation, based on a FCM pest risk analysis conducted by the European and Mediterranean Plant Protection Organization (EPPO, 2013), a systems approach for FCM has been developed. At an earlier stage of development, the systems approach was reported to include the following steps (Moore et al, 2016a): 1) official registration of orchards for participation in the systems approach; 2) compulsory production phase orchard sanitation, including the removal and destruction of all visibly damaged and infested fruit from trees and the removal and destruction of all fallen fruit from the orchard floor on at least a weekly basis; 3) Good Agricultural Practice for the control of FCM; 4) use of only registered

pre-harvest control measures in accordance with standardized crop production procedures; 5) orchard monitoring for FCM using pheromone-baited traps; 6) fruit infestation assessments during 12 wk prior to and during harvest with a threshold for additional pre-harvest control measures; 7) fruit infestation monitoring during the period of 4 wk prior to harvest to determine subsequent handling requirements; 8) post-picking, in-orchard grading out of potentially infested fruit; 9) postharvest fruit inspections for FCM infestation on delivery at the packinghouse, to determine subsequent handling requirements; 10) packinghouse grading of fruit on the packing line, involving the removal of any fruit appearing to be damaged or infested; 11) official inspection of a 2% fruit sample per pallet of fruit packed for export, with no tolerance for detected presence of live FCM; 12) prescription of shipping condition options for each export consignment according to compliance with preceding steps of the systems approach; 13) official phytosanitary certification of compliant consignments.

The standards (thresholds) for compliance with evaluations at various steps within the systems approach, were initially derived from practical experience obtained during the earlier stages of developing the systems approach, taking into consideration pest biology in combination with pre and postharvest pest management (Moore et al., 2016a). In development of the system, larval infestation of fruit was monitored in 33 citrus orchards throughout the season, both pre and postharvest (including post-harvesting and post-packing into export cartons), and it was noted that there was an improvement in the level of compliance with each of the sequential steps in the system, thus verifying that the grading and inspection thresholds were appropriate (Moore et al. 2016a). Moore et al. (2016a) reported that the level of risk mitigation assurance that compliance with the systems approach would provide, was calculated to be the following: the proportion of fruit that could potentially be infested with live FCM after application of the systems approach was determined to be 6 to 38 times less than the proportionate survival in a pest population exposed to a Probit 9 level efficacy stand-alone disinfestation cold treatment (Moore et al. 2016a), being 3 survivors in 100,000 at the 95% confidence level (Follett and Neven, 2006).

The key minimum compliance standards as measured in the system were given as: 1) a 4-wk average of 0.2 infested fruit per tree per week up to 4-wk prior to harvest, requiring additional controls if exceeded; 2) not more than a 4-wk average of 0.2 infested fruit per tree per week during the last 4 wk prior to or during harvest; 3) not more than 0.33% fruit infested on delivery to the packinghouse if inspections are non-destructive (superficial); 4) no infested fruit detected in a 2% sample taken from each pallet of fruit packed for export.

The objective of this study was to validate the system by applying it to 10 Nova mandarin orchards for the full duration of the 2015/16 citrus production season and to identify shortcomings in the system that could be corrected to improve the phytosanitary risk mitigation assurance provided by the system. Consequently, several improvements to the system and the equation for determining its level of phytosanitary risk mitigation are proposed. This improved system is currently being applied for exports of citrus from South Africa to the EU.

Objectives

The objective of this study was to validate the system by applying it to a range of Nova mandarin orchards for the full duration of the 2015/16 citrus production season. By so doing, any shortcomings with the system could be identified and further improvements made to the efficacy and accuracy of the system.

Material and methods

Trial sites

Ten orchards of Nova mandarins were selected in the Sundays River Valley (Table 1). Nova mandarins were selected for the trial as they are an important cultivar for export to countries which regulate FCM as a phytosanitary organism and Nova mandarins are susceptible to chilling injury, precluding the use of a stand-alone cold treatment as a disinfestation treatment.

Table 1. Details of Nova mandarin orchards in the Sundays River Valley, used for the FCM systems approach validation trials.

Orchard code	Coordinates	Year planted	Area (ha)	Tree spacing (rows x trees) (m)
DE7	33°28'36.38" S 25°33'34.30" E	1998	2.42	6.0 x 2.0
DE13	33°28'28,75" S 25°34'09.04" E	1997	2.52	5.5 x 2.0
DV3	33°27'13.12" S 25°33'27.15" E	1997	2.38	5.5 x 2.5
BD16	33°24'46.00" S 25°25'16.69" E	1998	1.52	5.0 x 2.0
L7	33°24'03.34" S 25°28'18.25" E	1999	3.04	6.0 x 2.0
S4	33°26'05.20" S 25°24'47.45" E	2000	1.33	5.0 x 2.0
R22	33°31'02.78" S 25°37'53.74" E	1999	1.7	5.0 x 2.0
Ha48	33°29'22.65" S 25°40'25.83" E	1999	0.61	5.0 x 2.0
The8	33°31'48.28" S 25°40'26.16" E	1997	2.25	5.0 x 2.5
P27	33°35'31.93" S 25°41'42.35" E	1998	3.15	5.0 x 2.0

Orchard monitoring

As per recommendation within the systems approach, one pheromone trap was hung in each orchard. These were yellow delta traps (210 x 197 x 143 mm (length x floor width x roof plane width)) with sticky floors and a Chempac FCM Lure (Chempac, Suider Paarl, South Africa). Traps were hung in the fifth tree of the fifth row of the orchard on the up-wind (southern) side of the orchard, at approximately 1.8 m above the ground (Moore 2016). Pheromone lures were changed every 12 wk. Traps were hung on 4 November 2015 and monitored each week on the same day until harvest (May 2016). All FCM adults caught in traps were recorded and removed.

On 31 December 2015, five data trees adjacent to the trap tree, on the inner-orchard side of the row, were marked with hazard tape and all fallen fruit under the trees were cleared. Thereafter, every week on the same day, starting from 7 January 2016, all fallen fruit under the five data trees were collected and inspected for FCM larval infestation, in accordance with the proposed systems approach (Moore et al. 2016a), based on the monitoring strategy recommended to growers (Moore 2016) and used for field evaluation of FCM insecticide efficacy trials (Moore et al. 2015a). Inspection was done by carefully inspecting and dissecting each fruit and determining whether the fruit was infested with a FCM larva and recording it as such. This fruit drop analysis is the most accurate method for monitoring FCM populations in the orchard, since all infested fruit would drop off the tree and the presence of infestation is obvious in such fruit (Moore et al. 2015a). Monitoring was continued until initiation of harvesting. However, only data from the final 12 wk before harvesting began were considered, as per the systems approach. Additionally, the infestation data from the final 4 wk before harvest were considered separately, as a potential guide to determine appropriate postharvest handling conditions during shipping.

Postharvest inspections

Harvesting of orchards occurred between 9 May and 13 June 2016. All but one of the orchards were harvested over a period of 3 to 6 days. The remaining orchard was harvested in a single day. On each day of harvest, a random sample of 300 fruit was taken from the picking bins on delivery to the packinghouse. Fruit were carefully inspected for signs of FCM larval penetration and infestation. This included any blemishes on or splitting of the fruit. All fruit bearing such signs were carefully dissected and inspected for infestation. All infestation was recorded, including the instar of the larva.

After the harvested fruit had been through the packinghouse process, including online grading entailing the removal of any fruit suspected of being infested, fruit were packed into export cartons. Approximately 3,200 fruit from each orchard were removed for further handling and inspection. Firstly, a sample of 70 fruit from each packed orchard was removed and inspected, in order to emulate the standard export procedure of a 2% sample from each pallet of fruit packed for export being officially inspected (Moore et al. 2016a). Only one such sample was taken from each orchard, even though there would normally be several pallets packed per

orchard under a commercial situation. Once again, only fruit bearing any possible signs of larval penetration or infestation were dissected and inspected further. All larvae found were recorded.

Thereafter, approximately half of the remaining fruit (approximately 1,600) were dissected and inspected for FCM infestation. Larvae were categorized as alive or dead and their instar was determined according to head capsule width (Daiber 1979; Hofmeyr et al. 2016).

Cold treatments

The remaining half of the fruit were subjected to simulated shipping at a temperature of 2°C (pulp temperature) for 18 d. This was conducted in a custom-built cold treatment chamber at Citrus Research International (CRI) in Port Elizabeth, Eastern Cape (5.0 m x 4.0 m x 2.4 m). The cold room was on a 6-h defrost cycle. A Brainchild data logger (Wika Instruments, South Africa) was used for recording temperatures in the cold room and inside the fruit, using 16 PT100 probes (Wika Instruments, South Africa) with a stated accuracy of 0.3 °C. Probes were calibrated before each treatment using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reached equilibrium (Grout et al. 2011; Moore et al. 2016b). A thermometer immersed in the melting ice was used to confirm the temperature. Three calibration runs were conducted and the mean result for each probe was used for correction purposes. Calibration was performed immediately before initiation of trials. Most probes were found to be 100% accurate. Inaccuracy was never measured at more than 0.01 °C and such probes were recalibrated to be fully accurate. Two probes were used to measure air temperatures at each of the inlet and outlet of the cooling coil. Ten probes were inserted into “indicator” fruit to measure pulp temperature. These fruit were placed in an approximately regular fashion amongst the trial fruit. Four “floating” probes were used in each new batch of trial fruit placed into the cold room, to measure the drop in temperature in fruit from ambient to the target temperature. Throughout, temperatures were recorded at 10 min intervals and averaged for the 14 probes used in the fruit. Mean temperature and mean maxima and minima were calculated. Cartons of fruit were covered with blankets to insulate them from rapid temperature fluctuations, particularly during the defrost cycle in the cold-rooms and in the event of cold-room doors being opened to remove samples. When the temperature in the fruit dropped to the target temperature, recorded by at least 50% of the probes, treatment duration was deemed to commence. Cooling was then adjusted slightly if necessary to achieve the target temperature. Fruit were removed after the designated duration and kept at 25 °C, uncontrolled humidity and natural daylight for 24 h before evaluation.

Fruit were carefully dissected and larvae were identified under magnification as alive or dead and their instar determined according to head capsule width. Larvae were determined to be dead when they displayed a brown discoloration and there was no movement after repeated prodding (Moore et al. 2016a).

Chilling damage

After completion of the cold treatment, approximately 50 fruit that had been exposed to the cold treatment and 50 fruit that had not been (control fruit), from each orchard, were evaluated for possible chilling damage. However, this was only done seven days after completion of the cold treatment, so that any chilling damage that had occurred could develop fully and become more visually conspicuous. Chilling symptoms were considered to be a brown discoloration of the flavedo, accompanied by pit-like depressions (Lafuente and Zacarias 2006). Chilling damage was categorized from 0 to 3, based on necrotic surface and intensity of browning (Lafuente et al. 2003): 0 (no damage), 1 (< 10% of surface area affected), 2 (10-25% of surface area affected) and 3 (> 25% of surface area affected) (Fig. 1). A chilling injury index, CI index, was determined according to the following formula: $CI\ index = \frac{\sum(CI\ scale\ (0-3) \times \text{number of fruit in each class})}{\text{total number of fruit estimated}}$ (Lafuente et al. 2003). This was also converted to a percentage chilling injury.

Packinghouse grading efficacy

The efficacy of the packinghouse grading process was previously tested, thus determining the mean reduction in fruit infestation from delivery to packinghouse to being packed for export. However, due to subsequently increased awareness of the phytosanitary importance of FCM, the efficacy of the packinghouse grading process was again examined.

Sixteen orchards, packed in six different packinghouses were used for this trial. All orchards were Valencia (either Midnight or Delta) oranges (Table 2). After harvest, an estimated 600 fruit were randomly sampled from picking bins on delivery to the packinghouse from each orchard. After packinghouse sorting, grading and being packed for export, a further estimated 600 Class 1 fruit (i.e. fruit of the highest quality) were sampled. All fruit sampled were thoroughly inspected for signs of FCM infestation. This was done by carefully dissecting the fruit, as described by Moore et al. (2015a), and inspecting for FCM larvae. The following measurements were recorded: number of fruit infested, the number of infested fruit with externally visible signs of infestation, and the larval instar.

Table 2. Orchards and packinghouses used to test the efficacy of the packinghouse grading process in detecting and removing FCM infested fruit.

Packinghouse	Farm	Orchard	Cultivar	Number of fruit inspected	
				Prepacking	Post-packing
SK	TC	17	Midnight	568	615
	TC	49	Delta	645	630
SH	FPBM	4	Delta	568	630
	LM	51	Midnight	567	630
	Kp	23	Midnight	657	630
SS	BFJ	100	Delta	655	628
	RFE	34	Midnight	382	600
	W	19	Midnight	610	517
W	W	20	Midnight	610	620
	Ad	12	Delta	469	630
	Uf	62	Midnight	382	629
Uf	Ha	16	Midnight	463	543
	JAB	30	Delta	590	615
	PSB	10	Midnight	612	616
PSB	Lh	19	Midnight	589	722
	Dh	5	Midnight	734	420
	LGV	22	Midnight	587	649

Improvement in calculating the level of phytosanitary risk mitigation provided by the systems approach

Previously, the equation to determine the maximum infestation level of fruit packed for export was given as:

$$I = ([S \times (1 - [D \times H])] + S) \times [1 - G],$$

where I = maximum infestation level, S = inspection standard on delivery to packinghouse; D = proportion of infested fruit detectable on delivery to packinghouse; H = human efficiency factor (as a proportion); and G = grading efficacy in the packinghouse grading (Moore et al. 2016a). Improvements were made to this methodology for calculating the level of risk mitigation assurance provided by the systems approach. Firstly, this was done by expressing S as a proportion, rather than a percentage, as was incorrectly done previously. Secondly, variance and hence standard deviations were calculated where appropriate and included in the equation, thus calculating of an overall standard deviation for the maximum potential infestation level of fruit packed for export. Additionally, revised values for the proportion of infested fruit detectable on delivery to the packinghouse and the packinghouse grading efficacy were used in the equation. Thereafter, a revised value for the effect of the 2% official post-packing inspection was included. The maximum potential infestation level before shipping, used to determine the mortality required and therefore shipping temperature required was reduced by the proportion of first and second instars in the population, as these are proven to be more cold-susceptible than the largest three instars and as shipping temperatures required are based on their efficacy against only the most cold-tolerant instars. Finally the formula used for calculating the post-shipping maximum potential infestation with live FCM was modified to provide an improved comparison with the risk mitigation provided by a stand-alone, Probit 9 level efficacy, disinfestation treatment. Full details of these improvements are provided under Results.

Results

Orchard monitoring

Moth catches in pheromone traps were low in all orchards, ranging from zero moths for the full duration of the monitoring period in two of the orchards to a mean (\pm SE) of 2.24 ± 0.40 moths per trap per week in the orchard with the highest catches (Table. 3). The highest number of moths caught in a trap in one week was nine (in orchard Ha48), lower than the action threshold (i.e. ≥ 10 moths per trap per week) that used to be recommended when management of FCM was conducted from an economic, rather than a phytosanitary, perspective (Moore et al. 2008). However, due to the greater stringency in FCM management, aimed at zero tolerance, this threshold has not been in use for many years now. Due to this zero tolerance, there is now no trap-based action threshold and traps are used purely for management purposes such as improved timing of spray application (Grout and Moore, 2015).

Table 3. FCM adults caught in a pheromone trap in each of 10 Nova mandarin orchards from 11 November 2015 to 25 May 2016.

Orchard code	Total moths caught	Mean moths per trap per week (\pm SE)
DE7	6	0.21 ± 0.08
DE13	6	0.21 ± 0.14
DV3	1	0.03 ± 0.03
BD16	12	0.41 ± 0.13
L7	9	0.31 ± 0.10
S4	0	0
R22	0	0
Ha48	65	2.24 ± 0.40
The8	19	0.65 ± 0.23
P27	5	0.18 ± 0.09

In only one of the orchards (L7) was FCM infestation of fruit recorded at any stage during the season (Table 4), confirming the low presence of the pest, as indicated by the low trap catches, and also possibly a low susceptibility of the cultivar to infestation by FCM. The proposed FCM systems approach recommended that if a threshold of a 4-wk rolling average of 0.2 infested fruit per tree per week is surpassed, then an additional registered control measure must be applied. This occurred on two occasions in orchard L7 (Fig. 2). The farmer was informed after the first incident on 11 March 2016 and he responded by spraying Cryptex (Andermatt, Switzerland), a formulation of the *Cryptophlebia leucotreta* granulovirus (Moore et al. 2015a). The second exceedance occurred on 1 April. However, the systems approach states that the intervention threshold will only again apply to the next 4-wk period commencing five weeks after the registered control measure was applied, thus providing sufficient time for the efficacy of the control measure to be detected through a reduction in FCM infestation. Therefore the necessity for an additional treatment was not indicated by the second exceedance.

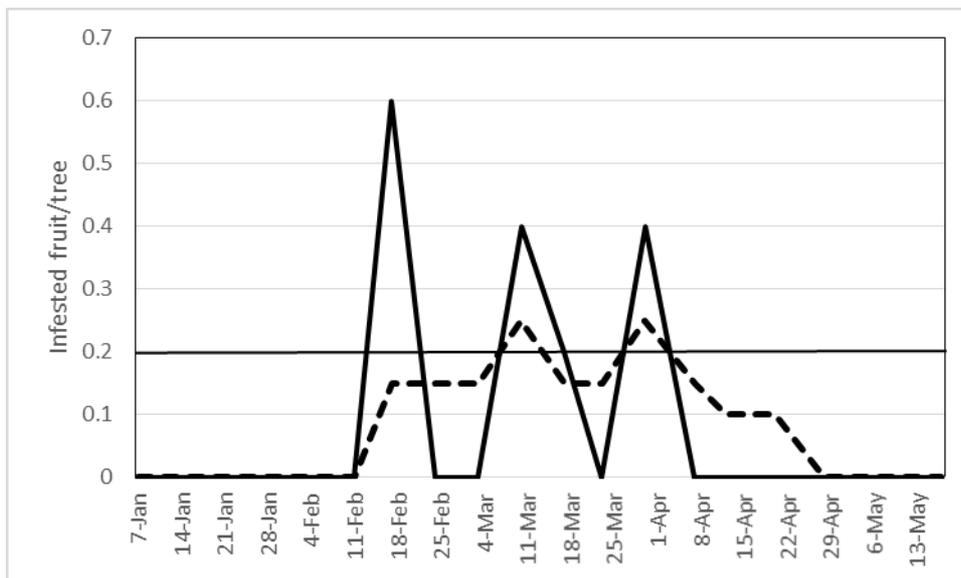


Figure 2. Average number of FCM infested fruit per tree per week in orchard L7, Nova mandarins. Solid line is average per tree per week; dashed line is the 4-wk rolling average per tree per week; horizontal line at 0.2 indicates the proposed action threshold, which applies to the 4-wk rolling average. Harvesting was initiated on 16 May 2016, therefore the 4-wk preharvest period, during which postharvest handling conditions are determined, began on 21 April 2016.

Postharvest inspections

The first postharvest inspection to be conducted was on delivery of fruit to the packinghouse. One infested fruit out of the 300 inspected was recorded from each of three orchards (BD16, Ha48 and The8) and three infested fruit were recorded from orchard L7 (Table 4). As the threshold at this inspection point was no more than one infested fruit (0.33%) (Moore et al. 2016a), only orchard L7 exceeded the threshold with an infestation level of 1.00%. The four orchards from which infestation was recorded in fruit on delivery to the packinghouse were also the four orchards in which the highest trap catches were recorded in the field (Table 3).

After the grading and treatment process of the packinghouse, no infested fruit were recorded in any of the 70 fruit (approximately 2%) samples taken from the cartons packed for export (Table 4).

Of the fruit cut from each orchard pre-cold treatment (between 998 and 2,504 fruit per orchard), FCM infestation was recorded from five of the nine remaining orchards (Table 4) (L7 already having been excluded from less stringent handling options during shipping). In four of the five orchards, infestation was very low, between one to two infested fruit, which was well below the maximum of 0.419% infestation that the systems approach was previously designed to achieve at this point (Moore et al. 2016a). Infestation was higher than this, at 0.50%, in Ha48. However, it must be noted that this calculation has now changed, by using the appropriate formula as given by Couey and Chew (1986), as explained below for the improvement in calculating the assurance provided by the systems approach.

Cold treatments

The mean temperature recorded over the full duration of the cold treatments from the 10 probes inserted into the “indicator” fruit was $1.98 \pm 0.00^\circ\text{C}$. The mean minimum and mean maximum daily temperatures recorded over this time were $1.72 \pm 0.00^\circ\text{C}$ and $2.18 \pm 0.00^\circ\text{C}$ respectively. After cold treatment at 2°C for 18 d, inspections for infestation were again conducted and were recorded in fruit from five of the nine orchards (Table 4). Three of these five orchards were the same ones in which infestation was recorded before the cold treatment was applied ((BD16, S4 and Ha48). Infestation was again below the 0.419% level in four of the five orchards, and again higher than this, at 0.68%, in Ha48. When the pre and post-cold treatment infestation figures were combined, infestation was recorded in seven out of the nine orchards, with the level of infestation in all of the orchards, except Ha48, which was 0.60%, being well under 0.419%. Only 34.78% of the larvae were fourth and fifth instars (Table 5), recognized as being the most cold-tolerant instars (Myburgh 1965;

Boardman et al. 2012; Moore et al. 2016b). All of the larvae found post-cold treatment were dead (Table 4), confirming the efficacy of the treatment as a component within the systems approach.

Table 4. FCM larval infestation of fruit from 10 Nova mandarin orchards at various stages from pre-harvest to packed for export.

Orchard code	Date harvesting initiated	Ave. number infested fruit/tree/week in orchard (pre-harvest)		Infested fruit postharvest (%)							
		During 12 wk before harvest	Final 4 wk before harvest	Packinghouse delivery ¹	Packed for export (and number of fruit inspected)						
					Simulated official inspection ²	Before cold treatment	After cold treatment ⁴	Overall			
DE7	27 May 2016	0	0	0	0	0.08 ³	(1,273)	0	(1,382)	0.04	(2,655)
DE13	26 May 2016	0	0	0	0	0	(1,963)	0.05	(1,949)	0.02	(3,912)
DV3	27 May 2016	0	0	0	0	0	(1,577)	0	(1,294)	0	(2,871)
BD16	17 May 2016	0	0	0.33	0	0.08	(2,504)	0.24	(2,501)	0.17	(5,005)
L7	16 May 2016	0.083	0	1.00	0	-	-	-	-	-	-
S4	2 June 2016	0	0	0	0	0.10	(1,902)	0.06	(1,625)	0.08	(3,527)
R22	27 May 2016	0	0	0	0	0.06	(1,726)	0	(1,696)	0.03	(3,422)
Ha48	26 May 2016	0	0	0.33	0	0.50	(1,920)	0.68	(2,059)	0.60	(3,979)
The8	27 May 2016	0	0	0.33	0	0	(998)	0	(1,055)	0	(2,053)
P27	27 May 2016	0	0	0	0	0	(1,019)	0.10	(948)	0.05	(1,967)

¹All larvae were alive.

²70 fruit = ± 2% sample

³One larva, which was dead.

⁴All larvae were dead.

Table 5. FCM instars infesting fruit from 10 Nova mandarin orchards in postharvest inspections.

Inspection point		Instars					Total larvae
		1	2	3	4	5	
Packinghouse delivery		1	5	0	1	0	7
Packed for export: before cold treatment		1	4	4	3	4	16
Packed for export: after cold treatment		0	7	8	5	3	23
Total	n	2	16	12	9	7	46
	% of total	4.35	34.78	26.09	19.56	15.22	

Chilling injury

None of the control samples of fruit from all nine of the orchards from which fruit were cold treated, showed any damage or blemishes that resembled chilling injury. Chilling injury on cold-treated fruit (Fig. 3) ranged from 0 to 18.37% (Table 6). Total chilling injury for all of the fruit pooled (427 fruit) was 6.36% and the mean (\pm SE) chilling injury for the nine orchards was $5.15 \pm 1.84\%$.



Figure 3. Chilling injuries recorded on Nova mandarins.

Table 6. Chilling injury to Nova mandarins from nine orchards after exposure to 2°C for 18 d.

Orchard	n	Chilling injury index	Chilling injury (%)
DE7	57	0.017	1.75
DE13	49	0.286	18.37
DV3	49	0.020	2.04
BD16	48	0.021	2.08
S4	46	0.042	4.17
R22	49	0.039	3.92
Ha48	48	0	0
The8	50	0.100	8.00
P27	50	0.120	6.00

Packinghouse grading efficacy

It was previously reported that the mean reduction in fruit infestation from delivery to packinghouse to being packed for export, as a result of the packinghouse grading process was only $21.1 \pm 0.2\%$ (Moore et al., 2016a). Consequently, this figure was used in the equation developed to calculate the level of assurance provided by the systems approach up to the point of packing (Moore et al., 2016a). However, trials reported in this study demonstrated an improvement in this to $66.1 \pm 40.78\%$ (Table 7). This was in all likelihood a result of the increased awareness of the phytosanitary importance of FCM. From the 23 larvae found infesting fruit packed for export (post grading), relative proportions of the instars from first to fifth were 0%, 30.4%, 34.8%, 21.7% and 13.0%. This was not dissimilar to the relative proportions of FCM instars infesting fruit packed for export, reported by Moore et al. (2016a). It is also interesting to note that only five of the 17 orchards inspected had an infestation level higher than the maximum permissible level to avoid the most stringent handling conditions during shipping i.e. no more than 2 infested fruit in a sample of 600 (equating to $< 0.5\%$ infestation).

Table 7. Efficacy of the packinghouse online grading system for removing FCM infested Valencia oranges before packing.

Packinghouse	Farm	Orchard	Fruit infested prepacking (%)	Reduction in infestation post-packing (%)
SK	TC	17	0.18	100
	TC	49	0	-
	FPBM	4	0.35	54.92
SH	LM	51	0	-
	Kp	23	0.30	0
	BFJ	100	0.15	100
SS	RFE	34	0.79 ¹	100
W	W	19	0.33	100
	W	20	0.16	100
	Ad	12	0	-
Uf	Bb	62	1.31 ¹	87.85
	Ha	16	0	-
	JAB	30	0.68 ¹	28.05
PSB	Rd	10	0	-
	Lh	19	0	-
	Dh	5	0.54 ¹	56.31
	LGV	22	1.36 ¹	0
Mean ± SE				66.10 ± 40.78

¹Orchards which would have failed the packinghouse delivery inspection of no more than 0.33% fruit infested.

From these fruit samples, the proportion of infested fruit visually detectable before cutting of the fruit to confirm infestation, was also recorded. For fruit on delivery to the packinghouse, this was 81.81% and for fruit post-packing, this was 83.33%. As these figures did not differ meaningfully, they were combined in order to enlarge the data set. Additionally, data reported with a similar study (Moore et al., 2016a) was also used. However, this was only available for detectability post-packing, as a figure of 73.1%. Nevertheless, these figures were combined and the mean proportion of infested fruit visually detectable was calculated as 79.69 ± 12.27% (Table 8). This is very similar to the figure of 77.8% previously reported (Moore et al., 2016a), albeit calculated as an overall proportion, rather than a mean per packinghouse, and can thus be used in calculating the assurance provided by the systems approach.

Table 8. Proportion of infested fruit visually detectable before cutting of fruit to confirm infestation, conducted with fruit samples from a total of seven packinghouses on two different occasions (2013 and 2017), both on delivery of fruit to the packinghouse and post-packing.

Year	Packinghouse	Number of fruit inspected	Number of fruit infested	Infestation externally detectable (%)
2013 ¹	SK	3,224	8	75.00
	SH	2,669	13	76.92
	Uf	2,712	17	70.59
	SC	3,909	14	71.43
2017	SK	3,656	5	80.00
	SH	3,767	5	100
	SS	982	3	66.67
	W	3,456	4	100
	Uf	3,222	16	68.75
	PSB	4,929	24	87.50
Mean ± SE				79.69 ± 12.27%

¹Reported in Moore et al. (2016a).

Improvement in calculating the assurance provided by the systems approach

Variance and standard deviation of values was not previously considered in the equation to determine the maximum infestation level of fruit packed for export,

$$I = ([S \times (1 - [D \times H])] + S) \times [1 - G] \quad \text{[equation 1]}$$

These have now been calculated and included for *D* (proportion of infested fruit detectable on delivery to packinghouse) and *G* (grading efficacy in the packinghouse grading) and for the final calculation of *I* (maximum infestation level). As previously reported, *H* (human factor – conservative and arbitrary assumption of the efficiency of the person conducting the inspection) remains 0.5 (50%). *D* changes slightly from 0.778 (77.8%) to 0.797 (79.7%), as new data have been included and it is now calculated as a mean of all of the samples inspected (described in Moore et al. (2016a)). The standard deviation for *D* is calculated as 0.123. The results from the latest packinghouse grading efficacy trial (Table 6) allow *G* to be improved to 0.661 (66.1%). Additionally, *G* is also calculated as a mean value, allowing the standard deviation to be calculated as 0.408. *S* (inspection standard on delivery to packinghouse) was previously given as 0.0033 (0.33%), but can vary, based on the size of the packinghouse delivery sample and on the infestation threshold permitted. This is envisaged to include the following options: no more than 2 infested fruit in 600, no more than 1 infested fruit in 600, no more than 1 infested fruit in 1,000, no more than 1 infested fruit in 1,900 and no more than 1 infested fruit in 2,800. However, other sample sizes and infestation thresholds can be used, and the appropriate ones selected depending on the shipping temperature to be used and the larval mortality attributable to the temperature and the minimum potential duration of the shipping journey to market. Although the other values in the equation are considered relatively fixed, there is a large degree of flexibility for the *S* value. Additionally, the appropriate equation for calculating the 95% confidence level (*C* = 0.95) should be used (Couey and Chew 1986). For one infested fruit in a sample, this would be:

$$C = 1 - (1 - p_u)^n - np_u(1 - p_u)^{n-1} \quad \text{[equation 2]}$$

for two infested fruit in a sample, this would be:

$$C = 1 - (1 - p_u)^n - np_u(1 - p_u)^{n-1} - \frac{1}{2}n(n-1)p_u^2(1 - p_u)^{n-2} \quad \text{[equation 3]}$$

where *C* is the confidence level, which we choose to be 95% (0.95), *n* is the sample size (number of fruit inspected) and *p_u* is the proportion of fruit within the sample that are infested. If *C* and *n* are known, then *p_u* is calculated iteratively i.e. by trial and error (Couey and Chew, 1986). For example, if *C* ≥ 0.95 and *n* = 600 and our maximum level of infestation permissible is 1, then we use equation 2, which calculates *p_u* to be 0.008 (i.e. 5 infested fruit in 600). If *C* ≥ 0.95 and *n* = 600 and our maximum level of infestation permissible is 2, then we use equation 3, which calculates *p_u* to be 0.012 (i.e. 7 infested fruit in 600). The value calculated for *p_u* in equations 2 and 3 is therefore effectively the value that is used for *S* in equation 1.

Consequently, the equation to determine the maximum infestation level of fruit packed for export as a proportion, remains as previously reported (Moore et al., 2016a), but with certain altered values with improved reliability. As an example, if the shipping temperature selected would result in a level of mortality which is adequate to reduce a 2 in 600 fruit infestation level (threshold) in the carton to a phytosanitarily acceptable level, then the equation would be populated as follows:

$$I = ([0.012 \times (1 - [0.797 \times 0.5])] + 0.012) \times (1 - 0.661) \\ = 0.0063$$

Additionally, the standard deviation (σ) was calculated as the square root of the variance, which was derived from the definition of the variance of a function of random variables (Bain and Engelhardt, 1991) as follows:

$$\begin{aligned} \text{Var}(I) &= \text{Var}(2S - S \times H \times D + S \times H \times D \times G - 2S \times G) \\ \sigma_I^2 &\square \sigma_D^2 \left(\frac{\partial I}{\partial D} \right)^2 + \sigma_G^2 \left(\frac{\partial I}{\partial G} \right)^2 + \rho_{DG} \sigma_D \sigma_G \left(\frac{\partial I}{\partial D} \right) \left(\frac{\partial I}{\partial G} \right) \\ \sigma_I^2 &\square \sigma_D^2 [S \times H (G - 1)]^2 + \sigma_G^2 [S (H \times D - 2)]^2 \end{aligned} \quad \text{[equation 4]}$$

Where $\frac{\partial I}{\partial D} = -S \times H + S \times H \times G$, $\frac{\partial I}{\partial G} = S \times H \times D - 2S$ and

$$\rho_{DG} \sigma_D \sigma_G \left(\frac{\partial I}{\partial D} \right) \left(\frac{\partial I}{\partial G} \right) = 0 \quad \text{assuming G and D are independent.}$$

The σ of I would therefore be calculated as 0.0076. Adding this σ to the value calculated for I (0.0063) would effectively give a maximum infestation level of 0.0139 (1.39%) in the example given.

Thereafter, a 2% sample is officially inspected from each pallet of fruit packed for export. Only pallets of fruit in which no FCM are detected in these inspections would be exportable within the systems approach. Twenty pallets are loaded into a shipping container which normally constitutes a consignment of export fruit. A batch of 20 pallets sampled and inspected in this way (2 cartons per pallet) would provide a sample of 2,880 fruit, assuming there are 72 fruit per carton and 70 cartons per pallet, which is common for oranges packed for export. This would provide an assurance at the 95% confidence level that no more than 0.0205 (2.05%) of the fruit would be infested (Couey and Chew 1986), based on the previously used 79.7% detectability. This is not as effective as the previously calculated 0.181% infestation (Moore et al. 2016a). However, the calculation is more accurate as it now includes a 95% confidence limit, calculated according to Couey and Chew's (1986) equation for zero infestation in the sample:

$$p_u = 1 - (1 - C)^{1/n} \quad \text{[equation 5].}$$

As the packinghouse inspection and the grading process plus the post-packing official inspection are two independent measures, the overall risk mitigation up to this point can be calculated as the product of these independent measures (FAO 2002). Therefore, the previously calculated 0.0139 (1.39%) maximum potential infestation level would be reduced to 0.000017 (0.0017%).

Thereafter the systems approach includes the temperature conditions during shipping. However, not all instars are equally cold-susceptible. It has already been demonstrated that fourth and fifth instars are the most cold-tolerant (Myburgh 1965; Boardman et al. 2012; Moore et al. 2016c). Third instars are marginally less cold tolerant (Moore et al. 2016b). However, whereas there is a low level of survival of third, fourth and fifth instars after 18 d at 2°C, there is no survival of first and second instars (Moore et al. 2016b). FCM larvae infesting citrus fruit packed for export, 35.4% were first and second instars (Moore et al. 2016a). In the current study, no first instars were recorded infesting fruit packed for export. However, second instars made up 30.4% of all FCM larvae infesting fruit, thus supporting the finding in the previous study. However, as the total number of FCM larvae recorded infesting fruit packed for export in the current study was only 23, it is deemed disproportionate to obtain a mean from this and the 133 larvae recorded in the previous study. Consequently, the two figures were added, such that first and second instars made up 34.6% of all larvae. As first and second instars have been shown to be notably more cold-susceptible than fourth and fifth instars and as all cold treatment efficacy has been assessed using the most cold-tolerant instars, the maximum potential infestation level can be reduced from the previously calculated 0.000017 by 0.346 (i.e. the proportion of first and second instars in the population) to 0.000014 (0.0014%). This would equate to 1.4 infested fruit per 100,000 fruit, which is a lower level of infestation than the efficacy provided by a treatment achieving the Probit 9 standard (Follett and Neven, 2006), which is survival of no more than 3 individuals in a sample of 100,000 treated.

However, whereas a systems approach ensures no more than a certain level of infestation with live insects, a standalone treatment ensures a certain level of efficacy i.e. mortality, in this case at the Probit 9 level. Consequently, a further adjustment must be made to ensure that the systems approach does mitigate risk to the same extent as a standalone treatment. If it is assumed that infestation of a fresh product by a quarantine organism at time of export does not exceed 2%, then this is the level of infestation against which the Probit 9 efficacy treatment is employed. Consequently, the survival in a sample of 100,000 fruit after application of a Probit 9 treatment will actually be 0.06 individuals (3 x 0.02 (2%)), which is 95.7% less than the 1.4 infested

fruit per 100,000 achieved with the systems approach. Consequently, a final step is required in the systems approach to reduce the potential for infestation with live larvae by a further 95.7%. This is therefore the efficacy required from the shipping conditions.

Mortality in excess of this level is achieved with a temperature of 1°C for 14 d or 2°C for 16 d (Moore et al. 2016b). Recent unpublished data have also demonstrated that this can be achieved with a temperature of 3°C for 16 d or 4°C for 22 d. Further work will be required to determine the efficacy of a more extensive range of temperatures and durations.

Moore et al. (2016b) reported that 69.27% of larvae surviving a cold treatment of 2°C for 18 d, still died post the cold treatment. Death always occurred in the larval stage. If this is included in the final mortality figure achieved, then the direct mortality required from the shipping temperature in order to reduce the maximum possible infestation level in fruit, achieved by the systems approach up to that point, to a level equivalent to the Probit 9 standard, can be reduced further according to the following equation:

$$D = (P - F) / - ([100 - P] / 100) \quad [\text{equation 6}]$$

Where D = direct mortality required from the cold treatment, P = the post-cold mortality (69.27%) and F = the final overall mortality required. However, this equation will not be used in calculating the overall risk mitigation provided by application of the systems approach

In summary, there are five equations and two further calculations for determining the risk mitigation provided by the systems approach:

- Equation 1 calculates the maximum infestation level of fruit packed for export (I).
- Within equation 1, the inspection standard on delivery to packinghouse (S) is substituted with p_u , calculated from equation 2 or equation 3, depending on whether the inspection standard is 1 or 2 infested fruit.
- The standard deviation of I , calculated using equation 4, is added to the maximum infestation level of fruit packed for export (I), calculated from equation 1 (= A).
- The maximum infestation level of a consignment of fruit after official inspection is determined according to equation 5 and this value is multiplied by A (= B).
- B is reduced by the proportion of first and second instars determined to be infesting a sample of fruit at the time of packing i.e. a 34.6% reduction (= C).
- C is then adjusted for comparability with the efficacy provided by a Probit 9 standalone treatment (3 survivors in 100,000) assuming that such treatment was applied to a consignment of fruit in which 2% of fruit were infested (i.e. $3 \times 0.02 = 0.06$).
- Finally, the level of mortality required from the final step in the systems approach i.e. the handling conditions during shipping, is calculated as the percentage reduction from C to 0.06.

Discussion

A systems approach developed by Moore et al. (2016a) for mitigation of risk associated with FCM in citrus fruit exported from South Africa, as an alternative to stand-alone cold treatment, was applied to 10 Nova mandarin orchards in the Eastern Cape Province of South Africa for the full duration of the 2015/16 season. This was done in order to validate the systems approach reported in Moore et al. (2016a). Firstly, the systems approach involved weekly monitoring of FCM levels in orchards, most importantly the monitoring of fruit infestation. If a threshold of an average of 0.2 infested fruit per tree per week, over a 4-wk rolling period, was surpassed during the period 12 wk prior to start of harvest, the systems approach stipulated that a registered control measure must be applied. This only occurred in one of the 10 orchards (orchard L7). Secondly, the systems approach stipulated that if an average of 0.2 infested fruit per tree per week was exceeded over a 4-wk rolling period during the 4 wk preceding commencement of harvesting and during harvesting, then the orchard must be shipped at a temperature capable of inducing total mortality, such as those determined by Moore et al. (2017). In the case of orchard L7, despite the control measure being applied to the orchard, infestation of fruit in the orchard still exceeded the stipulated threshold during the last 4 wk before harvest. By using a 4-wk rolling period average, rather than a weekly average, the application of a treatment was delayed by 3 wk. The stipulated threshold was first surpassed on 17 February, but the 4-wk rolling average was only surpassed on

11 March (Fig. 2). Additionally, as the second exceedance of the 4-wk rolling average threshold was surpassed within 5 wk of the treatment application, it was not necessary to apply a second treatment, according to the systems approach. However, if a weekly average, rather than a rolling average, was used, a second treatment would have been required on 1 April (Fig. 2). Consequently, an intervention threshold based on weekly average fruit infestation, rather than a 4-wk rolling average, would have resulted both in a treatment being applied earlier and a second treatment being applied. Making such a change to the systems approach, would reduce the likelihood of a compliant orchard becoming non-compliant during the last 4 wk before harvest and would thus be an improvement to the systems approach. Although the first exceedance of the intervention threshold occurred 14 wk before harvesting began, therefore outside of the 12 wk threshold period, it is still highly probable that the grower would have applied a control measure, as the exact harvesting date is not known until shortly before harvesting, due to variable and unpredictable fruit maturation. Consequently, growers are recommended to initiate this monitoring, including application of thresholds, 16 wk before the projected harvest date. It is also important to point out that the systems approach does not dictate that a treatment must only be applied if the fruit infestation threshold is surpassed. Farmers are trained and exhorted to follow a preventative treatment program for FCM (Moore 2016; Grout and Moore, 2015). If this is done and the fruit infestation threshold of an average of 0.2 infested fruit per week is still surpassed, then the systems approach requires an additional control measure to be employed, regardless of what other control measures might previously have been used.

The systems approach proved effective in determining whether fruit from orchards should be shipped at colder temperatures due to higher risk of infestation in packed fruit (orchard L7) or verifying that they could be shipped under less severe conditions. There appeared to be one aberration to this i.e. orchard Ha48. This orchard did not surpass any of the pre or postharvest infestation thresholds (Table 4), but infestation of fruit in the carton still exceeded the maximum potential infestation of 0.419% predicted by the systems approach. However, as demonstrated in the improved calculation of the assurance provided by the systems approach, the maximum potential infestation of fruit in the carton has now been adjusted to 1.39%. Consequently, the infestation of 0.60%, recorded in a sample of 3979 fruit in the carton did not exceed the level predicted by the systems approach. Nevertheless, this orchard was the only one that was harvested in a single day and consequently, the only orchard from which only one 300 fruit sample was taken for inspection on delivery to the packinghouse. The other orchards were harvested over 2 to 6 days, thus the total number of fruit sampled and inspected per orchard on delivery to the packinghouse was between 600 and 1800 fruit per orchard. If one permits a threshold of 0.33% infestation, a 300 fruit sample provides assurance of detecting a 1.7% infestation with a 95% confidence level, whereas a 600 fruit sample provides assurance of detecting a 1.2% infestation with a 95% confidence level (Couey and Chew 1986). Consequently, it may be prudent to increase the minimum sample size for packinghouse delivery inspection from 300 to 600 fruit per orchard. If the threshold for this inspection remains no more than 0.33% fruit infested, this equates to no more than 2 infested fruit in 600 (or otherwise stated as < 0.5% infestation).

Calculation of the maximum potential infestation level is sensitive to altering the sample size for packinghouse delivery inspection. By increasing the sample size (assuming the infestation threshold remains low), the maximum potential infestation level is reduced, thus requiring a reduced level of mortality from the shipping temperature in order to achieve Probit 9 standard for the systems approach. This is illustrated by the following example, assuming 100% detectability. If the threshold used is no more than one infested fruit and the sample size was 600 fruit, the maximum infestation predicted would be 0.009 ± 0.011 (mean $\pm \sigma$), requiring a shipping temperature that would result in 96.3% mortality. If the sample size is increased to 1,000 fruit, maximum infestation becomes 0.003 ± 0.003 , requiring a shipping temperature that would result in 87.7% mortality. This is therefore a useful tool for chilling injury susceptible cultivars, enabling retention of the requisite level of phytosanitary risk mitigation assurance from the systems approach with shipping temperatures that are less prone to causing chilling injury.

In addition to mortality resulting directly from the cold treatment component in the systems approach, it was shown that a further 69.27% of surviving larvae failed to complete their development and died before pupation. Although measures that do not kill pests, but reduce their potential for entry or establishment in some other way, can be included in a systems approach (FAO 2002), reliance on mortality after an on-arrival inspection

can be problematic for compliance verification by the importing country. Consequently, this post-shipping mortality has not been included in calculating the level of compliance assurance provided by the systems approach, but does provide an additional level of security to the systems approach.

The following improvements are proposed for the systems approach for FCM for citrus exports from South Africa described by Moore et al. (2016a): 1) Infestation thresholds for orchard inspections should be per week, rather than a 4-wk rolling average. 2) Packinghouse delivery inspection samples should consist of at least 600 fruit. 3) Size of the fruit sample inspected on delivery to the packinghouse has a marked effect on the level of risk mitigation of the systems approach and thus the efficacy (larval mortality) required from the shipping temperature. Consequently, practicable combination of sample size and shipping temperature should be provided for in the systems approach. A set of formulae has been reported in this study to provide for this. 4) Values for the proportion of infested fruit detectable on delivery to packinghouse and the grading efficacy in the packinghouse should be revised according to the results reported in this study. Detectability was determined as 79.7% and grading efficacy as 66.1%. 5) Standard deviations for the proportion of infested fruit detectable on delivery to packinghouse and grading efficacy in the packinghouse should be included in the calculation of the maximum possible infestation level of fruit packed for export in order that the standard deviation thereof be calculated. 6) The maximum potential infestation after 2% post-packing inspection, for inclusion in the systems approach calculation, should cater for variable levels of infestation detected and confidence limits for the indicated infestation thresholds. 7) The mortality required by the shipping temperature should be calculated on the level of fruit infestation of only the most cold-tolerant instars (third, fourth and fifth). 8) The calculation of the level of phytosanitary assurance derived from the systems approach should include a conversion factor to make comparison with the efficacy of a standalone Probit 9 level disinfestation treatment more reliable.

Conclusions

In summary, we have identified improvements to the systems approach, which will improve the level of phytosanitary risk mitigation assurance attributable to the systems approach. A set of formulae have been developed to implement the improvements. The end product is a systems approach that provides a risk mitigation measure that can be used as a quantified equivalent alternative to the widely used standalone cold treatments. Implementation of the systems approach in international trade protocols can greatly enhance international citrus trade opportunities, especially with citrus types that are more sensitive to chilling injury.

Future research

No further research is planned on this project. However, the systems approach is being commercially applied in the citrus industry during the 2017/8 citrus production and packing season. It may become evident during or after the season that further improvements to the system are required.

Technology transfer

Presentation at all regional grower meetings have conducted widespread and intensive communication of the system, in the form of the FCM risk management system (FMS) during every spring IPM and Disease Management roadshow and every Postharvest roadshow for the last four years. Additionally, it has been presented at the DAFF special markets regional roadshow and the PPECB annual regional meetings. Additionally, it has been communicated to the industry through three Cutting Edges and through dissemination of the FMS itself.

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2.2.7 FINAL REPORT: FCM population ecology in citrus orchards: the influence of orchard age.
Project 1114 (2015/6 – 2017/8) by S Albertyn, M Hill (Rhodes University) and S D Moore (CRI)

Summary

Anecdotal reports in the South African citrus industry claim higher populations of false codling moth (FCM), *Thaumatotibia (Cryptophlebia) leucotreta* (Meyr) (Lepidoptera: Tortricidae), in orchards during the first three to five harvesting years of citrus planted in virgin soil, after which, FCM numbers seem to decrease and remain consistent. Various laboratory studies and field surveys were conducted to determine if, and why juvenile orchards (four to eight years old) experience higher FCM infestation than mature orchards (nine years and older). In laboratory trials, Washington Navel oranges and Nova Mandarins from juvenile trees were shown to

be significantly more susceptible to FCM damage and significantly more attractive for oviposition in both choice and no-choice trials, than fruit from mature trees. Although fruit from juvenile Cambria Navel trees were significantly more attractive than mature orchards for oviposition, they were not more susceptible to FCM damage. In contrast, fruit from juvenile and mature Midnight Valencia orchards were equally attractive for oviposition, but fruit from juvenile trees were significantly more susceptible to FCM damage than fruit from mature trees. Artificial diets were augmented with powder from fruit from juvenile or mature Washington Navel orchards at 5%, 10%, 15% or 30%. Higher larval survival of 76%, 63%, 50% and 34%, respectively, was recorded on diets containing fruit powder from the juvenile trees than on diets containing fruit powder from the mature trees, at 69%, 57%, 44% and 27% larval survival, respectively. Bioassays were conducted to determine if differences in plant chemistry between fruit from juvenile and mature trees will have an impact on the susceptibility of FCM to entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF) and *Cryptophlebia leucotreta* granulovirus (CrLeGV). No significant differences in the susceptibility of larvae reared on diets containing 15% fruit powder from juvenile and mature trees to EPN and EPF were recorded. Mortality of neonate larvae was significantly lower when placed on diets containing 15% fruit powder from mature trees (45% mortality) than diets containing 15% fruit powder from juvenile trees (61% mortality), after larvae ingested the lowest virus concentration tested, being 2×10^4 OBs/ml. Data collected from field surveys showed significantly lower egg parasitism, virus infection of larvae and EPF occurrence in juvenile orchards than mature orchards. Egg parasitism was between 11% and 54% higher in mature orchards than juvenile orchards, with the exception of Mandarins during 2015, where egg parasitism was slightly higher in juvenile orchards, but not significantly so. A significantly higher proportion of larvae retrieved from mature orchards (7% of larvae) were infected with CrLeGV than larvae retrieved from juvenile orchards (4% of larvae). A significantly higher occurrence of EPF was recorded in non-bearing and mature orchards, with 40% and 37% occurrence respectively, than in juvenile orchards, with 25% occurrence recorded. EPF occurrence in juvenile orchards increased significantly by 16% to 32% from the first to the third year of sampling. In contrast to results recorded in laboratory trials, similar or higher pest pressure in juvenile orchards than mature orchards did not always result in significantly higher levels of FCM damage under field conditions. FCM damage in juvenile orchards may have been lower than expected, as greater extremes of temperature and lower humidity were recorded in juvenile orchards, which would increase larval mortality. Results of this study showed that juvenile and mature orchards are significantly different and should be managed differently. A comprehensive report on this study is available as a PhD thesis by the first author.

Opsomming

Anekdotiese verslae is gemaak van hoë bevolkings van valskodlingmot, (VKM) *Thaumatotibia leucotreta*, gedurende die eerste 3-5 oes jare van sitrus geplant in onversteurde grond, waarna VKM getalle verminder. Verskeie laboratoriumstudies en veldopnames is uitgevoer om te bepaal of en waarom jong boorde (vier tot agt jaar oud) hoër VKM-besmetting as volwasse boorde (nege jaar en ouer) ervaar. In laboratoriumproewe is Washington Navel-lemoene en Nova Mandaryne vanaf jongbome getoon om aansienlik meer vatbaar te wees vir VKM-skade en aansienlik meer aantreklik te wees vir eierlegging in beide keuse- en geen-keuse proewe as vrugte van volwasse bome. Alhoewel vrugte van jong Cambria Nawelbome aansienlik aantrekliker was as volwasse boorde vir eierlegging, was hulle nie meer vatbaar vir VKM-skade nie. In teenstelling hiermee was vrugte van jong en volwasse Midnight Valencia-boorde ewe aantreklik vir eierlegging, maar vrugte van jong bome was aansienlik meer vatbaar vir VKM-skade as vrugte vanaf volwasse bome. Kunsmatige diëte is aangevul met 5%, 10%, 15% of 30% poeier, gemaak van vrugte vanaf jong of volwasse Washington Nawel-boorde. Hoër oorlewing van larwes (76%, 63%, 50% en 34% onderskeidelik), is aangeteken op diëte wat vrugtepoeier vanaf jong bome bevat het as op die diëte wat vrugtepoeier vanaf vollwasse bome bevat het (69%, 57%, 44% en 27% larwe oorlewing, onderskeidelik). Biototse is uitgevoer om vas te stel of verskille in plantchemie tussen vrugte van jong en volwasse bome 'n impak op die vatbaarheid van VKM vir entomopatogeniese nematodes (EPN), entomopatogeniese swamme (EPS) en *Cryptophlebia leucotreta* granulovirus (CrLeGV) sal hê. Geen beduidende verskille in die vatbaarheid van larwes op diëte wat 15% vrugte poeier bevat het vanaf jong en volwasse bome tot EPN en EPF infeksie is aangeteken nie. Mortaliteit van pasuitgebroeide larwes was aansienlik laer as dit op diëte geplaas is wat 15% vrugtepoeier van volwasse bome bevat het (45% mortaliteit) as diëte wat 15% vrugtepoeier vanaf jong bome bevat het (61% mortaliteit), nadat larwes die laagste viruskonsentrasie wat getoets is (2×10^4 OBs / ml) ingeneem het. Data wat vanaf

veldopnames ingesamel is, het aansienlik laer eierparasitisme, virusinfeksie van larwes en EPS-voorkoms in jong boorde getoon as volgroeide boorde. Eiersparasitisme was tussen 11% en 54% hoër in volwasse boorde as jong boorde, met die uitsondering van Mandaryne gedurende 2015, waar eiersparasitisme effens hoër was in jong boorde, maar nie beduidend nie. Mortaliteit van larwes weens CrleGV besmetting was aansienlik hoër in volgroeide boorde (7% van larwes besmet) as in jong boorde (4% van larwes besmet). 'n Aansienlik hoër voorkoms van EPS is aangeteken in nie-draende en volwasse boorde, met onderskeidelik 40% en 37% voorkoms, as in jong boorde, met 25% voorkoms aangeteken. EPS-voorkoms in jong boorde het aansienlik toegeneem vanaf 16% gedurende die eerste jaar van evaluering tot 32% gedurende die derde jaar. In teenstelling met resultate wat in laboratoriumproewe aangeteken is, het soortgelyke of hoër plaagdruk in jong boorde as volgroeide boorde nie altyd aansienlik hoër vlakke van VKM-skade onder veldtoestande tot gevolg gehad nie. VKM skade in jong boorde was dalk laer as wat verwag is, aangesien groter uiterstes van temperatuur en laer humiditeit in jong boorde aangeteken is, wat mortaliteit sou verhoog. Resultate van hierdie studie het getoon dat jong en volwasse boorde aansienlik anders is en dus anders bestuur moet word. 'n Omvattende verslag oor hierdie studie is beskikbaar as 'n PhD tesis deur die eerste outeur.

Introduction

Anecdotal reports in the citrus industry have observed higher populations of false codling moth, (FCM) *Thaumatotibia (Cryptophlebia) leucotreta* (Meyr) (Lepidoptera: Tortricidae) during the first three to five harvesting years of citrus planted in virgin soil, after which, FCM numbers seem to stabilise (Dave Gerber, personal communication). This population increase of FCM in young citrus orchards is in contrast to what has been observed for codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) populations in young apple orchards. Codling moth population increase in young apple orchards is severely limited by a lack of protective pupation sites (Wearing and Skilling, 1975). A lack of pupation sites is however not a limiting factor for FCM as larvae pupate in the soil and only in extremely rare cases in fruit (Newton, 1998). FCM can cause major economic losses and is considered one of the most important citrus pests in South Africa (Moore *et.al.*, 2004). This study has helped us to gain a deeper understanding of FCM ecology in young citrus orchards and to possibly find ways of preventing this initial population increase from occurring, which will help producers to get a more rapid return on investment. The aim of this project was to compare how population numbers of FCM and their natural enemies change within the different orchard age groups during a three year period. A comprehensive report on this study is available as a PhD thesis by the first author.

Objectives

- Record and compare the numbers of FCM, CrleGV, EPN, EPF and parasitoids of newly planted, juvenile (2 – 4 years) and mature (9 years and older) orchards.
- Quantify and compare nitrogen, fibre and gross calorie content of fruit from young trees to fruit from older trees.
- Compare FCM infestation, larval growth, pupal weight and fecundity on diets of wild host plants, fruit from juvenile trees and fruit from mature trees.
- Determine if FCM reared on diets low in nutrients is more susceptible to pathogens.
- To ultimately determine why FCM populations are higher in young orchards and what practices could possibly be implemented to keep population levels stable.

Materials and methods

FCM pheromone traps were set out in newly planted, juvenile and mature orchards as well as bush-veld sites and inspected weekly. Fruit were inspected fortnightly for signs of egg parasitism and collected weekly to determine FCM infestation. Any larvae found in fruit were placed individually into glass tubes with artificial diet to detect possible infection of larval parasitoids and CrleGV.

To test for the presence of EPN and EPF, soil samples were collected every second month. Entomopathogenic nematodes and fungi were recovered from soil samples by using the insect-baiting technique. Soil was placed into 250-ml plastic containers and baited with 3 FCM and 3 mealworm larvae. Samples were examined every

second day for the presence of dead larvae and/or pupae for 2 weeks. Insects possibly infected with EPN or EPF were placed on White traps to harvest any emerging entomopathogenic nematodes. Larvae were sterilised with 70% ethanol before placing larvae on White traps, to prevent external fungal growth of saprophytic fungi on cadavers. Sporulating larvae and/pupae were removed from White traps and placed on an appropriate agar medium to isolate cultures. To determine that fungi are in fact entomopathogenic and not saprophytic, FCM were inoculated with fungal spores and incubated in moisture chambers at 22°C. Petri-dishes were examined daily for dead larvae, which were then placed onto agar until sporulation was detected again.

To test the sensitivity of EPF to fungicides, normal doses of fungicide were mixed with agar. Spores were collected from EPF and added to the medium. Mycelial growth, spore counts and germination percentage after exposure to each fungicide were recorded.

Oviposition preference of FCM and fruit susceptibility of fruit from juvenile and mature trees was determined. Oviposition preference was determined in both choice and no choice trials. Trials were conducted in large mesh oviposition cages. Four gravid females were released per trial just before dusk to allow time for oviposition to occur overnight. Fruit were inspected the following morning and the number of eggs per fruit was recorded. Fruit susceptibility was determined by placing four neonates onto each fruit. The fruit were then kept at 25 °C for 20 days to allow larvae to penetrate fruit and develop. Fruit that showed signs of decay before 20 days had passed were removed and dissected to determine and record FCM infestation. After 20 days, oranges were inspected externally for any signs of penetration marks and then dissected carefully to search for larvae or signs of larval damage. The instar and number of larvae retrieved from fruit were recorded.

The nutritional value of fruit from juvenile and mature trees was evaluated. Sufficient samples of >200g dry weight were collected, ground to a fine powder and analysed by standard techniques. Differences in pupal and larval weights and fecundity of FCM reared on diets containing 15% fruit powder from mature and juvenile trees were determined. Differences in the volatile profiles of fruit from juvenile and mature Washington Navel trees were also determined with Gas chromatography (GC)/mass spectrophotometry.

The susceptibility of FCM reared on diets containing 15% fruit powder from mature and juvenile trees to EPF, EPN and CrleGV was also determined. To evaluate susceptibility to EPF, fifth instar larvae were added to Petri dishes with sieved, autoclaved sand, mixed with 5 ml suspension of distilled water and conidia (10^7 conidia per ml) and incubated for seven days. After seven days, pupae were removed from Petri dishes and moved to glass vials containing sterilised sand. Vials were plugged with cotton wool to prevent emerging moths from escaping. Ten days after the first adult emerged, the number of live moths, dead moths and pupae which failed to eclose were recorded. Susceptibility of FCM larvae and pupae to EPN was determined by exposing insects individually with the required concentration of nematodes in 50 µl of water. After inoculation, plates were placed inside plastic containers, lined with moistened paper towels and closed with the lid to maintain high humidity levels of approximately 95% RH. Plastic containers were then incubated in a dark growth chamber at 25 °C for 48 h, after which FCM mortality was determined by means of gentle prodding. Droplet feeding bioassays were conducted to evaluate susceptibility to CrleGV. Before droplet feeding bioassay trays were filled with a thin layer of artificial diet containing 15% fruit powder from either juvenile or mature trees. Larvae were droplet fed with three different concentrations of virus inoculum. The virus occlusion bodies (OBs) were serially diluted in sterile microfuge tubes and distilled water to obtain final concentrations of 2×10^4 , 5×10^5 and 7×10^6 OBs/ml. Thereafter, 1% Brilliant blue R dye (USB Corporation, United States of America) was added to each virus suspension. Numerous virus suspension droplets of 2 µl each were placed onto the Parafilm M® to allow neonate FCM to feed without drowning. Control larvae were fed with sterile distilled water containing 1% blue dye only. After larvae had finished feeding on the various virus-dye suspensions, they were removed and placed individually into bioassay cells containing the respective diets. Larval mortality was determined after eight days.

Results and discussion

False codling moth oviposition preferences and host susceptibility: the influence of orchard age

Results of this study have shown that fruit from juvenile trees were significantly more attractive for oviposition than fruit from mature trees of the same cultivar, with the exception of Midnight Valencia oranges. However, although care was taken not to harvest fruit from damaged branches, this exception may be due to damage caused to trees by frost followed by warm winds (per obs, W. Kirkman, pers comm), which could have changed the volatile composition of the Midnight Valencia fruit used in the experiment. Volatiles from fruit from juvenile Washington Navel orchards peaked at significantly higher levels (pico amp) for a total of 11 volatile compounds recorded in SPME-GC/MS detection, than volatiles from fruit from mature trees harvested from the same farm. The higher emission of such volatile compounds could explain why fruit from juvenile trees are preferred by FCM for oviposition above fruit from mature trees. The study also showed that juvenile citrus trees are more susceptible to FCM infestation than mature trees, but the degree of vulnerability varies depending on cultivar.

The influence of nutritional differences between fruit from juvenile and mature citrus trees on false codling moth susceptibility to entomopathogens

Results of this study showed no significant differences between the susceptibility of FCM reared on diets containing 15% fruit powder from mature Washington Navel trees, diets containing 15% fruit powder from juvenile trees or a control diet which contained no fruit powder, to either EPF or EPN.

Significantly lower larval mortality was recorded in mature tree diets at the lowest virus dose of 2×10^4 OBs/ml compared to the juvenile tree diets.

The influence of orchard age on FCM ecology

Higher mean ranks moth counts were recorded in juvenile and mature orchards during all three sample years compared to non-bearing orchards and refugia. Moth catches were still significantly lower in the non-bearing orchard group during 2017 (trees now juvenile) even though it was their first fruit bearing year. No significant differences in mean ranks of moth catches were measured between juvenile and mature orchards during any of the three sampling years. Significantly higher trap counts recorded in either juvenile or mature trees were not necessarily linked to higher egg counts or higher FCM damage. Although fruit from juvenile trees have been shown to be preferred above fruit from mature trees for oviposition, this was not always so in the field. However, mean egg counts in juvenile orchards were higher in Washington Navels and significantly higher in Midnight Valencias during 2017, than in mature orchards of the same cultivars and from the same farms.

Egg parasitism was consistently higher (between 10.75% and 53.75% higher) in mature orchards than juvenile orchards and significantly so in most trials with the exception of Mandarins during 2015, where egg parasitism was slightly higher in juvenile orchards than mature orchards, but not significantly so. In contrast to what was expected, significantly higher mean counts of viable eggs in juvenile orchards compared to mature orchards were not necessarily linked to significantly higher FCM damage and vice versa. During 2016, similar FCM damage levels were recorded in juvenile and mature Midnight Valencia orange orchards, even though the mean numbers of viable eggs were significantly higher in mature orchards. During 2016, significantly higher mean counts of 0.41 viable eggs per tree per week were recorded in juvenile Navel orange orchards, compared to mean egg counts of 0.21 viable eggs recorded in mature orchards. However, FCM damage recorded in juvenile Navel orange orchards was higher than damage recorded in mature orchards, but not significantly so. Similar results were recorded in Washington Navel orchards during 2017. Results of this study showed that mean temperatures recorded in juvenile orchards were between 0.22 °C and 0.52 °C higher than mean temperatures recorded in mature orchards. Maximum temperatures were between 1.52 °C and 4.55 °C higher and minimum temperatures between 0.46 °C and 0.94 °C lower in juvenile orchards compared to mature orchards. Mean humidity recorded in juvenile orchards was also between 1.96% and 2.93% lower than mean humidity recorded in mature orchards.

The influence of orchard age on the ecology of entomopathogenic fungi, entomopathogenic nematodes and ants

Beauveria bassiana was isolated significantly more often from both mealworm (84.94% of isolates) and FCM (94.17% of isolates) than any other fungal species. Only 0.24% of soil samples collected during this study tested positive for EPN. The low occurrence of EPN reported in this study could possibly be due to the close proximity of orchards sampled. No difference in ant activity was recorded in juvenile and mature orchards. No significant difference in EPF occurrence was recorded in refugia compared to citrus orchards. Contrary to the hypothesis, EPF occurrence was significantly higher in non-bearing and mature orchards compared to juvenile orchards. Non-bearing orchards may possibly have higher EPF occurrence than expected, as no fungicides were applied during the first two sampling years. No significant difference in EPF occurrence was recorded between mature and non-bearing orchards. EPF occurrence in juvenile orchards increased significantly from the first sampling year to the third growing season of this study.

Influence of orchard age on the tolerance of entomopathogenic fungi to fungicides

The response of *B. bassiana* isolates tested in this study was variable. On non-amended media, all three fungicides significantly reduced spore viability in *B. bassiana* isolates collected from all three orchard maturity groups. No growth was recorded for any isolates grown on media amended with Benomyl. Limited growth and sporulation was recorded in isolates collected from non-bearing and juvenile orchards when grown on media amended with Pennfluid. Isolates collected from mature orchards were unable to grow on media amended with Pennfluid. Vegetative growth and sporulation was significantly reduced in isolates collected from non-bearing and juvenile orchards when grown on media amended with Fungaway while no significant reduction in vegetative growth and sporulation was recorded in isolates collected from mature orchards. On non-amended media, brief exposure to Fungaway had no significant effect on vegetative growth or sporulation of isolates collected from non-bearing orchards. In contrast, Fungaway significantly reduced vegetative growth and sporulation in isolates from juvenile orchards. Interestingly, brief exposure to Fungaway significantly reduced vegetative growth in isolates collected from mature orchards, but significantly increased sporulation. Brief exposure to Pennfluid significantly reduced vegetative growth and sporulation in isolates collected from juvenile and mature orchards. Pennfluid had no significant effect on sporulation of isolates collected from non-bearing orchards, but reduced vegetative growth significantly after brief exposure to the fungicide. Brief exposure to Benomyl significantly reduced vegetative growth and sporulation in isolates collected from juvenile orchards, while no significant effect on vegetative growth and sporulation was recorded in isolates collected from mature orchards. Benomyl had no significant effect on vegetative growth of isolates collected from non-bearing orchards, but did reduce sporulation significantly after brief exposure to the fungicide. In general, *B. bassiana* isolates from juvenile orchards were more sensitive to fungicides than isolates from non-bearing and mature orchards. It is not clear why isolates from juvenile orchards were more sensitive to fungicides and it may simply be incidental, possibly due to the relatively limited extent of the survey.

Conclusion

Through this study it is concluded that great advances have been made towards understanding the physiological and ecological differences between juvenile and mature citrus orchards. The knowledge gained from this study shows that juvenile and mature orchards are significantly different. Therefore, changes in pest management are required to improve FCM control (and possibly control of other pests) in juvenile citrus orchards. However, in order to improve FCM control in juvenile citrus orchards, further trials are required to determine exactly what those changes should be. Since juvenile orchards will deliver a lower yield than mature orchards, citrus producers should also determine if the cost involved in improving FCM control in juvenile orchards is financially viable. For example, it may be more financially rewarding to improve FCM control in high value citrus varieties such as Mandarins than Navel oranges. This study suggests that FCM control in juvenile orchards will be improved by releasing parasitoids and applying UV sensitive microbial control agents more frequently than in mature orchards. The efficiency of these biological control agents may be improved by covering juvenile orchards with nets. However, the use of nets to improve FCM control is not recommended until the effect of nets on the ecology of FCM and other citrus pests is determined. The qualities of juvenile orchards which will improve or decrease their susceptibility to FCM in comparison to mature orchards are summarised in Table 1.

Table 1. List of factors in juvenile orchards which increase or decrease susceptibility to FCM in comparison to mature orchards.

Increase	Decrease
Fruit more susceptible to FCM damage	Smaller tree size improves pesticide coverage
Fruit more attractive for oviposition	Lower average humidity increases mortality of FCM eggs and neonate larvae
Less protection against UV radiation for UV sensitive microbial control agents	Higher exposure to temperature extremes increases FCM mortality
Less suitable microclimate for parasitoids viz. lower egg parasitism	
Higher average temperatures expedite FCM larval development	

Future research

Future research should be conducted to determine if juvenile citrus trees are possibly also more susceptible to other citrus pests and diseases. For example, citrus fruit from juvenile orchards may also be more susceptible to post-harvest decay than fruit from mature orchards. It should also be determined at what age trees reach peak immunity and if immunity can be improved by optimising fertilisers to enhance plant defence. To improve pest control in juvenile orchards of crops which have been shown to be more attractive for oviposition than mature orchards, future research could aim at developing control products that repel the desired pest insect. Covering orchards with nets will improve microbial control in juvenile orchards. However, studies have shown that covering orchards with nets will have a significant impact on the microclimate of orchards, which include higher mean humidity levels (Solomakhin & Blanke 2007) and cooler mean temperatures (Kührt *et al.* 2006, Solomakhin & Blanke 2007, Tanny *et al.* 2009). Future studies should be conducted to determine the influence of nets on the ecology of tree crop pests and their natural enemies. Trials should also be conducted to determine if the adverse effects of nets on orchard microclimates can be reduced by only covering the roof of orchards. Only covering the roof of orchards will also reduce the cost of nets, making it a more viable option for improving FCM control in juvenile orchards.

Technology transfer

- Preliminary results were presented at the 2015 ESSA conference in Grahamstown.
- Oral presentation presented at the SIP annual meeting in Tours France and the Citrus Research Symposium in Drakensburg during 2016.

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

Project RCE 1 (2015/16 – 2017/18) by Michael Jukes, Caroline Knox, Martin Hill (Rhodes University) and Sean Moore (CRI)

Summary

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

Opsomming

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

nuwe biologiese plaagdoder kan vorm en ook in kombinasie met CrleGV gebruik kan word om beter beheer van VKM in die veld te behaal. 'n Omvattende verslag van die volgedige studie is beskikbaar in die PhD tesis van die eerste outeur.

Introduction

The Baculoviridae is a large family of insect viruses known to infect several insect families such as Lepidoptera, Diptera and Hymenoptera. These viruses contain a double stranded DNA genome of between 80 and 180 kbp encoding for 90 to 180 genes. Genomic analysis of baculoviruses has identified 30 core genes present in all known baculovirus isolates, with more than 60 complete genomes available in GenBank. Baculoviruses were initially arranged into two genera based upon their morphological characteristics, these being the Nucleopolyhedrovirus (NPVs) and the Granulovirus (GVs), however they have more recently been re-arranged into 4 genera the alphabaculoviruses, betabaculoviruses, gammabaculoviruses and the deltabaculoviruses based upon genetic analysis. All GV's fall into the genus Betabaculovirus, infecting a variety of lepidopteran hosts, while NPVs are distributed among the remaining three genera with the alphabaculoviruses having lepidopteran hosts, gammabaculoviruses having hymenopteran hosts and the deltabaculoviruses having dipteran hosts.

Due to the narrow host range of baculoviruses, research has been primarily motivated by the ability to use these viruses to control agricultural insect pests. More than 50 baculovirus species have been registered for use as biopesticides against insect pests worldwide. Many more NPVs and GV's have been isolated followed by genetic and biological characterisation for use as biopesticides. Cryptogran is a commercial biopesticide available in South Africa and is used in the control of the citrus pest *Thaumatotibia leucotreta* (previously *Cryptophlebia leucotreta*), more commonly known as the false codling moth (FCM). It is a baculoviruses based biopesticide which contains a commercially produced strain of the *Cryptophlebia leucotreta* granulovirus (CrleGV). Other similar CrleGV-based biopesticides on the market are Cryptex and Gratham. FCM is a major pest in the citrus industry, resulting in reduced crops as well as affecting the ability to export fruit to the international market.

Recent experiments conducted by Sczewczyk (unpublished) at the University of Gdansk and Medical University of Gdansk in Poland, in collaboration with Citrus Research International, indicated the presence of a previously unknown NPV in homogenates of virus infected FCM cadavers. Although this is the first ever record of an NPV infecting a *Thaumatotibia* or *Cryptophlebia* species, it appears to be genetically similar to *Adoxophyes honmai* NPV (AdhoNPV), the Japanese summer fruit tortrix. This NPV was detected with next generation sequencing of a virus infected FCM homogenate sample whereby large sections of sequences similar to the AdhoNPV genome were sequenced alongside the genome of CrleGV. More recent analysis of this novel NPV genome has identified it as a new species of NPV and it has been named ThleNPV. The presence of this NPV in FCM larvae is interesting since this virus has not previously been identified in *Thaumatotibia leucotreta* and offers the potential to produce a second independent NPV based biopesticide, should resistance to the GV present in any of the CrleGV products (Cryptogran, Cryptex or Gratham) be reported. Furthermore, the effect of a joint infection of CrleGV and ThleNPV on a single FCM host can also be determined with a mixed infection of these two viruses, possibly producing a more lethal biopesticide, offering the potential to produce a mixed GV/NPV biopesticide for the control of FCM. Such a product would almost certainly increase citrus production and exports, particularly in regions and cultivars that experience high pest (FCM) pressure.

This study was based on the observation that virus infected FCM larvae were infected with an NPV (like AdhoNPV), in addition to CrleGV, which was isolated and characterised genetically and biologically and could subsequently be developed into an effective biopesticide. A comprehensive report of the complete study is available in the PhD thesis of the first author.

Objectives

A – To confirm the presence of NPV in FCM larvae by performing a bioinformatics analysis of available NPV and GV genome data to determine unique and conserved regions in NPVs.

B – To develop a conventional PCR based method for the detection of the NPV and CrleGV in a mixed baculovirus sample using the bioinformatics analysis described in A.

C – To develop a quantitative PCR (qPCR) assay to quantify the ratio of NPV to GV in homogenates from virus infected FCM larvae.

D – To develop a method for the isolation and purification of the GV and NPV from infected larvae for morphological examination and downstream applications.

E – To evaluate the biological activity of the novel NPV on a laboratory culture of FCM. Furthermore, bioassays using various ratios of mixed CrleGV and NPV will also be carried out to determine whether the virus either improves or reduces the virulence of CrleGV.

F – To obtain a fully annotated genome sequence for the novel NPV.

G – To perform a phylogenetic analysis using all available NPV genome sequences to confirm the taxonomy of the isolated novel NPV.

H – Achievement of the above objectives will lead to bulking up of the NPV for field trials targeted against FCM in citrus orchards.

Materials and methods

Research on baculovirus isolation and genetic and biological characterisation is well established at Rhodes University. Protocols for occlusion body purification from infected insects, PCR amplification and analysis of viral genes, morphological characterisation by TEM and viral DNA profiling have been optimised over the past years for both GVs and NPVs. The host laboratories have all the facilities required for the proposed research, including constant environment rooms for insect cultures, a molecular biology laboratory with essential equipment, and a TEM unit. Facilities and software for computer-based analysis of viral genomes have been acquired.

A – Data for the bioinformatics study was acquired from GenBank and our collaborators at the University of Gdansk and Medical University of Gdansk in Poland. All alignments, analysis and primer design were performed using Geneious R7 and Primer3 software. Several sets of PCR primers were designed to target specific regions of CrleGV and ThleNPV for use in both qPCR analysis as well as for multiplex PCR analysis allowing the detection of the NPV and GV in a given sample.

B – PCR reactions were carried out on Bio-Rad mini and Life technologies SimpliAmp thermal cyclers. PCR cycles were set up and optimised using standardised PCR protocols and ready-mix PCR reagents. Samples of virus infected FCM larvae were tested for the presence of the novel ThleNPV and that of CrleGV. This required the development of a sucrose cushion purification method which has allowed for the purification of a mixture of both the NPV and GV.

C – A qPCR assay to estimate the relative proportions of NPV and GV in virus infected larvae was developed using a high-performance KAPA SYBR® FAST qPCR Kit for gene amplification analysis and a Bio-Rad Opticon thermal cycler. Target regions for amplification were chosen based on the bioinformatics analysis and results obtained from objective B.

D – Virus samples were purified using sucrose cushions and differential centrifugation protocols as previously developed in our laboratory. Virus samples were morphologically characterised using transmission electron microscopy and virus purity evaluated using a multiplex PCR assay developed during objective B.

E – Bioassays were carried out in triplicate across a range of 6 virus concentrations and a control. Each assay was evaluated by probit analysis. Treatments evaluated consisted of CrpeNPV and CrleGV alone along and two mixtures of NPV:GV at a ratios of 25:75 and 75:25.

F –The complete genome sequence was determined using data generated by Prof. Sczewczyk’s group at the University of Gdansk and Medical University of Gdansk. Additional regions independently sequenced were aligned against the NPV genome using Geneious R7 software.

G – The completed NPV genome was phylogenetically analysed in MEGA 7. This analysis incorporated sequences from 57 other baculoviruses to produce a maximum likelihood tree. A bootstrap method was used to determine the statistical accuracy of the tree.

H – CrpeNPV and CrleGV were evaluated in the field, both alone and in combination according to protocols described by Moore et al (2004& 2015).

Results and discussion

Objective / Milestone	Achievement
A. Bioinformatics analysis	
A.1. Design of primers for the detection of AdhoNPV and CrleGV (Lef-4 Primers).	Partly achieved, primers were designed using genome data for CrleGV and AdhoNPV (Available on GenBank). AdhoNPV primers were unable to detect the NPV
A.2. Design of primers for the detection of the NPV and CrleGV (qPCR primers).	Achieved, new primers were designed using genome data for CrleGV (Available on GenBank) and the NPV (Provided by Sczewczyk).
B. PCR detection method.	
B.1. NPV and CrleGV detection.	Achieved, primers were tested using DNA extracted from OBs which were purified from FCM larval homogenates. Both the GV and NPV were detected.
B.2. Multiplex PCR using a combination of Lef-4 and qPCR primers.	Achieved, A multiplex PCR reaction was designed which enables samples to be screened for the NPV and CrleGV in a single PCR reaction.
C. Develop qPCR.	
C.1. qPCR primer testing.	Achieved, qPCR primers successfully produced amplicons for both CrleGV and the NPV.
C.2. qPCR test analysis	Achieved, qPCR was successfully carried out on the NPV and CrleGV test samples.
D. Isolation method.	
D.1. Sucrose cushion purification method.	Achieved, a sucrose purification method was developed which allows for the purification of the GV and NPV from FCM homogenates. NPV and GV particles observed under TEM.
D.2. Codling moth infection assay	Achieved, pure NPV was obtained by infecting codling moth with mixed GV:NPV resulting in pure NPV OBs obtained
D.3. Bulking up of NPV and GV OBs	Achieved, sufficient GV and NPV OBs have been obtained

E. Biological assays	
E.1. Pure NPV and GV on FCM	Achieved, bioassays using FCM neonate larvae are complete with the LC ₅₀ and LC ₉₀ calculated for both the GV and NPV.
E.2. Mixed NPV and GV on FCM	Achieved, bioassays using FCM neonate larvae with mixtures of the NPV and GV are complete with the LC ₅₀ and LC ₉₀ calculated.
F. Genome sequence of the NPV	Achieved, in co-operation with UG&MUG the fully annotated genome sequences for CrpeNPV including gene parity plots and the genome map have been generated.
G. Phylogenetic analysis	Achieved, the phylogenetic analysis of CrpeNPV is complete and has been included in a manuscript for publication.
H. Field Trials	Achieved, two sets of field trials were completed using pure GV and NPV as well as specific mixtures of the two. Data analysis is complete

Objective A: Literature review complete. Bioinformatics study of AdhoNPV complete

The purpose of the literature review is to review available information regarding the viruses concerned and techniques which may be used throughout this project. This output is ongoing and will likely continue as the project progresses, new results are obtained and new articles are published.

The bioinformatics study of AdhoNPV and CrleGV was intended to provide the necessary understanding of these viruses genomic structure providing information required for upcoming objectives. The information obtained was applied in the design of sets of primers for use in PCR for the detection of CrleGV and an NPV similar to AdhoNPV in FCM homogenate samples later in the project. An initial alignment of the reference genomes available for each of these viruses on the NCBI's GenBank was performed in MEGA 5.2. The results from this analysis did not produce any significant information regarding the genome organization possibly due to a lack of data for the comparison. As such the alignment was expanded to include two additional NPVs and two GVs which have been shown to be closely related (Jehle *et al.*, 2006). Again the results were somewhat uninformative and consequently an alternative approach was taken. Analysis of the two reference genomes for AdhoNPV and CrleGV was carried out using the Artemis Comparison Tool (ACT) which identified multiple short nucleotide regions which were common to each virus yet have undergone inversions and/or rearrangements. These unique rearrangements provided a starting point for the development of primers for PCR which could then be tested on DNA extracted from OBs purified from FCM homogenates. Three regions have been identified in the genome sequence for each virus allowing the design of several AdhoNPV and CrleGV primers which are specific to each virus respectively. Target regions currently include the Lef-4-IAP and Lef-4-VP39 regions in the CrleGV and AdhoNPV genomes respectively which are inverted and rearranged in each of the viruses. Figure 1 below shows the targeted regions in the CrleGV and AdhoNPV genomes, with the CrleGV primers named CrleGV-L4 and the AdhoNPV primers names AdhoNPV-L4. These primers were synthesised and tested during objective B.

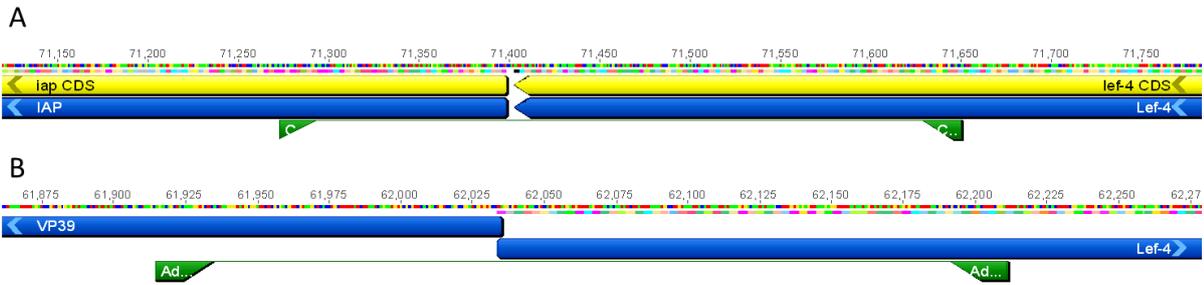


Figure 1. Schematic showing the forward and reverse primer binding regions in the A) CrleGV and B) AdhoNPV genomes.

In June 2015, our collaborators in Poland at the University of Gdansk and Medical University of Gdansk provided us with the complete genome sequence of the NPV detected in CrleGV preparations. By this time, the virus had been identified as a novel NPV and was referred to as *Thaumatotibia leucotreta* Nucleopolyhedrovirus (ThleNPV) with the previous identification as a member of AdhoNPV shown to be incorrect. This NPV has instead been recognised as a unique virus which is closely related to AdhoNPV. As such new primers were designed using the supplied genome sequence of ThleNPV as well as the reference genome available for CrleGV on GenBank. The design process ensured that the primers were not only specific to each virus, but could also be used in further analysis such as the qPCR experiments which will be performed in the second half of 2015. This required the primers to have similar binding temperatures, produce amplicons of similar sizes while ensuring specificity. Figure 2 below shows the targeted regions in the CrleGV and ThleNPV genomes with the CrleGV primers named CrleGV-qPCR and the ThleNPV primers named ThleNPV-qPCR.

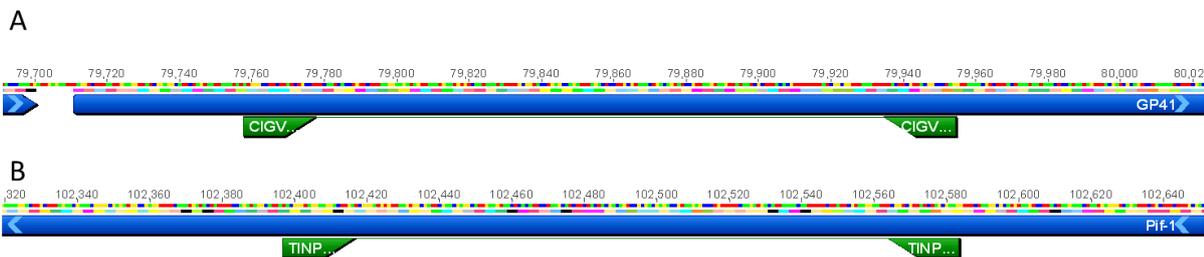


Figure 2. Schematic showing the forward and reverse primer binding regions in the A) CrleGV and B) ThleNPV genomes.

Objective B: Primers designed and tested. Infected larvae tested for NPVs

In order to evaluate the primers, purification of OBs was required from which DNA could be extracted and used as template for the PCR reactions. Progress has been made regarding objective D with various approaches evaluated for the purification of the NPV and GV particles from CrleGV preparations. The first approach attempted to use a standard glycerol gradient ultra-centrifugation purification method, with the resulting fractions from the gradient examined by transmission electron microscopy (TEM) for NPV and GV particles. The typical appearance of a virus band in the glycerol gradient was not observed and examination of the gradient fractions by TEM indicated either an absence of GV and/or NPV particles or very low concentrations of these particles. Fractions which had had viral particles detected were used for DNA extractions with a low quantity of DNA successfully obtained (Figure 3A). The CrleGV-L4 and AdhoNPV-L4 primers were tested on DNA extracts with only the CrleGV primers and the positive control producing amplicons (Figure 3B). This indicated the presence of CrleGV in the samples, with the AdhoNPV primers indicating either a lack of NPV DNA or an inability to bind to the genome due to low primer specificity.

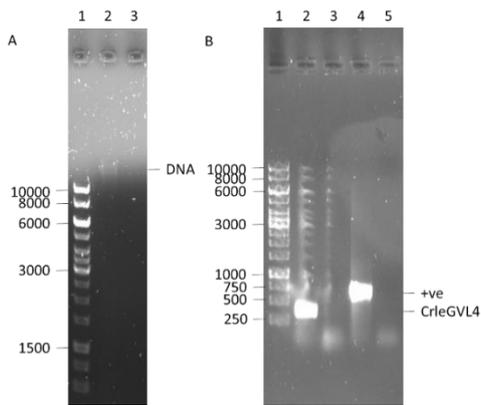


Figure 3. Agarose gel images of A) DNA extracted from gradient purified OBs, lane 1: 1Kb GeneRuler, lane 2: genomic DNA and B) PCR amplification of CrleGV and AdhoNPV Lef-4 regions, lane 1: 1Kb GeneRuler, lane 2: CrleGV-L4 amplicon, lane 3: AdhoNPV-L4 amplicon, lane 4: positive control, lane 5: no template control.

A second approach was attempted in order to obtain higher yields of GV and NPV virus particles by using low and high speed centrifugation steps before purification through a sucrose cushion. An initial purification of OBs was carried out using a 50% sucrose cushion with TEM images showing the presence of both GV and NPV particles (Figure 4A). This purification method was further examined and optimised using various densities of sucrose (0%, 30%, 50% and 70%) to determine which provides the highest yield of GV and NPV OBs. High yields are essential for downstream applications such as the biological assays. TEM images have been obtained for the 0% and 30% (Figure 4B and C) cushions which showed fewer particles than was observed when using the 50% cushion. Additionally, DNA was extracted from OBs obtained from each sucrose cushions and analysed by gel electrophoresis (Figure 5). DNA is visible along the top of the gel although the concentrations obtained were low, with the OBs purified through a 50% sucrose cushion providing the highest DNA yield. Each lane shows 1 μ l of DNA out of a total extraction volume of 20 μ l.

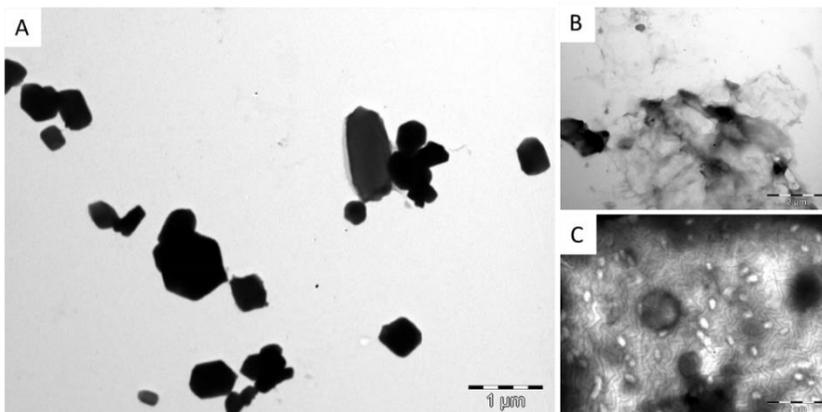


Figure 4. Transmission electron microscope images of NPV and GV particles purified through A) a 50% sucrose cushion, B) a 0% sucrose cushion C) a 30% sucrose cushion.

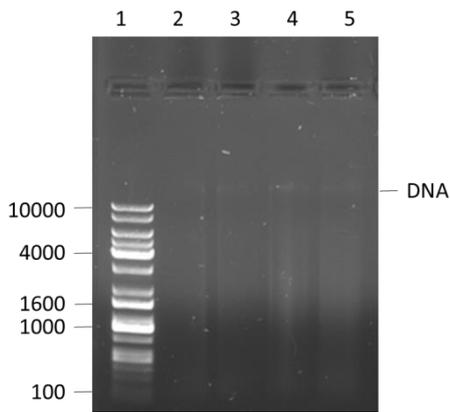


Figure 5. Agarose gel images of DNA extracted from purified OBs using various sucrose densities, lane 1: KAPA universal ladder, lane 2: 0% sucrose cushion, lane 3: 30% sucrose cushion, lane 4: 50% sucrose cushion, lane 5: 70% sucrose cushion.

By comparing the available TEM images obtained for the 0%, 30% and 50% sucrose cushions, it was determined that the 50% produced the highest concentration of both the NPV and GV particles and as such DNA extracted from OBs obtained through the 50% cushion were used in a multiplex PCR reaction to confirm the presence of CrleGV and ThleNPV using the CrleGV-L4 and ThleNPV-qPCR primer sets. The results for this experiment are shown in Figure 6 below. The primer design allows for each amplicon to produce a uniquely sized band for each virus and can consequently be used as an efficient screening protocol. The CrleGV-qPCR primer set was also tested and has shown a positive result (data not shown).

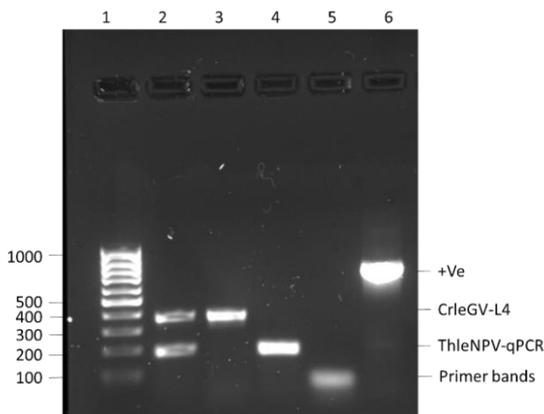


Figure 6. Agarose gel of the multiplex PCR reaction. Lane 1: 100bp GeneRuler, lane 2: Multiplex reaction, lane 3: CrleGV-L4 amplicon, lane 4: ThleNPV-qPCR amplicon, lane 5: no template multiplex control, lane 6: positive control.

Objective C: Development and optimization of qPCR assay for the NPV and CrleGV complete

Both of the primer sets required for qPCR have been tested and were able to produce the required amplicons for each virus. DNA extracted from GV and NPV OBs obtained during objective D were used to evaluate the qPCR protocol. For each virus, a standard was prepared using a 6-fold serial dilution of DNA extracted from a known quantity of OBs. The standard was run alongside an “unknown” sample and a NTC sample. For the purpose of this test, the quantity of template in the “unknown” sample was known and used to evaluate how accurate the test was. Results obtained for each qPCR run showed that test were completed as expected producing amplification graphs (Figure 7a and 7b) and a standard curve (Figure 7c and 7d) for each virus.

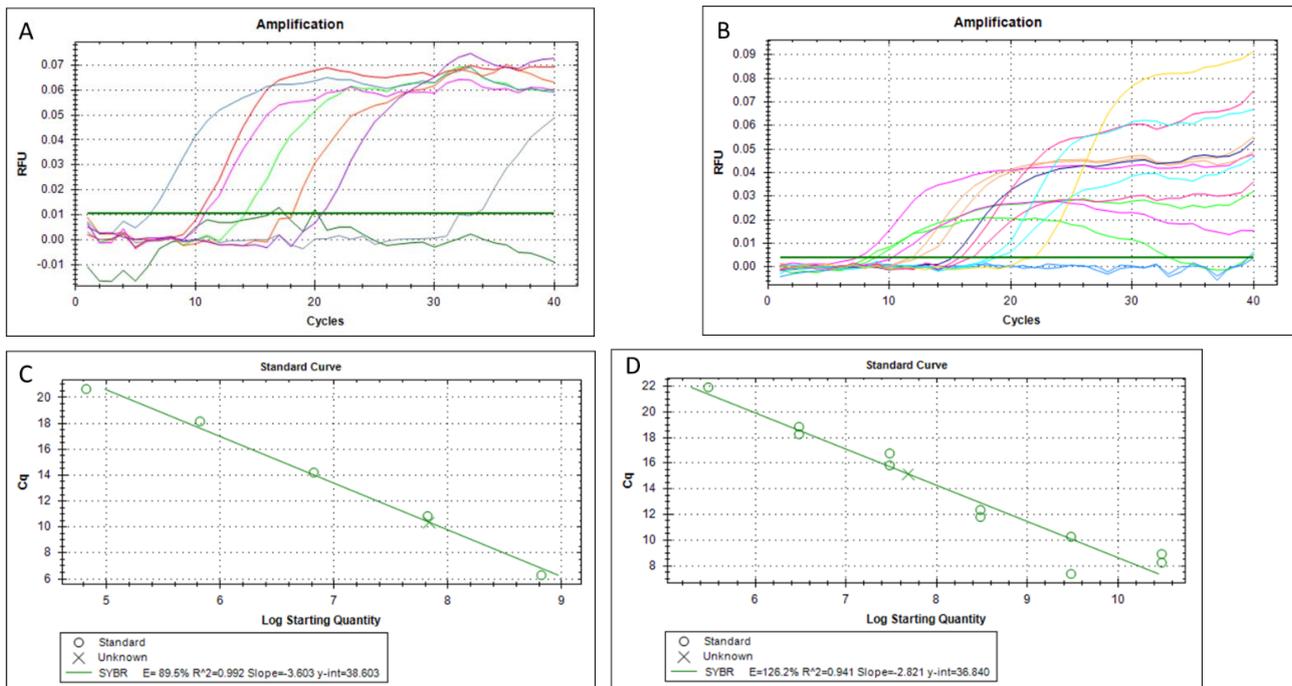


Figure 7. Amplification and standard curve graphs produced by qPCR analysis of test samples. A) CrleGV amplification graph B) ThleNPV amplification graph C) CrleGV standard curve and D) ThleNPV standard curve. Standards marked with an “o” and unknown test samples with an “x”.

The concentration of OBs used to prepare each of the “unknown” test samples was accurately determined by qPCR. For each virus, the standard curve was satisfactorily produced with each sample used to produce the standard curve indicated by an “o” and the “unknown” sample indicated by an “x”. This test demonstrated the accuracy of qPCR as well as ensured the protocol was correct and repeatable.

Objective D: To develop a method for the isolation and purification of the GV and NPV from infected larvae for morphological examination and downstream applications.

As mentioned above for objective B, a sucrose purification method was developed in order to obtain NPV and GV OBs. This method allowed for mixed NPV and GV OBs to be obtained (Figure 4), however, sufficiently pure samples of the NPV could not be produced using this method. As such an alternative approach was developed whereby mixed NPV and GV samples were used in a codling moth (CM) (*Cydia pomonella*) infection assay. This infection assay was carried out in triplicate using 24 neonate CM larvae per virus or control treatment which were reared on an artificial diet in 24 well plates. Each well contained a diet plug which was surface inoculated with either 50 μ l of the virus mixture (2.04×10^9 OBs/ml) for the virus treatment or 50 μ l of ddH₂O for the control treatment. After the inoculation had dried sufficiently, larvae were transferred to the plate with one larva per diet plug and left at 25 °C. The assays were evaluated at 7 and 14 days post infection, with the observed mortality levels shown in figure 8 below.

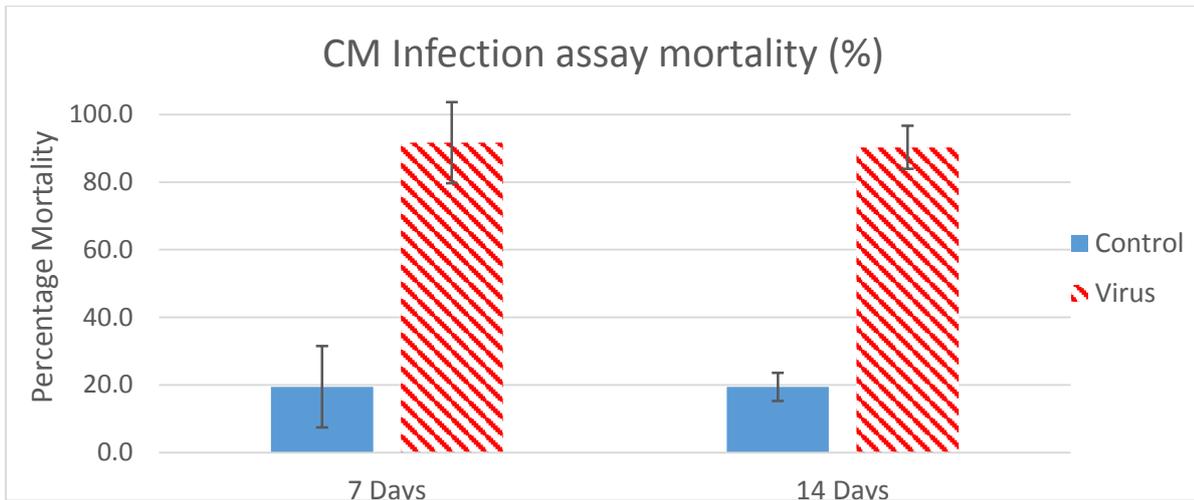


Figure 8. Observed percentage mortality in the codling moth infection assay, treated with a mixture of NPV and GV OBs. The assay was evaluated at 7 and 14 days post infection with blue bars representing the control larvae and red hatchet bars representing the virus infected larvae.

The assay showed that the virus treatment resulted in a significant increase in larval mortality when compared to the control treatment. This result indicated that either both or one of the viruses were able to infect the CM larvae and increasing the observed mortality. Two larval cadavers were collected from this infection assay and are referred to as C1 and C6. These cadavers underwent a crude OB purification process with purified samples used in a subsequent DNA extraction. Extracted DNA was analysed using the multiplex PCR protocol developed under objective B with only the NPV detected in sample C6. However, additional OBs were required before further analysis could be performed, with the use of neonate larvae proving problematic for the bulking up of the virus as few cadavers could be collected and any that were, were not ideal for OB purification. To resolve this, a single set of 24 5th instar CM larvae were reared. These larvae were transferred to a 24 well plate containing artificial CM diet with 12 wells surface inoculated with 20 µl of a 1:10 dilution of the C6 OBs (3.09×10^8 OBs/ml) and the remaining 12 with 20 µl ddH₂O. Fourteen days post infection, 10 of the virus treated larvae had died while only 1 of the control larvae had died. A total of 8 virus infected cadavers were collected, with OBs successfully purified and imaged by TEM (Figure 9A). Furthermore, these extracted NPV OBs (Figure 9B) and additional GV OBs (Figure 9C) which were purified from FCM larval cadavers through a glycerol gradient were sectioned before being imaged by TEM. The NPV was identified as a single nucleopolyhedrovirus (SNPV), while a few GV particles were observed to have multiple nucleocapsids in individual OBs shown by the arrow in figure 9B, possibly as a result of an assembly error.

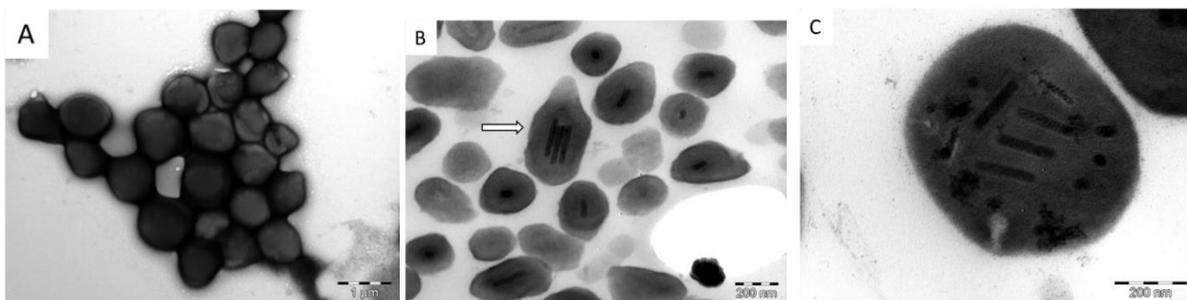


Figure 9. TEM images of A) purified NPV particles obtained from CM assay B) sectioned GV particles purified from FCM larvae by glycerol gradient purification with the arrow indicating an OB observed with multiple nucleocapsids and C) sectioned NPV particles obtained from 5th instar CM larvae.

An additional discovery was made shortly after the NPV was purified from CM larvae. The complete genome sequence of *Cryptophlebia peltastica* single nucleopolyhedrovirus (CrpeSNPV) had recently been assembled here at Rhodes University and was compared to the complete genome sequence of ThleNPV. This analysis showed 100 % identity between these two viruses indicating that they were in fact the same virus. A multiplex

PCR analysis of DNA extracted from OBs obtained from FCM homogenate samples from 2015, 2012, 2009, 2006, 2003 and 2000 was carried out to determine when the NPV entered the FCM samples. This analysis showed that the NPV was introduced into the FCM samples between 2013 and 2015 (Figure 10). This time span coincides with the timing of a new litchi moth (LM), *Cryptophlebia peltastica*, colony having been established from which CrpeSNPV was originally isolated. This result, along with additional PCR analysis on the 2013 and 2014 samples (data not shown) indicated that the NPV likely originates from the LM colony, and as such is hence forth referred to as CrpeSNPV rather than ThleSNPV. Furthermore, with the complete genome sequence analysis confirming the NPV as CrpeNPV, purified stocks of CrpeNPV OBs were provided by T. Marsberg which had been purified from infected LM cadavers. These OBs, along with purified GV OBs have been used in downstream processes such as the biological assays.

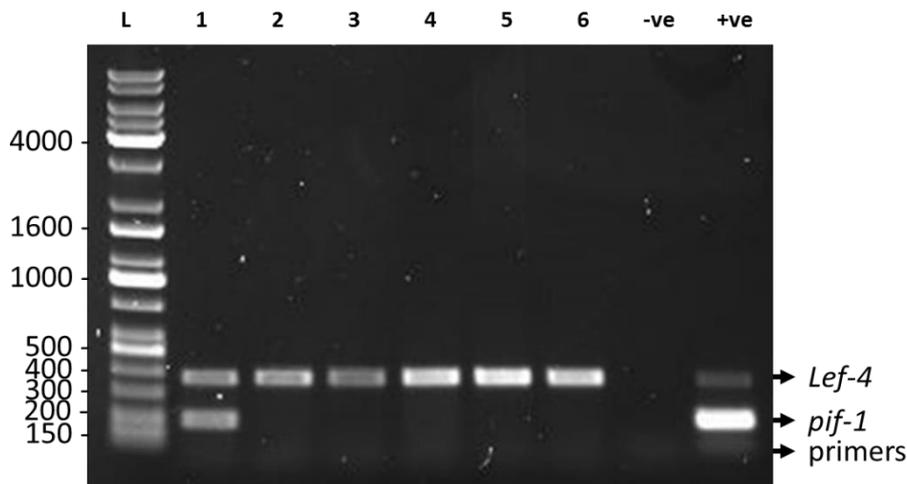


Figure 10. Multiplex analysis using DNA extracted from OBs obtained from FCM homogenate samples from various years. L: KAPA universal ladder, Lane 1: 2015, Lane 2: 2012, Lane 3: 2009, Lane 4: 2006, Lane 5: 2003, Lane 6 : 2000, -ve: NTC and +ve: positive multiplex control.

Objective E: To evaluate the biological activity of the novel NPV on a laboratory culture of FCM. Furthermore, bioassays using various ratios of mixed CrleGV and NPV will also be carried out to determine whether the virus either improves or reduces the virulence of CrleGV.

Bioassays using pure CrpeNPV and pure CrleGV on neonate larvae have been completed. Early tests were performed to determine the optimal range of OB concentrations to be used in each assay. Doses ranging between 1.6×10^4 and 1.5×10^6 were prepared and used in the subsequent assays. The pure NPV and GV assays consisted of 6 treatments along with a control each applied to 24 neonate FCM larvae. Artificial FCM diet was inoculated using a surface dose method with biological assays carried out in triplicate.

Analysis of biological data indicates LC_{50} values of 1.17×10^5 and 1.23×10^5 OBs/ml for CrleGV and CrpeNPV respectively. An LC_{90} value of 3.0×10^6 and 2.75×10^6 OBs/ml has also been determined for CrleGV and CrpeNPV respectively. Mixtures of CrpeNPV and CrleGV at ratios of 1:3 and 3:1 have also been completed. These biological assays were carried out on FCM neonate larvae using the same procedure as the pure assays. Analysis of the biological data from the mixed assays indicates LC_{50} values of 8.55×10^4 and 7.95×10^4 OBs/ml for the 1:3 and 3:1 (CrpeNPV:CrleGV) assays respectively. LC_{90} values of 1.07×10^6 and 7.18×10^5 OBs/ml for the 1:3 and 3:1 (CrpeNPV:CrleGV) assays respectively.

Mixtures of the viruses showed decreased LC_{50} and LC_{90} values compared to the pure virus LC values. Interestingly the CrpeNPV dominant mix showed the lowest LC_{50} and LC_{90} values. These results suggest that mixtures of the virus may provide an improvement in the LC_{50} and LC_{90} values when testing against neonate larvae. Furthermore, antagonistic and synergistic effects were also investigated in the mixed virus treatments using the Thames-Bakuniak graphical method shown in figures 11 and 12 (Lara-Reyna et al 2003).

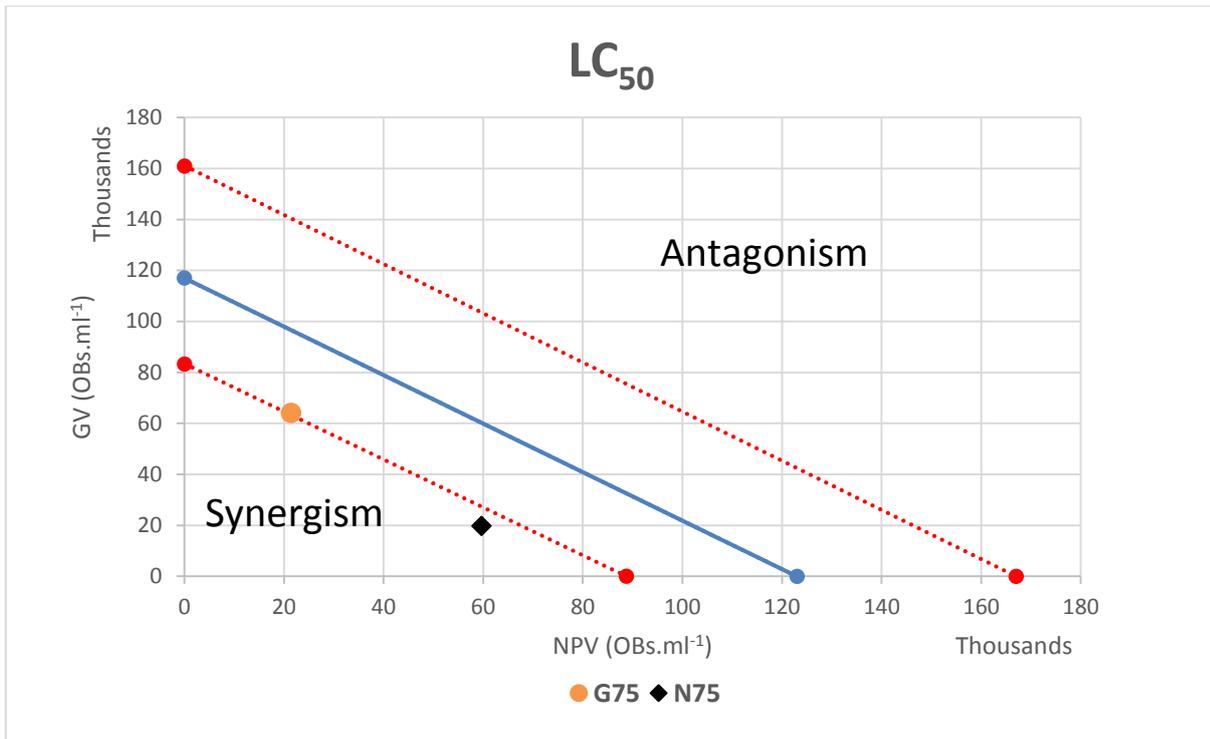


Figure 11. Thames-Bakuniak plot of the synergistic interaction between CrpeNPV and CrleGV at LC₅₀. The solid line indicates the equitoxic line with the 95 % upper and lower fiducial limits shown by the dotted lines. The GV and NPV dominant mixtures are shown as a circle and diamond respectively.

At the LC₅₀ values (Figure 11), both mixtures performed better than each virus in isolation however the GV dominant mixture was identified to have an additive effect falling within the concentration limits for each virus. The NPV dominant mixture was identified to have a synergistic effect at the LC₅₀ values improving the virulence beyond the limits of the viruses when used in isolation.

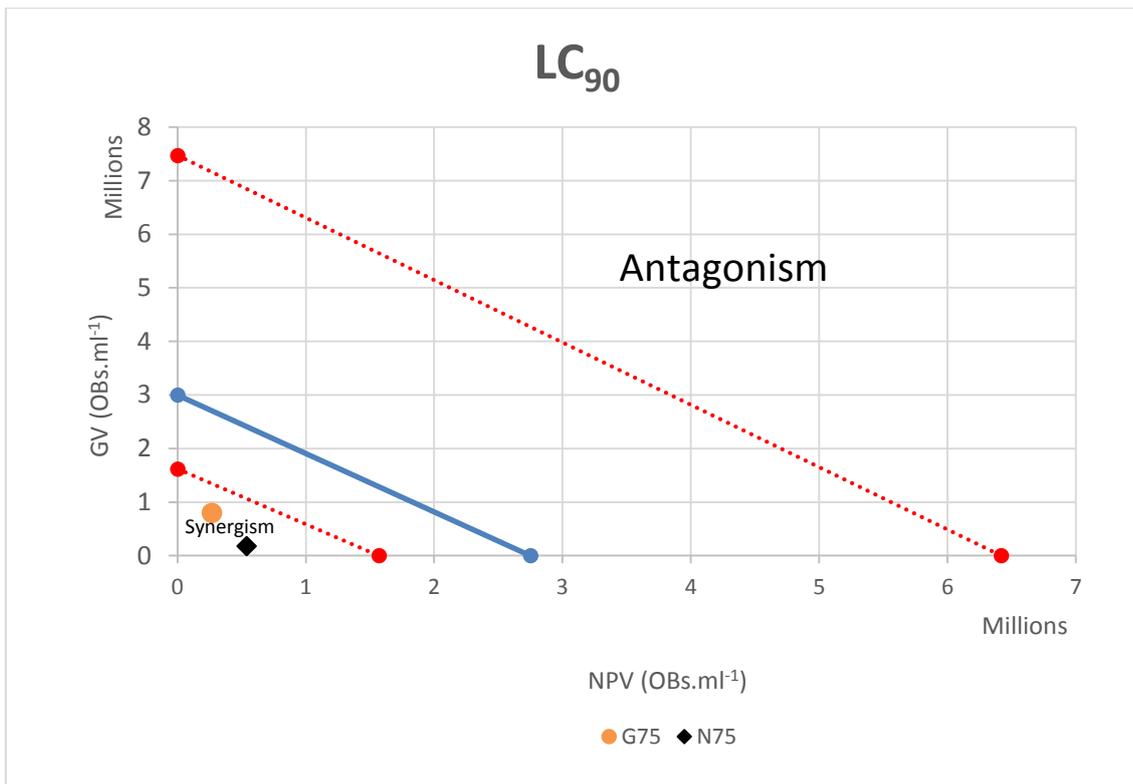


Figure 12. Thames-Bakuniak plot of the synergistic interaction between CrpeNPV and CrleGV at LC₉₀. The solid line indicates the equitoxic line with the 95 % upper and lower fiducial limits shown by the dotted lines. The GV and NPV dominant mixtures are shown as a circle and diamond respectively.

At the LC₉₀ values (Figure 12), both mixtures again performed better than each virus in isolation. Unlike at the LC₅₀ values, both mixtures were identified to have a synergistic effect showing improved virulence beyond the fiducial limits of the viruses when used in isolation.

Objective E: Sequential passage of CrpeNPV and CrleGV in FCM

An additional experiment was completed to further examine mixtures of CrleGV and CrpeNPV in FCM larvae. Each virus was used alone and in combinations to treat sets of FCM 2nd and 3rd instar larvae. Recovered virus was used to inoculate four successive sets of FCM larvae. Following passage of the virus through FCM five times, OB composition was examined using the mPCR and qPCR techniques developed at the start of the project. The results indicated that CrleGV quickly becomes the dominant virus in mixed treatments, displacing CrpeNPV by the third passage. Additionally, FCM larvae treated with only CrpeNPV were measured to have CrleGV present by the second passage possibly due to presence of covert infections. CrpeNPV was isolated from the final passage OB mixtures using codling moth larvae. This enabled sequencing of the complete genome to determine whether recombination between CrleGV and CrpeNPV may have occurred during co-infection. Multiple alignment of the genome sequences generated did not show any changes in these sequences.

These results suggest that vertical transmission of CrleGV and CrpeNPV in FCM, tends towards CrleGV becoming the major component in infected larvae. Furthermore, field applications using combinations of these viruses may result in decreased levels of CrpeNPV secondary infection due to CrleGV dominance.

Objective F: To obtain a fully annotated genome sequence for the novel NPV

Jan-Mar 2017:

Objective F: PCR amplify regions missing in the preliminary genome data for the NPV

An additional discovery was made shortly after the NPV was purified from codling moth larvae. The complete genome sequence of CrpeNPV had recently been assembled at Rhodes University and was compared to the complete genome sequence of CrpeNPV generated at the University of Gdansk. This analysis showed almost complete identity between these two viruses indicating that they were likely the same virus. Two regions were identified to be different between these assemblies which were further examined by generating specific primer sets followed by PCR amplification and sequencing. The results obtained indicated a slight assembly error in the CrpeNPV genome assembled at Rhodes which once corrected showed complete agreement between both assemblies.

Apr-Jun 2017:

Objective F: Sequence PCR products and complete assembly of CrpeNPV

These two regions were identified between the Polish generated sequence (labelled ThleNPV below) and the South African generated sequence (labelled CrpeNPV below), the J-Domain and MR regions, to have low coverage and required additional PCR amplification and sequencing. Both regions were successfully amplified, sequenced and aligned to the CrpeNPV genome sequence.

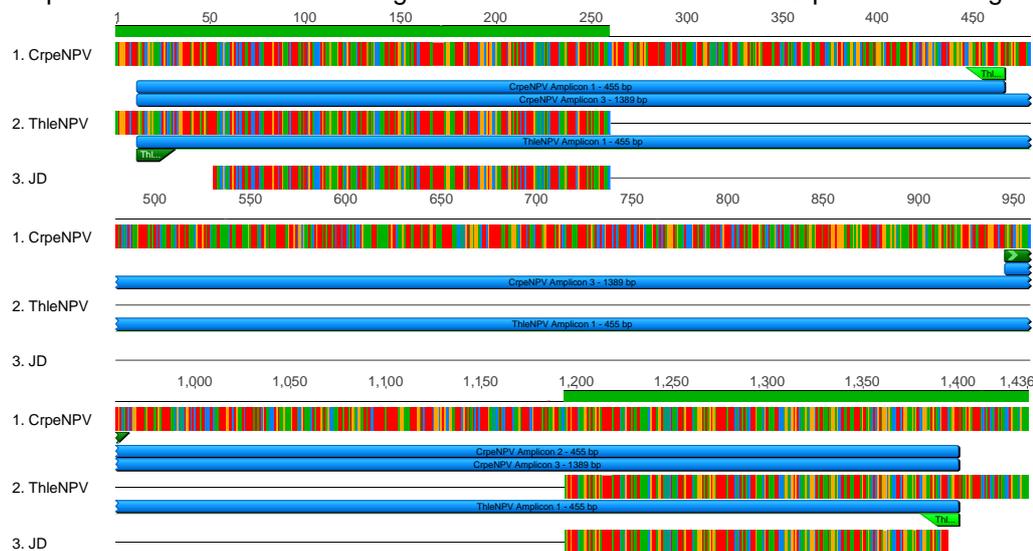


Figure 13. Pairwise alignment of the J-Domain sequence to the respective regions in ThleNPV and CrpeNPV. The green bar shows alignment identity with the forward and reverse oligonucleotide binding regions annotated on the sequence.

The alignment of the J-Domain sequence confirmed the Polish assembly of the genome sequence, indicating that a large deletion was present in the J-Domain region. This result was used to correct the consensus genome sequence for CrpeNPN.

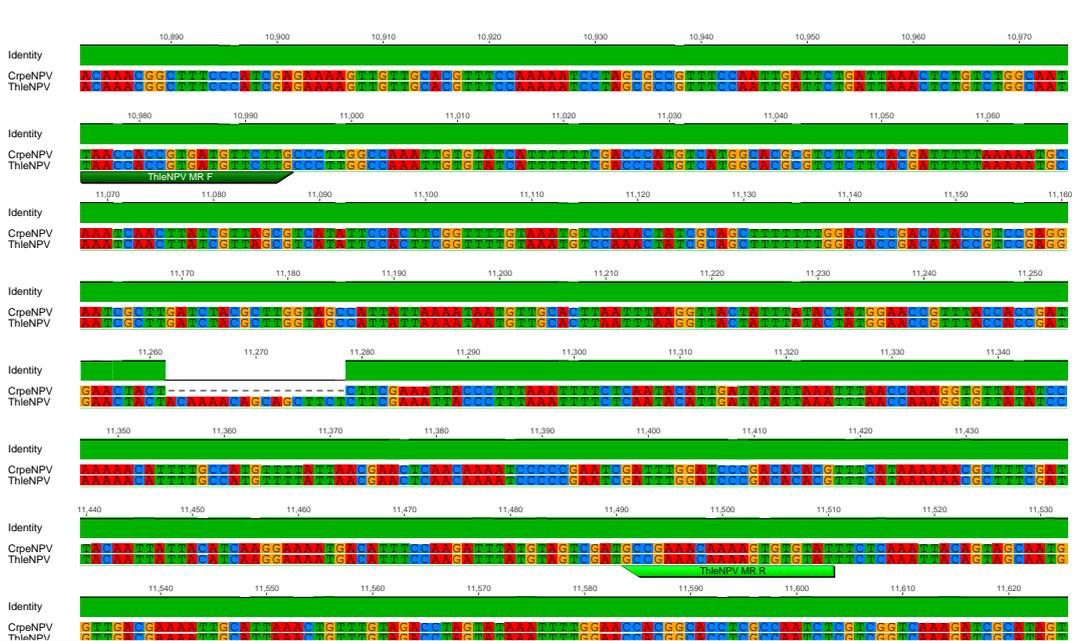


Figure 14. Pairwise alignment of the MR amplicon to the respective regions in ThleNPV and CrpeNPV. The green bar shows alignment identity with the forward and reverse oligonucleotide binding regions annotated on the sequence.

Alignment of the MR amplicon sequence to the complete genome sequences indicated that the South African sequence assembly correctly represented this region, whereas the polish assembly identified a small deletion in the genome. These results were again used to correct the consensus sequence for the complete CrpeNPV genome. All other regions, when compared by pairwise alignment, showed complete agreement.

Objective G: To perform a phylogenetic analysis using all available NPV genome sequences to confirm the taxonomy of the isolated novel NPV

Jul-Sep 2017:

Objective G: Complete the bio-informatic analysis comparing the CrpeNPV genome to all other known NPV genomes along with phylogenetic analysis

A phylogenetic analysis of the CrpeNPV genome was completed by comparison of all 37 core genes to the respective genes from 58 other baculoviruses. Sequences were analysed by Maximum likelihood using Mega 7 (Figure 15).

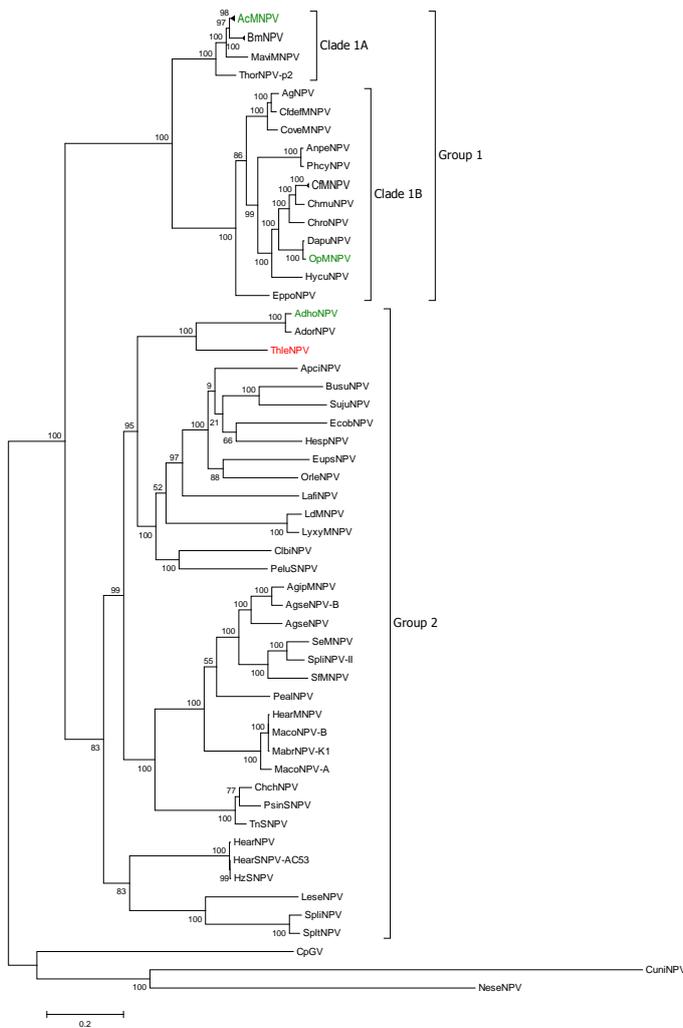


Figure 15. Molecular phylogenetic analysis by the Maximum Likelihood method based on 37 concatenated core gene amino acid sequences from 58 baculoviruses. AdhoNPV, OpMNPV and AcMNPV are shown with green text. ThleNPV (now CrpeNPV) is shown in red text.

The analysis showed CrpeNPV to group closely with AdhoNPV and AdorNPV, falling within the group 2 alphabaculoviruses.

Objective H – bulk up NPV for field trials

Bio-informatic analysis of the complete genome sequence of CrpeNPV was performed using a variety of web and software applications including pBLAST, HMMER, MEGA 7 and Geneious R8/R10. ORFs in the genome were identified and annotated with potential homologous proteins identified using pBLAST and HMMER. Genome parity plots were generated against AcMNPV, OpMNPV and AdhoNPV. Phylogenetic analysis of CrpeNPV was conducted using the 37-core gene amino acid sequences against 58 baculovirus. The results show that CrpeNPV groups most closely with AdhoNPV and AdorNPV all of which fall within the group 2 alphabaculovirus clade. To confirm whether CrpeNPV represents a novel species, kimura-2-parameter distances were calculated using concatenated *lef-8*, *lef-9* and *polh* nucleotide sequences. This analysis indicated that the virus does indeed represent a distinct alphabaculovirus, most closely related to *Epipotia granitalis* NPV (EpgNPV) for which only partial gene sequences are available.

Objective H: Achievement of the above objectives has led to bulking up of the NPV for field trials targeted against FCM in citrus orchards

Oct-Dec 2017:

Objective H – Conduct field trials; Complete any remaining experiments or tasks. Complete thesis and begin revision

Field trials with both CrpeNPV and CrleGV along with mixtures of these viruses were conducted twice, first in December 2016 and again in April 2017. Treatments were applied in a randomised block format replicated ten times and evaluated over three weeks. Analysis of the resulting data did not show any significant improvement between the virus treatments and several other chemical treatments applied at the same time (Figure 16). This is believed to be due to unusually high levels of FCM because of increased fruit splitting earlier in the citrus season.

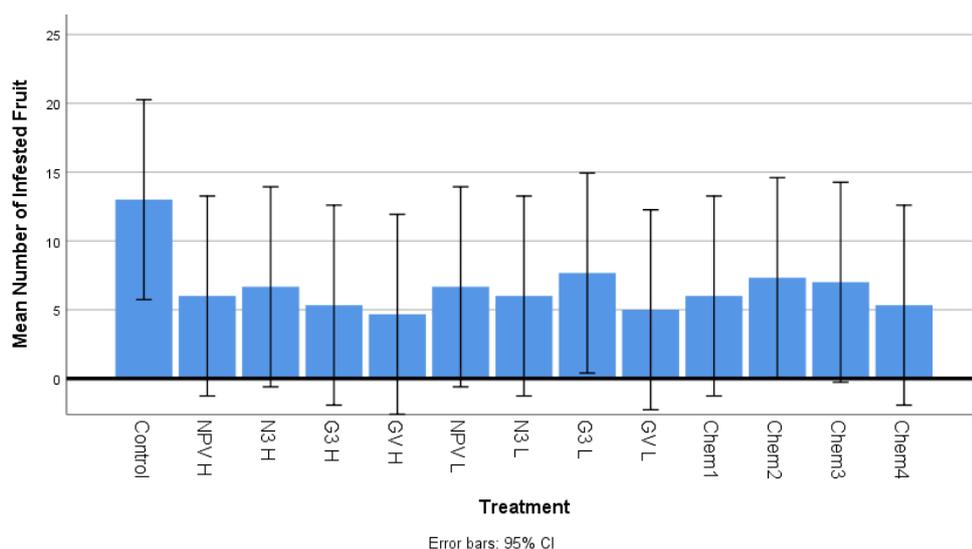


Figure 16. The mean number (std. error) of infested fruit per week for the Far Away Farm treatments after three weeks of evaluation. The virus treatments, each applied to ten trees, were CrpeNPV (NPV), NPV dominant (N3), GV dominant (G3) and CrleGV (GV) at high (H) and low (L) concentrations. A chemical treatment was also applied at four concentrations Chem1 (lowest) to Chem4 (highest).

Conclusion

To date, the NPV detected using next generation sequencing in FCM homogenates by our collaborators in Poland has been observed by TEM in the samples provided. Furthermore, a purification method has been developed which allows for the NPV particles to be recovered. DNA extracted from recovered particles has been used in PCR analysis with primers specifically targeting each virus producing amplicon. Furthermore, a multiplex PCR assay has been developed which can be used to screen samples for the presence or absence of either of these viruses. Pure NPV was later obtained by infecting codling moth larvae with a mixture of both viruses, with only the NPV able to infect this host. Cadavers were collected and used to purify OBs for examination by TEM followed by the extraction of DNA and subsequent PCR analysis. Additionally, the NPV was identified as CrpeNPV, originating from a litchi moth colony. This identification has enabled a sufficient amount of NPV OBs to be obtained which were used in downstream applications, such as the bioassays. Evaluation of the biological activity of pure NPV and pure GV is complete. Bioassays utilising various mixtures of the NPV and GV have also been evaluated using two combinations of 3:1 and 1:3 of the GV and NPV respectively. Here some improvement was observed with improved LC_{90} values obtained. The qPCR assay has been successfully used on DNA acquired from each virus with accurate OB measurements made on “unknown” test samples. Furthermore, a serial passage of the viruses in isolation and in mixtures was performed and the ratio of each virus measured using qPCR in the subsequent infections. It was observed that CrleGV became the dominant virus in all treatments with CrpeNPV levels gradually diminishing. Additionally, next generation sequencing data has also been generated from these passage assays and will be analysed to further investigate mixed virus infections. The milestones set out in the initial project proposal have been achieved with the results presented as a completed PhD thesis at Rhodes University.

Technology transfer

Thesis:

Jukes, M.D 2018. Baculovirus Synergism: Investigating Mixed Alphabaculovirus and Betabaculovirus Infections in the False Codling Moth, *Thaumatotibia leucotreta*, for Improved Pest Control. PhD Thesis, Rhodes University. pp. 1-211. Available at: <http://hdl.handle.net/10962/61797>

Conferences:

Jukes, M.D., Rabalski, L., Knox, C.M., Hill, M.P., Moore, S.D. and Szewczyk, B., 2017. Baculovirus synergy: mixed Alphabaculovirus and Betabaculovirus infections for the control of *Thaumatotibia leucotreta* in South Africa. Oral presentation at the IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" to be held at the Agricultural University of Georgia, Tbilisi, Georgia. 11-15 July 2017.

Jukes, M.D., Knox, C.M., Hill, M.P., Moore, S.D., Rabalski, L. and Szewczyk, B., 2016. Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control. Oral presentation at the International Congress on Invertebrate Pathology and Microbial Control and 49th Annual Meeting of the Society for Invertebrate Pathology conference held at Da Vinci Centre, Tours, France. 24-28 July.

Jukes, M.D., Knox, C.M., Hill, M.P., Moore, S.D., Rabalski, L. and Szewczyk, B., 2016. Baculovirus synergism: investigating mixed alphabaculovirus and betabaculovirus infections in the false codling moth, *Thaumatotibia leucotreta*, for improved pest control. Oral presentation at the 9th Citrus Research Symposium conference held at Champagne Sports Resort, Drakensberg, South Africa. 21-25 August.

Peer Reviewed Articles:

van der Merwe, M., Jukes, M., Rabalski, L., Knox, C., Opoku-Debrah, J., Moore, S., Krejmer-Rabalska, M., Szewczyk, B., Hill, M., 2017. Genome Analysis and Genetic Stability of the Cryptophlebia leucotreta Granulovirus (CrleGV-SA) after 15 Years of Commercial Use as a Biopesticide. Int. J. Mol. Sci. 18, 2327. doi:10.3390/ijms18112327. IF 3.226

Conference Bulletin:

Jukes, M.D., Rabalski, L., Knox, C.M., Hill, M.P., Moore, S.D. and Szewczyk, B., 2017. Baculovirus synergy: mixed Alphabaculovirus and Betabaculovirus infections for the control of *Thaumatotibia leucotreta* in South Africa. IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" bulletin. 129, pp.170-174.

Patents:

Moore, S.D., Hill, M.P., Knox, C.M., Marsberg, T., Jukes, M.D., Szewczyk, B., Rabalski, L. and Chambers, C., 2016. Biological control agent - Genetic Study of sNPV. South African Provisional Patent Application No. 2016/05197

Further objectives and work plan

All objectives have been completed.

Future research

Several future objectives were identified following the completion of this project. Evaluation of the biological activity of CrpeNPV and CrleGV alone and in mixtures indicated potential synergistic effects with improved virulence observed. Additional analysis and experiments should be carried out to better understand these interactions with the aim of identifying improved virulence and FCM mortality. Additionally, experiments were conducted to evaluate interactions between CrleGV and CrpeNPV during mixed infections. These experiments showed that CrleGV became the dominant virus in these mixed infections. This may have implications in the

field should CrpeNPV be used alongside or with CrleGV, potentially having adverse effects on non-synchronous applications and naturally occurring secondary infections.

Lastly, additional testing and evaluation of the multiplex PCR and quantitative PCR assays is necessary should these techniques be used in future experiments. Both techniques have potential application in the industry including in quality control protocols and for the screening and evaluating field and commercial samples.

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2.2.9 FINAL REPORT: Degreening of satsumas for identification of FCM infested fruit

Ad hoc project by Sean Moore, Wayne Kirkman (CRI) and Mellissa Peyper (RU)

Summary

This study was conducted to determine whether degreening of FCM-infested Satsumas would lead to detectable decay, enabling identification and removal of all FCM-infested fruit and thus removing the need for any cold shipping temperature. This is important, as the majority of Satsumas from South Africa are exported to the UK under the FCM risk management system (FMS), which requires a final step comprising cold shipping (3-4°C) to Europe. Degreening was found to increase fruit decay almost three-fold. However, it did not increase the overall detectability of FCM-infested fruit. Additionally, 76% of infested fruit showed no signs of decay after degreening and five out of 110 infested fruit showed no external signs of potential infestation after degreening. Consequently, degreening cannot be considered as a reliable tool for inducing decay of and therefore highlighting all FCM infested Satsumas, which will therefore not all be graded out efficiently in the packhouse.

Opsomming

Hierdie studie is uitgevoer om te bepaal of ontgroening van VKM-besmette Satsumas tot opletbare bederf sal lei, wat sal veroorsaak dat alle VKM-besmette vrugte geïdentifiseer en verwyder kan word en dus die behoefte

vir 'n koue verskeppings temperatuur sal verwyder. Hierdie is belangrik want die meerderheid Satsumas van Suid-Afrika word VK toe uitgevoer onder die VKM risikobestuur stelsel (FMS), wat 'n finale stap wat koue verskeping (3-4°C) Europe toe vereis. Ontgroening het vrugbederf amper drie-voudig vermeerder maar het nie die algehele opspoorbaarheid van VKM-besmette vrugte verhoog nie. Daarbenewens het 76% van besmette vrugte geen tekens van bederf na ontgroening gewys nie en vyf van die 110 besmette vrugte het geen eksterne simptome van moontlike besmetting na ontgroening gewys nie. Gevolglik kan ontgroening nie oorweeg word as 'n betroubare hulpmiddel om bederf van VKM-besmette Satsumas te verseker nie en dus om VKM-besmette vrugte uit te lig, wat gevolglik nie doeltreffend in die pakhuis uitgegradeer sal wees nie.

Introduction

As of 1 January 2018, the European Union (EU), South Africa's largest export market, officially regulated FCM as a phytosanitary pest. The directive states that if the country, area or place of production is not free of FCM, then an effective cold treatment or another effective treatment to ensure freedom from FCM must be applied. South Africa's answer to this has been to implement a systems approach in which the last step is a cold shipping temperature. However, certain citrus cultivars are susceptible to chilling injury and even the less severe cold treatments within the systems approach (3°C, 3.5°C and 4°C) may pose some risk of chilling injury. One of these cultivars is Satsuma mandarins. Degreening of Satsumas after harvest and before packing, using ethylene fumigation, is a common practice. Consequently, this study was conducted to determine whether degreening of FCM-infested fruit would lead to detectable decay, enabling identification and removal of all FCM-infested fruit and thus removing the need for any cold shipping temperature. This is important, as the majority of Satsumas from South Africa are exported to the UK.

Materials and methods

Three Satsuma trees were selected for the study in an orchard (no. 55) on Dunbrody Estates in the Sundays River Valley. These trees were planted in 1992 and irrigated via microjets. On 20 March 2017, 600 fruit on these three trees were numbered. Fruit numbered 1-300 were each inoculated with 5 neonate FCM larvae (obtained from River Bioscience, Addo, Eastern Cape). Fruit numbered 301-600 were left as untreated control fruit.

One hundred randomly chosen fruit from both the treated and untreated lots were harvested at 1 day after infestation (21 March 2017). The objective was to do the same at 3 days (23 March 2017) and 7 days (27 March 2017) after inoculation. However, it was not possible to collect the full 100 on these dates, as some of the fruit had dropped off the tree or could not be found. After harvest each fruit was visually evaluated for signs of FCM infestation or decay. Thereafter the fruit were subjected to degreening at Unifrutti Dunbrody Packhouse (Kirkwood, Eastern Cape). The degreening protocol was as follows: 72 hours at 19-21°C, 95% RH, a CO₂ content of 0.30% and an ethylene content of 1-2.5 ppm. Fruit was allowed to acclimate for 24 hours after degreening before assessment.

The post degreening evaluation was done by visually and assessing each fruit for external signs of infestation and decay and thereafter, destructively assessed for larval infestation. Larval instars were identified. Fruit with none of these symptoms were categorised as clean.

Results and discussion

Only four infested fruit were found amongst all of the untreated fruit (three in Day 1 fruit and 1 in Day 2 fruit), most likely being naturally infested in the field. None of these fruit showed signs of apparent infestation pre-degreening. However, post-degreening, all four showed signs of infestation, but only one of them was accompanied with any decay.

Results of FCM detectability pre- and post-degreening are provided in Table 1, whereas the details of post-degreening symptoms and infestation are provided in Table 2.

Table 1. Total treated (surface-inoculated with neonate larvae in the field) fruit showing signs of infestation and/or decay pre- and post-degreening and the actual number of infested fruit (post-degreening).

Days between infestation and picking	Total number of fruit	Pre-degreening			Post-degreening					
		Apparent infestation	Decay	Total apparent infestation and/or decay	Apparent infestation	Decay	Total apparent infestation and/or decay	Actual infestation	Fruit infested without decay	Fruit infested without any external sign of infestation
1	100	43	1	44	40	7	47	51	43	2
3	94	43	0	47	36	8	44	43	36	3
7	65	27	10*	42	14	17	33	16	5	0
Total	259	113	11	133	90	32	124	110	84	5

*All of these fruit also showed signs of apparent infestation.

Table 2. Total treated (surface-inoculated with neonate larvae in the field) fruit post-degreening with various symptoms and confirmed infestation.

Days between infestation and packing	No apparent infestation (clean)		Apparent infestation with no decay		Apparent infestation with decay		Decay with no other sign of infestation		Total number of infested fruit
	Total	Number Infested	Total	Number Infested	Total	Number Infested	Total	Number Infested	
1	53	2	40	40	7	7	0	0	51
3	50	3	36	33	8	7	0	0	43
7	33	0	14	7	17	11	1	1	16
Total	136	5	90	80	32	25	1	1	110

Conclusion

The most important results can be summarised as follows:

- Degreening increased decay almost three-fold.
- Degreening did not increase the overall detectability of FCM-infested fruit i.e. any symptoms on fruit that could indicate infestation, including penetration marks and decay.
- 76% of infested fruit showed no signs of decay after degreening.
- Five out of 110 infested fruit showed no external signs of potential infestation after degreening.
- All fruit infested 7 days before degreening showed external signs of potential infestation after degreening.

In conclusion, degreening cannot be considered as a reliable tool for highlighting all FCM infested Satsuma fruit. Although this could be considered a relatively small and unreplicated trial, it is sufficient to conclude that not all infested Satsuma mandarins will decay after degreening, and will therefore not all be graded out efficiently in packhouses.

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

Project 1039 (April 2012 – March 2015) by Sean Moore, Wayne Kirkman (CRI), Mellissa Peyper, Sonnica Albertyn, Tammy Marsberg (RU) and Vaughan Hattingh (CRI)

Summary

This project was initiated as a result of the announcement that the European and Mediterranean Plant Protection Organisation (EPPO) was conducting a Pest Risk Assessment (PRA) on FCM. This PRA was completed in September 2013, eventually leading to FCM being declared a regulated phytosanitary pest for the EU in July 2017, effective of January 2018. This affects all citrus, except lemons and certain limes, amongst a few other crops, being exported from Africa and Israel. Most work conducted in this project between 2012 and 2014 was aimed at demonstrating the efficacy of incomplete cold treatments for use as a step in a systems approach. Subsequently, studies were conducted to demonstrate that Probit 9 efficacy against fourth and fifth instar FCM, the most cold-tolerant larval life stages, could be achieved with improved postharvest cold treatments i.e. warmer temperatures and/or shorter duration. Research during the year in question was significantly delayed, due to CRI PE having to relocate to new premises. Despite this, further work on incomplete cold treatments, as a component in the systems approach, was resumed. The efficacy of each of 3°C, 4°C, 4.5°C and 5°C for periods ranging from 16 to 26 days was measured. Three replicates of the two warmer temperatures were conducted, whereas six replicates with the 4°C temperature were conducted, due to initial discrepancies in results. Additionally, three replicates with large numbers of naturally infested fruit were exposed to 4°C for 16 days in order to verify the results in artificial diet. Mortality of larvae at 3°C ranged from 96.3% to 100% for the durations of 16 to 26 days. Mortality of larvae at 4°C ranged from 75.76% to 99.84% for the 16 to 26 day durations. Results at 16 days was verified in fruit, with a mortality of 79.2%. Mortality at 4.5°C ranged from 89.35 to 96.88%. Mortality at 5°C ranged from 39.8 to 71.6% for durations of 19 to 22 days, and from 93.95 to 96.38% for durations of 23 to 26 days. Results from these new time-temperature combinations have been included in the FCM systems approach for chilling sensitive cultivars.

Opsomming

Hierdie projek is aangepak as gevolg van die aankondiging dat die Europese en Mediterreense Plantbeskermings Organisasie (EPPO) besig was met 'n Plaaig Risiko Analise (PRA) op VKM. Hierdie PRA is in September 2013 voltooi wat uiteindelik gelei het tot die verklaring van VKM as 'n gereguleerde fitosanitêre plaag vir die EU in Julie 2017, van toepassing van 1 Januarie 2018. Hierdie is van toepassing tot alle sitrus, behalwe suurlemoene en sekere lemmetjies, tussen sekere ander gewasse, wat uit Afrika en Israel afkomstig is. Meeste van die werk in hierdie projek wat tussen 2012 en 2014 uitgevoer is het die doeltreffendheid van onvolledige koue behandelings as 'n stap in 'n stelselsbenadering gedemonstreer. Daarna is studies uitgevoer om te bewys dat Probit 9 doeltreffendheid teen vierde en vyfde instar VKM, die mees koue tolerante

lebensstadiums, met verbeterde na-oes koue behandelings bereik kon word d.w.s. warmer temperature en/of korter blootstelling. Gedurende die betrokke navorsingsjaar is werk beduidend vertraag omdat die CRI PE kantore na 'n ander perseel toe moes skyf. Ondanks hierdie vertraging, is verdere werk met onvoldoende kouebehandelings, as 'n komponent in 'n stelselsbenadering, voortgesit. Die doeltreffendheid van elk van 3°C, 4°C, 4.5°C en 5°C vir tydperke wat van 16 tot 26 dae geduur het is gemeet. Drie replikate van die twee warmer temperature is uitgevoer en ses replikate met die 4°C temperatuur is uitgevoer, as gevolg van oorspronklike wisselvalligheid in resultate. Boonop is drie replikate met groot getalle natuurlik besmette vrugte uitgevoer teen 4°C vir 16 dae om die resultate in kunsmatige dieet te verifieer. Mortaliteit van larwes teen 3°C het van 96.3% tot 100% gewissel vir tye van 16 tot 26 dae. Mortaliteit van larwes teen 4°C het gewissel van 75.76% tot 99.84% vir tye van 16 tot 26 dae. Resultate teen 16 dae is in vrugte geverifieer, met 'n mortaliteit van 79.2%. Mortaliteit teen 4.5°C het gewissel van 89.35 tot 96.88%. Mortaliteit teen 5°C het gewissel van 39.8 tot 71.6% vir tye van 19 tot 22 dae, en vanaf 93.95 tot 96.38% vir tye van 23 tot 26 dae. Resultate van hierdie nuwe tyd-temperatuur kombinasies is nou ook in die VKM stelselsbenadering ingesluit vir koue gevoelige kultivars.

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

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

Summary

Several years ago it was discovered, almost accidentally, that 7-vinyldecyl acetate 1 (7-VDA) was capable of preventing adult false codling moth (FCM) males from locating virgin females. Consequently, we decided to examine this further with a view to developing a novel mating disruption, or rather a mating inhibition, technology. In the previous research year it was determined that a combination of 10% 7-VDA and 90% FCM pheromone was the most effective in reducing mating of FCM. Consequently, release rate trials were conducted with this combination and with pure FCM pheromone in novel polyethylene dispensers, recording a release rate of approximately 3 ug per day, which is what is required in the field. A field trial was initiated in November 2017. Four treatments were used: untreated control, FCM pheromone, 7-VDA (10%) + FCM pheromone (90%) and Splat. Each treatment was replicated twice in a randomised block design, each to a 1 ha block of Navel orange trees on Boerboom Farm in the Sundays River Valley. Splat was applied by River Bioscience (in November and January) and FCM pheromone and 7-VDA + FCM pheromone were applied at 1 dispenser per tree i.e. 555 dispensers per hectare each (only in November). One FCM pheromone trap was simultaneously hung in the centre of each replicate and monitored weekly. The trial was monitored until the end of February (15 weeks). A total of 300 moths were caught in the untreated control, 17 in the FCM pheromone treatment, 10 in the 7-VDA + FCM pheromone treatment and 20 in the Splat treatment. Infestation was monitored during January and February. Splat reduced infestation by 50%, FCM pheromone by 23% and 7-VDA + FCM pheromone not at all. Therefore, infestation results do not concur with trapping results. The field trial should thus be repeated. However, this project has now come to an end.

Opsomming

Jare gelede is dit ontdek, amper toevalig, dat 7-vinieldesielasetaat 1 (7-VDA) die vermoë het om volwasse valskodlinmot (VKM) mannetjies te verhoed om ongepaarde wyfie motte te vind. Daarom het ons besluit om hierdie verder te ondersoek met die moontlikheid van 'n oorspronklike paringsontwrigting – of liever paringsverhoeding – tegnologie te ontwikkel. In die vorige navorsings jaar is dit bepaal dat 'n kombinasie van 10% 7-VDA en 90% VKM feromoon die mees doeltreffend was om VKM paring te verminder. Gevolglik is vrystellings tempo proewe met hierdie kombinasie en met suiwer VKM feromoon uitgevoer in oorspronklike poliëtileen vrystellers, wat 'n vrystellings tempo van omtrent 3 ug per dag getoon het, presies wat in die veld nodig word. 'n Veldproef is in November 2017 geïnisieer. Vier behandelings is gebruik: onbehandelde kontrole, VKM feromoon, 7-VDA (10%) + VKM feromoon (90%) en Splat. Elke behandeling is twee keer herhaal in 'n gerandomiseerde blokontwerp, elk op 'n 1 hektaar blok van Nawellemoenbome op Boerboom Plaas in die Sondagsriviervallei. Splat is deur River Bioscience toegedien (in November en Januarie) en VKM feromoon en 7-VDA + VKM feromoon is teen 1 vrysteller per boom toegedien dws. 555 vrystellers per hektaar

(net in November). Een VKM feromoon vrysteller is gelyktydig gehang in die middel van elke replikaat en weekliks gemonitor. Die proef is tot die einde Februarie gemonitor (15 weke). In totaal is 300 motte in die onbehandelde kontrole gevang, 17 in die VKM feromoon behandeling, 10 in die 7-VDA + VKM feromoon behandeling en 20 in die Splat behandeling. Besmetting is gedurende Januarie en Februarie gemonitor. Splat het besmetting met 50% verminder, VKM feromoon met 23% en 7-VDA + VKM feromoon glad nie. Daarom stem besmettings resultate nie met lokval resultate saam nie. Die veldproef behoort dus herhaal te word, alhoewel hierdie projek nou tot einde gekom het.

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

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

Summary

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

Opsomming

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

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

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

Summary

A Solid Phase Microextraction (SPME) probe has been shown to effectively trap as well as concentrate headspace volatile compounds surrounding intact fruit. Gas Chromatography – Mass Spectrometry (GCMS) analysis was conducted on five major volatile compounds of interest previously shown to be released by FCM infested oranges. These major volatile compounds are D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. In the 2017 season, GCMS trials were conducted on Washington and Witkrans Navel oranges, as well as Mor Mandarins and Midnight and Delta Valencias. The significantly different ratios between D-limonene and Naphthalene found in infested Witkrans Navels was not evident in 2017. This was mainly due to variability in D-limonene levels in all cultivars, which can most likely be ascribed to the extremely unusual climactic conditions in the Eastern Cape, which resulted in excessive splitting and fruit drop. The ability of an electronic nose to detect FCM infested fruit was further investigated. Trials were conducted on Washington Navel oranges, infested 2, 6 and 10 days previously. The electronic nose could detect 70, 90 and 90 percent of infested fruit respectively for the three treatments, with 20% false positives, which was similar to results on Lane Late Navel oranges the previous season. The reaction of individual sensors in the electronic nose was examined, and a new array of sensors, comprising of the most sensitive ones, is due to be manufactured. W Kirkman visited the University of California – Davis, to be trained on Differential Mobility Spectrometry (GCDMS). Trials to detect phytophthora in rhododendron plants were successful, and it is intended to test the unit on FCM infested fruit in the future. W Kirkman will visit the Division of Mechatronics, Biostatistics and Sensors, University of Leuven in Belgium in August to evaluate their Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) technology to detect FCM.

Opsomming

Daar is gewys dat Soliede Fase Mikro-ekstraksie (SPME) hoofruim vlugtigsteowwe rondom vrugte effektief kan opvang en konsentreer. Gaskromatografie-Massaspektrometrie (GCMS) analise is gedoen op D-limonien, 3,7-dimetiel-1,3,6-oktatrien, (E)-4,8-dimetiel-1,3,7-nonatrien, kariofileen en naftaleen, wat in 'n vorige studie uitgewys is as die vyf belangrikste vlugtigsteowwe wat deur VKM-besmette vrugte afgeskei word. In 2017 is GCMS analise uitgevoer op Washington en Witkrans Nawellemoene, Mor mandaryne, asook Miknight en Delta Valencias. Gedurende die 2017 seisoen was geen statisties-betekenisvolle verskille in die verhouding van D-

limonien en naftaleen in besmette Witkrans Nawellemoene aangeteken nie. Dit was hoofsaaklik as gevolg van groot variasie in die vlakke van D-limonien in alle kultivars, wat heelwaarskynlik veroorsaak is deur buitensporige veranderde klimatiese toestande wat gelei het tot grootskaalse vrugbars en vrugval in die Oos-Kaap. Verdere proewe is gedoen om te kyk of 'n elektroniese neus tussen besmette en gesonde vrugte kon onderskei. Analise is gedoen op Washington Nawellemoene wat 2, 6 en 10 dae vroeër besmet is. Die elektroniese neus kon onderskeidelik 70, 90 en 90 persent van besmette vrugte uitken, met 20% valspositiewes. Die reaksie van elke individuele sensor is ondersoek, en 'n nuwe groep sensors wat bestaan uit die mees sensitiewe sensors gaan vervaadrig word. W Kirkman het die Universiteit van Kalifornia-Davis besoek vir opleiding op hulle Differentiële Mobiliteitsspektrometrie (GCDMS) eenheid. Proewe op phytophthora-besmette rhododendron plante was suksesvol, en daar is beplan om te kyk of die eenheid tussen VKM-besmette en gesonde vrugte kan onderskei. W Kirkman sal besoek aflê by die Divisie van Megatronika, Biostatistieke en Sensors, Universiteit van Leuven in België in Augustus, om hulle Geselekteerde loon Vloeibare Massaspektrometrie (SIFT-MS) technologie te evaluëer vir VKM-opsporing.

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

Project 1117 (2015/16-2018/19) by P. Mwanza, G. Dealtry, M. Lee (NMU), S. Moore (CRI)

Summary

Baculovirus biopesticides offer a more environmentally friendly approach to combat crop pests and are used as part of an integrated pest management programme (IPM) to combat these crop pests. On citrus, formulations of the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) are used in the control of the false codling moth (FCM). Three CrleGV products, Cryptogran®, Cryptex® and Gratham® are currently registered for use on citrus in South Africa. Ultraviolet (UV) radiation is the most important factor affecting the persistence of baculovirus biopesticides in the field. Under field conditions the half-life of baculoviruses, varies from 10 h to 10 d and in the absence of any form of UV protection, the average half-life is around 24 h. In this study, the existence of CrleGV-SA that is naturally resistant to UV is being investigated. Samples of CrleGV-SA were repeatedly exposed to UV, propagated in FCM fifth instars and re-exposed to UV. A total of five exposure cycles were completed. Samples were exposed to UV in a Q-SUN Xe-3HC Xenon Test Chamber (Q-LAB), with parameters set to simulate normal sunlight. According to literature, this is the minimum time required to detect UV resistance. Completed bioassay data from the first three cycles is still inconclusive. After completion of bioassays from exposure cycles four and five, it may become more evident whether UV-resistant virus has been successfully isolated. Following confirmation of presence of UV resistant CrleGV, molecular techniques will be used to identify differences between the resistant and non-resistant virus. In the same study, various potential UV-protectants are being tested. These were exposed to UV in the test chamber for 24 hours. Bioassays are being carried out to investigate if they have a protective effect against UV.

Opsomming

Bakulovirus biologiese plaagdoders voorsien 'n meer omgewingsvriendelike benadering om gewasplae te bestry en word as deel van 'n geïntegreerde plaagbestrydings (IPM) program gebruik om hierdie plae te bestry. Op sitrus, formulasies van die bakulovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) word vir die beheer van die valskodlingmot (VKM) gebruik. Drie CrleGV produkte, Cryptogran®, Cryptex® en Gratham® is tans vir gebruik op sitrus in Suid-Afrika geregistreer. Ultraviolet (UV) bestraling is die belangrikste faktor wat die nawerking van die bakulovirus in die veld benadeel. Onder veld omstandighede wissel die half-lewe van bakuloviruse van 10 ure tot 10 dae en in die afwesigheid van enige vorm van UV beskerming, is die gemiddelde half-lewe omtrent 24 ure. In hierdie studie word die voorkoms van CrleGV-SA wat natuurlik bestand is teen UV ondersoek. Monsters van CrleGV-SA is herhaaldelik aan UV blootgestel, in vyfde instar VKM gepropageer en weer aan UV blootgestel. In totaal is vyf blootstellings siklusse voltooi. Monsters is aan UV in 'n Q-SUN Xe-3HC Xenon Test Chamber (Q-LAB) blootgestel, met parameters wat gestel is om normale sonlig te simuleer. Volgens die literatuur is hierdie die minimum tydsduur nodig om enige UV weerstand op te spoor. Voltooid bioassay data van die eerste drie siklusse is nogsteeds onoortuigend. Na voltooiing van bioassay van blootstellings siklusse vier en vyf sal dit moontlik meer duidelik word of UV-bestande virus wel suksesvol

geïsoleer is. Na bevestiging van teenwoordigheid van UV-bestandheid sal molekuleêre tegnieke gebruik word om verskille tussen die bestande en nie-bestande virus te identifiseer. In dieselfde studie word verskeie moontlike UV-beskermers getoets. Hierdie is aan UV in die toets kamer vir 24 ure blootgestel. Biototse word uitgevoer om te bepaal of hulle enige beskermende effek teen UV het.

2.2.15 **PROGRESS REPORT: Potential of novel products, including a novel nucleopolyhedrovirus, for control of FCM**

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

Summary

On 14 July 2017 FCM was declared a regulated pest by the EU. Consequently, it has become extremely important to develop and test a full suite of control options for FCM. Recently, a novel nucleopolyhedrovirus (NPV) was discovered infecting both litchi moth and FCM. As it appears that the litchi moth is the natural host, the virus has been named the *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). Laboratory bioassays have indicated that the NPV, has a relatively broad host range, being similarly virulent to FCM as to litchi moth. Consequently, for a second season, field trials were conducted with different concentrations of CrpeNPV. It was also applied in combination with the FCM granulovirus, CrleGV (Cryptogran), as laboratory bioassays indicated some synergism between these two viruses, particularly at a 3:1 ratio of CrpeNPV:CrleGV. Additionally, some experimental chemical and biological insecticides were included in trials on the request of the manufacturers or suppliers of the products. Three field trials were conducted. The first was applied to an orchard of Navel oranges in the Sundays River Valley in December 2017 and evaluated for 8 weeks. The treatments were as follows: two different concentrations of CrpeNPV, a combination of CrpeNPV and Cryptogran, Cryptogran, Methoxyfenozide (Runner) and several experimental chemical treatments. Cryptogran and CrpeNPV both reduced infestation by 58%, Runner by 67% and one of the experimental treatments by more than 90%. The second trial was conducted in another Navel orange orchard in Sundays River Valley in February 2018. The same virus treatments were used as well as Broadband, EcoBb and three biological experimental treatments at different rates. Results were rather variable and unconvincing, as FCM infestation was very high and trees appeared stressed. A third trial was conducted, in a Navel orange orchard, but near Clanwilliam in the Western Cape. Evaluations are currently underway.

Opsomming

Op 14 Julie 2017 is VKM deur die EU as 'n geregleerde plaag verklaar. Gevolglik het dit uiters belangrik geword om 'n volle reeks beheer opsies vir VKM te ontwikkel en te toets. Onlangs is 'n nuwe nukleopolihedrovirus (NPV) ontdek wat albei lietsjiemot en VKM besmet het. Omdat dit voorkom dat lietsjiemot die natuurlike gasheer is, is die virus die *Cryptophlebia peltastica* nukleopolihedrovirus (CrpeNPV) genoem. Laboratorium biotoetse het aangedui dat die NPV 'n relatiewe breë gasheer reeks het en sy virulensie teen VKM is omtrent gelyk aan die teen lietsjiemot. Gevolglik is veldproewe vir 'n tweede agtereenvolgende seisoen uitgevoer met verskillende konsentrasies van CrpeNPV. Dit is ook in kombinasie met die VKM granulovirus, CrleGV (Cryptogran), toegedien want laboratorium biotoetse het sinergisme tussen hierdie twee viruse aangedui, veral teen 'n verhouding van 3:1 van CrpeNPV:CrleGV. Daarbenewens is sekere eksperimentele chemiese en biologiese insekdoders in proewe ingesluit op versoek van die produk vervaardigers of verskaffers. Drie veldproewe is uitgevoer. Die eerste is in 'n boord Nawellemoene in die Sondagsriviervallei in Desember 2017 toegedien en vir 8 weke geëvalueer. Die behandelings is soos volg: twee verskillende konsentrasies van CrpeNPV, 'n kombinasie van CrpeNPV en Cryptogran, Cryptogran, Methoxyfenozide (Runner) en verskeie eksperimentele chemiese behandelings. Cryptogran en CrpeNPV het albei besmetting met 58% verminder, Runner met 67% en een van die eksperimentele behandelings met meer as 90%. Die tweede proef is in nog 'n Nawellemoenboord in die Sondagsriviervallei in Februarie 2018 uitgevoer. Dieselfde virus behandelings is gebruik, sowel as Broadband, EcoBb en drie biologiese eksperimentele behandelings teen verskillende dosisse. Resultate is ietwat wisselvallig en onoortuigend, waarskynlik omdat VKM besmetting baie hoog was en bome blykbaar gestres was. 'n Derde proef is uitgevoer, ook in 'n Nawellemoenboord, maar naby Clanwilliam in die Wes-Kaap. Evaluasies word tans uitgevoer.

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

Project 1162 (April 2017 – March 2019) by Sean Moore, Wayne Kirkman (CRI) and Mellissa Peyper (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. Consequently, these were tested as potential female attractants, ultimately for development for monitoring purposes. Firstly, release rate of the compounds, formulated with hexane, was determined in the laboratory at constant temperature. All compounds were found to volatilise within 2-3 days. Thereafter, FCM (yellow delta) traps with stick floors were loaded with filled dispensers and hung in random order in trees on the upwind side of a lemon orchard, each trap in a separate row. Six replicates were used per treatment. Approximately 400 moths were released 15 m downwind of each trap shortly before sunset. The trial was replicated three times. Only one of the treatments caught a single female, whereas all treatments caught some males. Future trials will focus on improving formulation of attractants.

Opsomming

VKM word tans in die veld gemonitor deur gebruik van lokvalle met 'n lokaas van gesintetiseerde wyfie mot seksferomoon, en lok dus net mannetjie motte. So 'n stelsel kan egter nooit akkuraat genoeg wees nie, want dit is die wyfies (nie mannetjies nie) wat eiers op die vrugte lê, wat lei tot die larwes wat die skade doen. Daarbenewens, blyk dit dat mannetjie motte groter afstande as wyfie motte vlieg en is daarom nie noodwendig verteenwoordigend van die wyfie populasie in the area nie. Identifikasie van 'n lokmiddel vir wyfies sal 'n meer akkurate moniterings tegniek moontlik maak. Vorige werk het 'n paar vlugtige stowwe en mengsels van verbindings geïdentifiseer wat belofte inhou vir aanlokking van volwasse wyfie VKM. Gevolglik is hierdie as moontlike wyfiemot lokmiddels getoets, met die uiteindeindelijke doel vir ontwikkeling vir monitoring doeleindes. Eerstens is die loslatingstempo van die verskillende middels, geformuleer met heksaan, teen konstante temperatuur bepaal. Alle middels het binne 2-3 dae vervlugtig. Daarna is VKM (geel delta) lokvalle met taai vloere met gevulde vrystellers gelaai en in ewekansige volgorde in bome aan die opwind kant van 'n suurlemoen boord gehang, met elke lokval in 'n aparte ry. Ses replikate per behandeling is gebruik. Omtrent 400 motte is 15 m windaf van elke lokval losgelaat, pas voor sonafgaan. Die proef is drie maal herhaal. Net een van die behandelings het 'n enkele wyfie gevang, waar alle behandelings 'n paar mannetjies gevang het. Toekomstige proewe sal op verbetering van formulasie van lokmiddels fokus.

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

Project 1164 (April 2017 – March 2019) by Sean Moore, Wayne Kirkman (CRI), Mellissa Peyper, Clarke van Steenderen, Tammy Marsberg, Martin Hill (RU), Nevill Boersma, Ciska Kruger, Craig Chambers and Sampie Groenewald (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 problems and possible problems have been identified and there is a continual pursuit to improve the quality and performance of the moths. One of these problems is the lack of activity of sterile moths at cooler temperatures, whereas wild moths remain active at these lower

temperatures. Previous work identified trehalose as an effective cryoprotectant for moths, if added to the larval diet. Consequently, a large scale field trial was conducted during autumn and winter of 2017, comparing recaptures of moths reared on a trehalose augmented diet with those reared on the normal diet. There was no difference in recaptures, but there were many confounding variables and moth quality was variable. Consequently, the trial is being repeated during 2018. Additionally, trials to test anoxia for immobilising sterile moths, as an alternative to cold are being conducted. However, not much progress has been made yet. Furthermore, a reliable quality control test to measure mating competitiveness of sterile moths is being developed. A laboratory trial, investigating the transfer of spermatophores from sterile and non-sterile male moths to the bursa copulatrix of female moths, indicated that sterile males were significantly less mating competitive than non-sterile males, therefore wild males. This stands to reason; however, it must be determined what an acceptable difference would be. Mating trials are now being conducted in field cages, to determine whether these laboratory trials are sufficiently indicative of mating competitiveness. In the laboratory trials, no mating preferences were found between sterile and non-sterile males in their choice of sterile and non-sterile females. Additionally, a bisexual release simulation resulted in a significantly lower overall mean egg viability than a sterile male-only release in the first generation, thus supporting the continuation of bisexual releases.

Opsomming

Die steriele insek tegniek (SIT) vir VKM is in sitrus in Suid-Afrika kommersieel geïmplementeer sedert 2007 met algemeen goeie sukses. 'n Paar probleme en moontlike probleme is egeer geïdentifiseer en daar is 'n voortdurende poging om die gehalte en vertoning van die motte te verbeter. Een van hierdie probleme is 'n vermindering in aktiwiteit van steriele motte teen koeler temperature, waar wilde motte teen hierdie temperature aktief bly. Vorige navorsing het trehalose geïdentifiseer as 'n doeltreffende kouebeskermer vir motte, as dit by die larwe dieet bygevoeg was. Gevolglik is 'n grootskaalse proef gedurende herfs en winter van 2017 uitgevoer, wat hervangs van motte wat op 'n tehalose dieet geteel is vergelyk met motte wat op die gewone dieet geteel is. Daar is geen verskil in hervangs nie, maar daar was heelwat verwarrende veranderlikes en mot gehalte was ook wisselvallig. Gevolglik word die proef gedurende 2018 herhaal. Daarbenewens is proewe uitgevoer om anoxia te toets, as 'n alternatief vir koue, vir immobilisering van steriele motte. Op hierdie stadium is daar nie veel vordering hiermee gemaak nie. Verder, word 'n betroubare gehalte beheer toets ontwikkel om paringsmededingendheid van steriele motte te toets. 'n Laboratorium proef wat die oordrag van spermatofore van steriele en nie-steriele mannetjie motte na die bursa kopulatriks van wyfie motte gemeet het, het aangedui dat steriele mannetjies betekenisvol minder paringsmededingend was as nie-steriele mannetjies, dus wilde mannetjies. Hierdie is vanselfspekend, maar dit moet bepaal word wat 'n aanvaarbare verskil sal wees. Parings proewe word nou in veldhokke uitgevoer, om te bepaal of hierdie laboratorium proewe aanduidend genoeg is van paringsmededingendheid. In die laboratorium proewe is geen paringsvoorkeure tussen steriele en nie-steriele motte gekry nie in hulle keuse van steriele of nie-steriele wyfies. Boonop het 'n biseksuele loslatings simulاسie tot 'n betekenisvol laer algemene lewensvatbaarheid van eiers gelei in vergelyking met loslating van net steriele mannetjies, in die eerste generasie. Hierdie het dus die voortsetting van biseksuele loslatings ondersteun.

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

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

Summary

The ability of a sniffer dog to detect FCM-infested fruit has been demonstrated with a 98.9% accuracy within a previously registered research project. This is substantially more accurate than the currently used human inspection system, which has a maximum potential of 77.8%. However, there are a couple of potential hurdles to the successful implementation of this within citrus packhouses. Firstly, inspection of cartons or pallets in the packhouse could be extremely labour intensive and may require several dogs and dog handlers in a large packhouse. Secondly, there may be regulatory restrictions on the presence of dogs in the packhouse. Both

of these can be overcome through the development of a remote vapour detection system involving a vacuum pump and absorbent filters for collection of odours. Odours can be collected from numerous cartons and/or pallets, marked and presented to a sniffer dog in any convenient location thereafter. To date, various options, for collection of volatiles, have been investigated, such as compressed air with an adjustable pressure valve that can reverse the pressured air into suction and also control the pressure. Subsequently, a battery operated vacuum pump, with apparently sufficient suction capacity, is being used. A series of different filters is being tested for absorbing volatiles extracted from infested and healthy fruit, and the sniffer dog, Max is being imprinted on the former.

Opsomming

Die vermoë van 'n snufferhond om VKM-besmette vrugte is in 'n vorige geregistreerde navorsingsproef met 'n 98.9% akuraatheid bewys. Hierdie is beduidend meer akuraat as die huidige menslike inspeksie sisteem wat gebruik word wat 'n maksimum vermoë van 77.8% het. Daar is egter twee potensiële struikelblokke tot die suksesvolle toepassing hiervan in sitruspakhuis. Eerstens kan inspeksie van kartonne of pallette in die pakhuis uiters arbeidsintensief wees en mag dalk verskeie honde en hondehandteerders benodig, veral in 'n groot pakhuis. Tweedens, mag daar dalk regulatoriese beperkings op die teenwoordigheid van honde in die pakhuis wees. Albei van hierdie kan deur die ontwikkeling van 'n afgeleë vlugtigestof opsporingsstelsel oorkom word, wat 'n vakuumpomp en absorberende filters gebruik vir die versameling van die vlugtige stowwe. Vlugtigestowwe kan van verskeie kartonne en/of pallette versamel word en gemerk word en daarna aan 'n snufferhond in enige geskikte perseel voorgestel word. Tot op hede is verskeie opsies vir die versameling van vlugtigestowwe ondersoek, soos onderdrukte lug met 'n verstelbare drukklep wat die onderdrukte lug kan insuig en ook die druk kan beheer. Meer onlangs word 'n battery-krag vakuumpomp, met waarskynlik voldoende suigings vermoë, gebruik. 'n Reeks verskillende filters word getoets vir absorpsie van vlugtigestowwe wat van besmette en gesonde vrugte geëkstrakteer word, en die snufferhond, Max, word op die eersgenoemde ingeprint.

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

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

Summary

This project was only funded to begin in April 2018. However, new nets were erected over several orchards on four different farms in winter 2017 and in order to not miss the opportunity to coincide the start of the trial with this, it was initiated earlier than planned. FCM levels in orchards under nets and in comparable orchards outside of nets were compared at four sites, both in traps and in fruit infestation. Management programmes in these orchards were very similar, including the release of sterile moths in both environments at two of the sites. Monitoring for FCM was conducted weekly and levels were not lower under nets. This may be related to high levels of FCM in these orchards before the nets were erected. This may change over time, as sterile moth recaptures and ratios of sterile to wild moths were higher under nets. These same orchards were scouted for mealybug and red scale infestation and thrips damage closer to harvest, with varying results. Evaluations are still ongoing.

Opsomming

Hierdie projek is net bevonds om in April 2018 te begin, maar nuwe nette is oor verskeie boorde op vier verskillende plase in die winter van 2017 opgerig. Om nie die geleentheid mis te loop om die begin van die proef met die oprig van die nette te koördineer nie is die proewe vroeër as wat beplan is geïnisieer. VKM vlakke in boorde onder nette en in vergelykbare boorde buite die nette by vier persele is vergelyk, albei wat lokval vangste en vrugbesmetting betref. Bestuursprogramme in hierdie boorde is vergelykbaar, insluitend die loslating van steriele motte in albei omgewings by twee van die persele. Monitoring vir VKM is weekliks uitgevoer en vlakke was nie laer onder nette nie. Hierdie kan wees as gevolg van hoë vlakke van VKM in hierdie boorde voor die nette opgerig was. Hierdie kan dalk oor tyd verander omrede steriele motte hervangste

en verhoudings van steriel tot wilde motte hoër was onder nette. Dieselfde boorde is vir witluis en dopluis besmetting en blaaspoetjies skade nader aan oestyd verken, met wisselvallige resultate. Evaluasies word nog voortgesit.

2.2.20 **PROGRESS REPORT: Using Carbon dioxide to shorten cold disinfestation treatments for internal pests of citrus fruit destined for Europe**

Project 1197 and PHI-3 03/2018 (2018/9 – 2019/20) by Tim Grout and Kim Stoltz (CRI)

Summary

This project was due to only start in April but it was accepted for surplus PHI funding from February and Valencia fruit in cold storage were available for comparisons between fumigation with CO₂ of 5th instar FCM in River Bioscience commercial media in glass jars, and larvae in fruit. Unfortunately, the gas did not penetrate the crust on the upper surface of the media in three trials conducted. Mean mortalities for 5th instars in media with gas only, cold only and gas plus cold were 6%, 10% and 13%, respectively, whereas mortalities in Valencia fruit for the same treatments were 39%, 12% and 67%, respectively. We are therefore dependent on the use of infested fruit for further trials, which will reduce the numbers tested, unless the more porous fruit fly diet proves practical for FCM.

Opsomming

Hierdie projek moest eers in April begin het maar dit is goedgekeur vir oorskot PHI bevonding van Februarie en Valencia vrugte was in kouestore beskikbaar vir verglykings tussen berokking met CO₂ van 5de instar VKM in River Bioscience se kommersiele dieet in glas buisies en larwes in vrugte. Ongelukkig het die gas nie die korsie op die boonste oppervlak van die dieet gepenetreer nie in drie proewe wat uitgevoer is. Gemiddelde mortaliteite van 5de instars in dieet met gas alleenlik, koue alleenlik en gas met koue was 6%, 10% en 13%, onderskeidelik, waar mortaliteite in Valencia vrugte vir dieselfde behandelings 39%, 12% en 67%, onderskeidelik was. Ons is daarom afhanklik van besmette vrugte vir verdere proewe, wat getalle wat getoets kan word sal verminder, behalwe as dit gewys kan word dat die meer poreuse vrugtevlieg dieet prakties bruikbaar vir VKM is.

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

Project 1163 (2017/8-2019-/20) by Marcel van der Merwe, Caroline Knox, Martin Hill (Rhodes University), Sean Moore (CRI)

Summary

Thaumatotibia leucotreta (false codling moth) is an indigenous pest of the citrus industry in southern Africa. The pest is highly significant as it impacts negatively on the export of fresh citrus from South Africa to international markets. To control *T. leucotreta* in South Africa, an IPM programme has been implemented. One component of this programme is the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV-SA). It has previously been reported that there is a mutualistic association between *Cydia pomonella*, also known as codling moth, and epiphytic yeasts. Laboratory assays and field trials show that combining yeast with *Cydia pomonella* granulovirus significantly increased larval mortality. We propose to determine which species of yeast occur naturally in *T. leucotreta* larvae and to examine whether any of these yeasts, when combined with CrleGV-SA, increase larval mortality. Navel oranges infested with *T. leucotreta* larvae were collected from orchards in Sundays River Valley and analysed for the presence of yeast. Four yeasts were isolated from *T. leucotreta* larvae and identified down to species level via PCR amplification and sequencing of ITS region and D1/D2 domain of the LSU. The yeast isolates were identified as *Meyerozyma caribbica*, *Pichia kluyveri*, *Pichia kudriavzevii* and *Hanseniaspora opuntiae*. A yeast preference assay was conducted on female *T. leucotreta* moths to examine whether any of the isolated yeast species affected their oviposition preference. *Pichia kudriavzevii* was shown to be the preferred yeast species for oviposition, as significantly more eggs were deposited on Navel oranges inoculated with this yeast compared to the other treatments. A detached fruit

bioassay was then performed to evaluate the efficacy of mixing *P. kudriavzevii* with CrleGV-SA. *Pichia kudriavzevii* was selected as it was demonstrated as having an effect on the oviposition preference of female *T. leucotreta* moths. Although an increase in larval mortality was observed between CrleGV-SA being applied alone and the yeast/virus mixture, this result was determined not to be statistically significant. Currently we are expanding the bioprospecting process to other geographically distinct citrus producing regions such as the Western Cape and Limpopo provinces. This may lead to the identification of unique yeast isolates that elicit stronger responses in *T. leucotreta* than those isolated thus far. Additionally, detached fruit bioassays are being conducted with varying yeast concentrations and the addition of molasses. The inclusion of molasses and lowering the yeast concentration may result in better mortality rates.

Opsomming

Thaumatotibia leucotreta (valskodlingmot) is 'n inheemse plaag van die sitrusbedryf in Suider-Afrika. Die plaag is baie belangrik, aangesien dit negatief impakteer op die uitvoer van vars sitrus vanaf Suid-Afrika na internasionale markte. Om *T. leucotreta* in Suid-Afrika te beheer, is 'n IPM-program geïmplementeer. Een komponent van hierdie program is die bakulovirus *Cryptophlebia leucotreta* granulovirus (CrleGV-SA). Daar is voorheen berig dat daar 'n mutualistiese assosiasie is tussen *Cydia pomonella*, ook bekend as kodlingmot, en epifitiese giste. Laboratorium toetse en veldproewe toon dat die kombinasie van gis met *Cydia pomonella* granulovirus aansienlik die larwe mortaliteit verhoog. Ons stel voor om te bepaal watter spesies gis natuurlik in *T. leucotreta* larwes voorkom en te ondersoek of enige van hierdie giste, wanneer dit gekombineer word met die CrleGV-SA, larwe mortaliteit verhoog. Navel lemoene wat met *T. leucotreta* larwes besmet is, is van boorde in Sondagsriviervallei versamel en ontleed vir die teenwoordigheid van gis. Vier giste is van *T. leucotreta* larwes geïsoleer en geïdentifiseer tot spesiesvlak via PCR versterking en volgordebepaling van ITS streek en D1 / D2 domein van die LSU. Die gis-isolate is geïdentifiseer as *Meyerozyma caribbica*, *Pichia kluyveri*, *Pichia kudriavzevii* en *Hanseniaspora opuntiae*. 'n Gispreferensassessering is uitgevoer op wyfie *T. leucotreta* motte om te ondersoek of enige van die geïsoleerde gisspesies hul eierleggings voorkeur beïnvloed het. *Pichia kudriavzevii* is getoon as die voorkeurgisspesie vir eierlegging, aangesien aansienlik meer eiers gelê is op Navel-lemoene wat met hierdie gis ingeënt is, in vergelyking met die ander behandelings. 'n Losstaande vrugte-biotoets is dan uitgevoer om die effektiwiteit van die vermenging van *P. kudriavzevii* met CrleGV-SA te evalueer. *Pichia kudriavzevii* is gekies omdat dit bewys is dat dit 'n effek het op die eierleggingsvoorkeur van wyfie *T. leucotreta* motte. Alhoewel 'n toename in larwe mortaliteit waargeneem word tussen CrleGV-SA alleen toegedien en die gis-virusmengsel, dit is bepaal dat hierdie uitslag nie statisties betekenisvol is nie. Tans brei ons die bioprospekteringsproses uit na ander geografies duidelike sitrusproduserende streke soos die Wes-Kaap en Limpopo provinsies. Dit kan tot die identifikasie van unieke gis-isolate lei, wat sterker reaksies in *T. leucotreta* ontlok as dié wat tot dusver geïsoleer is. Daarbenewens word losstaande vrugte biotoetse uitgevoer met verskillende gis konsentrasies en die toevoeging van melasse. Die insluiting van melasse en die verlaging van die gis konsentrasie kan tot hoër mortaliteit lei.

2.2.22 PROGRESS REPORT: Novel approaches to mating disruption of FCM

Project 1080 (2013/14 – 2018/19) by Martin Gilbert and Claire Love (CRI)

Summary

This project has been extended to enable a further experiment to be completed in August 2018. The aim of the project has been to investigate various approaches to the control of FCM. Aspects reported upon during the lifetime of the project have included: the augmentation of sterile releases with application of mating disruption products, the increasing of mating disruption product concentration in sprays to attempt better FCM control, and an investigation into the economic viability of local production of FCM pheromone. In 2017/18, an experiment was set up to compare the efficacy of different mating disruption products, some of which are relatively new to the citrus industry. The products included in the trial were RB Splat-FCM (River Bioscience), X-Mate FCM (Insect Science) and Isomate FCM (Nulandis) and these were compared with an untreated control block. The trial was carried out at Clanwilliam on Kleinvlei farm. Each treatment block comprised 1.5 ha with 3 replicates per treatment including the control block. FCM adult numbers were monitored with yellow delta pheromone traps. As the farm is within the Xsit moth release area both sterile and wild FCM could be monitored to measure the level of trap “shut-down” obtained with each mating disruption product. FCM fruit

infestation was monitored by collecting and analysing fallen fruit under five trees per block. The experiment has not yet been completed. Nevertheless, in terms of trap shut-down, Isomate performed the best with the lowest recapture of both sterile as well as wild FCM. Splat performed 2nd best followed by X-Mate. Unfortunately, due to the low number of wild moths, this pattern cannot yet be confirmed in terms of fruit infestation. The monitoring of both fruit fall and adult FCM will continue until harvest.

Opsomming

Die projek is uitgebrei om 'n addisionele proef in Augustus 2018 te laat voltooi. The doel van projek 1080 is om verskeie nuwe benaderings tot die beheer van VKM na te gaan. Aspekte waarvoor gerapporte is gedurende die leeftyd van die projek het die aanvulling van steriele loslatings met parings ontwinging produkte en die verhoging van parings ontwinging spuit konsentrasies ingesluit. 'N ondersoek oor die ekonomiese lewensvatbaarheid van plaaslike VKM feromoon produksie is ook uitgevoer. In 2017/18, 'n proef om verskeie parings ontwinging produkte, waarvan van hulle relatief nuut tot die die sitrus bedryf is, is opstel. The proef het die volgende produkte ingesluit: RB Splat-FCM (River Bioscience), X-Mate FCM (Insect Science) en Isomate FCM (Nulandis) sowel as 'n ongehandelde control blok. Die proef is by Kleinvlei plaas, Clanwilliam uitgevoer. Elke behandelde blok het uit 1.5 ha bestaan met drie replicate insluitend die kontrole. VKM getalle is met geel delta feromoon valletjies gemonitor. Aangesien die plaas binne die Xsit steriele mannetjie loslating area val, kon albei steriele en wild VKM volwassenes gemonitor word om die vlak van valletjie "shut-down" wat met elke afsonderlike parings ontwinging produk te kry. Vrugval as gevolg van VKM infestasië is ook deur manier van die versameling en ondersoek van vrugte wat geval het onder vyf data bome per blok. Die proef is nog nie voltooi. Nietemin, in terme van valletjie "shut-down", het Isomate die beste presteer met die laagste vangste van wild sowel as steriele VKM. Splat het tweede presteer gevolg deur X-Mate. Ongelukkig, as gevolg van lae getalle van wilde VKM motte, kan hierdie patroon nie in terme van vrug infestasië bevestig word nie. Die monitor van albei vrugval en volwasse VKM sal tot by oestyd voortdeur.

2.3 PROGRAMME: FRUIT FLY

Programme Coordinator: Aruna Manrakhan

2.3.1 Programme summary

Fruit flies remain pests of phytosanitary concern on export citrus. The four species problematic to citrus are: *Ceratitis capitata* (Mediterranean fruit fly or Medfly), *Ceratitis rosa* (Natal fly), *Ceratitis quillicii* (Cape fly) and *Bactrocera dorsalis* (Oriental fruit fly). There is a zero tolerance of these species in citrus destined for export markets. At citrus packhouses, presence of fruit flies in fruit consignments inspected by Perishable Products Export Control Board (PPECB) would lead to rejections of these consignments. Presence of some fruit fly species in an area could also impact on market access. On an annual basis, at least one fruit fly interception is recorded by the European Union on some citrus types exported from South Africa (mainly soft citrus and oranges). In those susceptible citrus types, fruit fly management needs to be further optimised to prevent rejections and interceptions.

There were eleven projects carried out under the fruit fly programme between April 2017 and March 2018. Two projects were on biology and ecology of Oriental fruit fly. One project addressed the ecology and development of the Natal fly and Cape fly in citrus. Four projects were on pre-harvest management of fruit flies which included monitoring and protein baiting. One project addressed the host status of export grade lemon for key fruit fly pests. Two projects addressed post-harvest cold treatments for fruit flies. In order to facilitate various research projects requiring reared fruit fly materials, the long term project on fruit fly rearing (2.3.5) was also maintained.

Movement and dispersal capacity of the Oriental fruit fly were determined in laboratory and field studies (2.3.2). Females of the Oriental fruit fly were found to fly further than males in the absence of hosts. In the presence of host plants, female dispersal was restricted. In laboratory and field studies, undamaged citrus was not found to be susceptible to infestation by Oriental fruit fly (2.3.6). In citrus, development of the pest did not occur when eggs were deposited in the flavedo region and also rarely occurred when deposited in the albedo region. Development of *B. dorsalis* to adulthood however occurred when eggs were deposited in the citrus pulp.

Orchard sanitation and management of other fruit infesting pests should be included in the management of the Oriental fruit fly.

In studies on Cape fly and Natal fly, Cape fly was found to be the dominant species in commercial citrus orchards in the northern areas of South Africa (2.3.9). Abundance of Natal fly was higher at low altitudes and in hotter and wetter regions. However, no natural infestation of citrus was recorded for either of the two species. When eggs of Cape fly and Natal fly were artificially inoculated in the pulp of citrus, both species developed at the same rates. Oranges was the most suitable citrus type for both species.

Efficacy and sensitivity of fruit fly monitoring systems were determined in field studies (2.3.3). The three-component Biolure was found to be most effective attractant for females of Medfly, Oriental fruit fly, Natal fly and Cape fly. Enriched Ginger Oil (EGO) was found to be an effective male lure for *Ceratitis* pests. The types of traps and dispensers used in trimedlure and methyl eugenol trapping systems influenced catches of males of Medfly and Oriental fruit fly respectively.

In studies on protein baiting, a new paper based bait station containing malathion was found to be effective for fruit fly control (2.3.7). The new bait station deployed at 200 units per ha had similar control efficacy as M3 bait stations deployed at 300 units per ha. Bait stations suppress fruit fly populations at a slower rate than bait sprays. As such in areas where fruit fly population pressure is high, timing of bait station application should be optimised. This is currently being investigated in a new project (2.3.11) which is being carried out in citrus orchards in the Western Cape. On application of bait sprays, incompatibility between copper applications and fruit fly baits is being investigated (2.3.8).

Studies on the host status of export grade Eureka lemon for fruit fly pests were completed (2.3.4). Export grade Eureka lemon from South Africa were found to be a non-host for Medfly, Natal fly, Cape fly and Oriental fruit fly.

As part of the development of cold treatments for postharvest disinfestation of fruit flies in citrus, the cold tolerances of immature stages of Medfly, Natal fly and Oriental fruit fly were determined. In the first project (2.3.10), at a target temperature of 4°C, Medfly was found to be more cold tolerant than Natal fly and Oriental fruit fly. In the second project (2.3.12) at a target temperature of -0.6°C, Medfly was found to be more cold tolerant than Oriental fruit fly. These results indicate that postharvest cold treatment schedules for Medfly should be effective for Natal fly and Oriental fruit fly.

Programopsomming

Vrugtevlieë bly plaë van fitosanitêre belang op uitvoer sitrus. Die volgende vier spesies is 'n probleem op sitrus: *Ceratitis capitata* (Mediterreense vrugtevlieg of *Medfly*), *Ceratitis rosa* (Nataalse vlieg), *Ceratitis quilicii* (Kaapse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg). Daar is 'n zero-toleransie vir hierdie spesies in sitrus wat vir uitvoermarkte bestem is. By sitruspakhuis sal die teenwoordigheid van vrugtevlieë in vrugbesendings wat deur *Perishable Products Export Control Board* (PPECB) geïnspekteer word, tot die afkeur van hierdie besendings lei. Die teenwoordigheid van sommige vrugtevlieëspesies in 'n area kan ook 'n impak op marktoegang hê. Op 'n jaarlikse basis word ten minste een vrugtevlieg onderskepping deur die Europese Unie op sommige sitrustipes wat vanaf Suid-Afrika uitgevoer word (hoofsaaklik sagte sitrus en lemoene), aangeteken. In hierdie vatbare sitrustipes moet vrugtevliegbestuur verder geoptimaliseer word ten einde afkeurings en onderskeppings te voorkom.

Daar is elf projekte tussen April 2017 en Maart 2018 onder die vrugtevliegprogram uitgevoer. Twee projekte was op die biologie en ekologie van die Oosterse vrugtevlieg. Een projek het die ekologie en ontwikkeling van die Nataalse vlieg en die Kaapse vlieg in sitrus aangespreek. Vier projekte was op die voor-oesbestuur van vrugtevlieë, wat monitering en proteïen lokaas-toediening ingesluit het. Een projek het die gasheerstatus van uitvoergraad suurlemoene vir sleutel vrugtevliegplae aangespreek. Twee projekte het na-oes koue-behandelings vir vrugtevlieë aangespreek. Ten einde verskeie navorsingsprojekte te fasiliteer wat geteelde vrugtevliegmateriaal benodig, is die langtermyn-projek (2.3.5) op vrugtevliegteling ook in stand gehou.

Beweging en verspreidingsvermoë van die Oosterse vrugtevlieg is in laboratorium- en veldstudies bepaal (2.3.2). Daar is bepaal dat wyfies van die Oosterse vrugtevlieg verder as mannetjies vlieg in die afwesigheid van gasheer. Die wyfies se verspreiding was egter in die teenwoordigheid van gasheerplante beperk. In laboratorium- en veldstudies was onbeskadigde sitrus nie vatbaar vir infestasië deur die Oosterse vrugtevlieg nie (2.3.6). Ontwikkeling van die plaag het nie voorgekom wanneer eiers in die 'flavedo' area geplaas is nie, en het ook skaars voorgekom wanneer in die 'albedo' area geplaas is. Ontwikkeling van *B. dorsalis* tot by volwassenheid het egter voorgekom wanneer die eiers in die pulp geplaas is. Boordsanitasie en -bestuur van ander vrugte-infesterende plae moet in die bestuur van die Oosterse vrugtevlieg ingesluit word.

In studies op die Kaapse vlieg en Natalse vlieg, was die Kaapse vlieg die dominante spesie in kommersiële sitrusboorde in die noordelike areas van Suid-Afrika (2.3.9). Volopheid van Natalse vlieg was hoër by lae hoogtes en in warmer en natter areas. Geen natuurlike infestasië van sitrus is egter vir enige van die twee spesies aangeteken nie. Wanneer eiers van die Kaapse vlieg en Natalse vlieg kunsmatig in die pulp van sitrus geïnokuleer is, het beide spesies teen dieselfde tempo ontwikkel. Lemoene was die mees geskikte sitrustipe vir beide spesies.

Effektiwiteit en sensitiwiteit van vrugtevlieg moniteringssisteme is in veldstudies bepaal (2.3.3). Die drie-komponent Biolure was die effektiwste lokmiddel vir wyfies van die Mediterreense vrugtevlieg, Oosterse vrugtevlieg, Natalse vlieg en Kaapse vlieg. *Enriched Ginger Oil* (EGO) was 'n effektiewe manlike lokmiddel vir *Ceratitis* plae. Die tipes lokvalle en vrystellers wat in trimedlure en metiel eugenol lokvalstelsels gebruik is, het die vangste van Mediterreense vrugtevlieg en Oosterse vrugtevlieg mannetjies onderskeidelik beïnvloed.

In studies op proteïene lokaas-toediening, was 'n nuwe papier-gebaseerde lokaasstasie, bevattende malathion, effektief vir vrugtevliegbeheer (2.3.7). Die nuwe lokaasstasie wat teen 200 dele per ha ontplooi is, het 'n soortgelyke beheer effektiwiteit gehad as M3 lokaasstasies wat teen 300 eenhede per ha ontplooi is. Lokaasstasies het vrugtevliegpopulasies teen 'n stadiger tempo onderdruk in vergelyking met lokaasspuiting. Sodanig is dit belangrik dat in areas waar vrugtevlieg populasiedruk hoog is, tydskedering van lokaasstasie toediening optimaal moet geskied. Dit word tans in 'n nuwe projek (2.3.11) wat in sitrusboorde in die Wes-Kaap uitgevoer word, ondersoek. Onverenigbaarheid tussen kopertoedienings en vrugtevlieg lokaasmiddels word tydens toediening van lokaasspuiting, ondersoek (2.3.8).

Studies op die gasheerstatus van uitvoergraad 'Eureka' suurlemoene vir vrugtevliegplae is voltooi (2.3.4). Daar is gevind dat uitvoergraad 'Eureka' suurlemoene vanaf Suid-Afrika nie 'n gasheer vir Mediterreense vrugtevlieg, Natalse vlieg, Kaapse vlieg en Oosterse vrugtevlieg is nie.

As deel van die ontwikkeling van koue-behandelings vir na-oes disinfestasië van vrugtevlieë in sitrus, is die koue-toleransies van onvolwasse fases van Mediterreense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg bepaal. In die eerste projek (2.3.10), by 'n teikentemperatuur van 4°C, is gevind dat Mediterreense vrugtevlieg meer koue-bestand as die Natalse vlieg en Oosterse vrugtevlieg was. In die tweede projek (2.3.12) by 'n teikentemperatuur van -0.6°C, is gevind dat die Mediterreense vrugtevlieg meer koue-bestand as die Oosterse vrugtevlieg was. Hierdie resultate dui aan dat na-oes koue-behandelingskedisules vir Mediterreense vrugtevlieg, effektief vir Natalse vlieg en Oosterse vrugtevlieg sal wees.

2.3.2 FINAL REPORT: Dispersal capacity of *Bactrocera dorsalis*

Project 1075 (Apr 2013 – Mar 2017) by C W Weldon (University of Pretoria), R Anguelov (University of Pretoria) and A Manrakhan (CRI)

Summary

The key outcome of this project was to establish the dispersal capacity of the oriental fruit fly, *Bactrocera dorsalis* with regard to environmental and physiological variables. Knowledge of the dispersal capacity of *B. dorsalis* represents a basic component of a systems approach to manage this pest because it delimits the area that needs to be quarantined and treated and the size of buffer zones surrounding pest-free areas.

Sterile *B. dorsalis* were used in mark-release-recapture experiments to determine the effects of fly maturity, sex and availability of host plants on dispersal. This study established that females fly further from the release point before being recaptured than males, and that this is particularly pronounced when females are released amongst non-host plants. Younger males disperse further than older males released from the same point at the same time.

To explore the physiological basis for field dispersal results, we also determined the influence of temperature on spontaneous locomotor activity and flight. *Bactrocera dorsalis* is largely inactive at temperatures lower than 20°C. The frequency and duration of walking and flight increases between 24-32°C. At a temperature of 36°C, the duration of resting increases, which may indicate the onset of thermal stress. When flies were young, the distance flown on "flight mills" did not differ between the sexes, but as flies aged, females flew further than males.

Fly age, sex and the proximity of host plants influences the distance that *B. dorsalis* travel from a point of origin. Based on observations of spontaneous flight in the laboratory, the dispersal ability of younger male flies may result from more short flights when temperatures are suitable. Despite this, it is clear that female *B. dorsalis* are stronger fliers than males, but their tendency for flight appears to be arrested if they are in an environment with host plants.

Opsomming

Die hoof doelwit van hierdie projek was om te bepaal wat die verspreidingsvermoë van die Oosterse vrugtevlieg, *Bactrocera dorsalis*, met betrekking tot omgewings- en fisiologiese veranderlikes is. Kennis van die verspreidingsvermoë van *B. dorsalis* verteenwoordig 'n basiese komponent van 'n sisteembenadering om hierdie plaag te bestuur omdat dit die area wat onder kwarantyn geplaas en behandel moet word, afbaken, asook die grootte van die buffersones wat plaag-vrye areas omring.

Steriele *B. dorsalis* is in merk-vrylaat-hervang proewe gebruik ten einde die effek van vlieg volwassenheid en geslag, en beskikbaarheid van gasheerplante, op verspreiding te bepaal. Hierdie studie het vasgestel dat wyfies verder van die vrylatingspunt af vlieg voordat hulle weer gevang word, as mannetjies, en dat dit veral duidelik is wanneer wyfies tussen nie-gasheerplante vrygelaat word. Jonger mannetjies versprei verder as ouer mannetjies wanneer vanaf dieselfde punt op dieselfde tyd vrygelaat word.

Ten einde die fisiologiese basis vir veld verspreidingsresultate te ondersoek, het ons ook die invloed van temperatuur op spontane lokomotoriese aktiwiteit en vlug vasgestel. *Bactrocera dorsalis* is grootliks onaktief by temperature laer as 20°C. Die frekwensie en duur van stap en vlieg neem tussen 24-32°C toe. By 'n temperatuur van 36°C neem die duur van rus toe, wat die begin van hittestres kan aandui. Wanneer vlieë jonk is, het die afstande wat op 'flight mills' gevlieg is, nie tussen die geslagte verskil nie, maar soos wat vlieë ouer geword het, het wyfies verder as mannetjies gevlieg.

Vlieg ouderdom, geslag en die nabyheid van gasheerplante, beïnvloed die afstand wat *B. dorsalis* vanaf 'n punt van oorsprong reis. Gebaseer op waarnemings van spontane vlug in die laboratorium, mag die verspreidingsvermoë van jonger manlike vlieë die resultaat wees van meer korter vlugte wanneer temperature geskik is. Ten spyte hiervan, is dit duidelik dat wyfie *B. dorsalis* sterker vlieërs is as mannetjies, maar hul geneigdheid om te vlieg, blyk gestrem te word as hulle in 'n omgewing met gasheerplante is.

Introduction

Knowledge of the dispersal capacity of *Bactrocera dorsalis* represents a basic component of a systems approach to suppress or eradicate incipient populations because it delimits the area that needs to be treated. In highly productive areas, delimitation of treatment areas based on a sound understanding of insect dispersal capacity leads to cost effective management actions.

Information on dispersal capacity of *B. dorsalis* would also provide a basis for determining width of buffer zones required around pest free areas or pest free places of production. In case of failure to eradicate *B. dorsalis* from the current quarantined areas in South Africa, maintenance of pest freedom in some areas might necessitate the establishment of surrounding buffer zones. Control and monitoring actions would have to be instigated in these buffer zones in order to isolate pest free areas from affected areas.

Further, knowledge of the dispersal capacity of *B. dorsalis* will benefit future attempts in South Africa to develop the Sterile Insect Technique (SIT) for this serious pest. SIT is an environmentally friendly and medically benign form of control that has been employed worldwide to effectively control a range of fruit fly species including *Bactrocera* species. The technique holds some promise for the control of *B. dorsalis*, particularly at the limits of its invaded range, but the effectiveness of SIT is affected by dispersal for a number of reasons: (1) long-distance dispersal by mated females into a target area will reduce the rate of wild population decline, (2) dispersal can lead to reinvasion of an area after eradication using SIT (as in other control techniques), (3) moderately high levels of dispersal can reduce spatial heterogeneity of sterile insects within the pest population, and (4) movement enables sterile insects to forage for essential resources and survive in the target area.

Stated objectives

The objectives of this project were to:

- A. Determine the optimal dose and fluorescent pigment colours required for marking *B. dorsalis* for use in MRR studies of dispersal.
- B. Establish the dispersal capacity of *B. dorsalis* with regard to temperature, rainfall, fly sexual maturity, and proximity of fruiting host plants.
- C. Determine the effect of temperature on locomotor activity, including flight, and the incidence of mating behaviour in *B. dorsalis*.
- D. Use mathematical models that incorporate knowledge of dispersal capacity of *B. dorsalis* to establish the spatial origin of incipient populations, width of buffer zones around pest free areas, and optimal spacing of sterile insect releases.

Materials and methods

A large, outbred, laboratory culture of *B. dorsalis* was established at the Research Services Division of the Department of Agricultural Research Services, Zimbabwe, and then at CRI Nelspruit. Flies used in the following laboratory studies were sourced from these cultures. Flies used in field studies were sourced from the Seibersdorf Laboratories of the International Atomic Energy Agency and sterilised with 90-100 Gy of gamma radiation.

Objective A: Optimisation of marking with fluorescent pigments

Six fluorescent pigment powder colours (Astral Pink 1, Blaze 5, Stellar Green 8, Lunar Yellow 27, Comet Blue 60 and Invisible Blue 70) were tested to determine their potential for marking *B. dorsalis*. Each pigment was applied at a concentration of 2, 4 or 6 g/l of pupae to 50 pupae placed in separate cages. A control group of 50 pupae to which no pigment was added was kept in a separate cage. Rate of full and partial eclosion were recorded for each pigment colour and dye concentration. Mark visibility of each pigment colour and concentration were determined at eclosion and 7, 14, 21 and 28 days after eclosion for 20 flies from each treatment under a dissecting microscope with blue/ultraviolet light source. Mortality in each cage was recorded daily for up to 21 days after emergence to assess the effect of pigment colour and concentration on survival. A total of three replicates were performed.

To ensure that pigment marks were retained and did not affect survival under field conditions, pupae were treated in the same manner, adults allowed to emerge and then placed in insect sleeves with food and water on branches of a guava tree. Flies in sleeves were checked daily for mortality over a period of 28 days. Visibility of pigment marks was determined on the day of eclosion and at 28 days after eclosion.

Objective B: Dispersal in relation to weather and host proximity

Dispersal of *B. dorsalis* was assessed in the Levubu area in Limpopo Province. The PhD student was based at in Nelspruit and Limpopo to permit this study.

Three trapping arrays were established, each with 100 traps covering an area of approximately 450 ha (4.0 × 1.5 km), within a matrix of fruiting and non-fruiting host and non-host plants. Traps were baited with methyl eugenol (ME) or Biolure three-part lure (50 of each) to permit recaptures of both males and females. Eight traps were scattered haphazardly at varying distances within a radius of 200 m of release points. Further out, trap clusters (2 ME, 2 BioLure) sampled flies at different distances from the release points.

Newly-emerged flies and 10 day-old flies were released concurrently (~5,000 of each) at two points within each trapping grid: one release point within a host crop, the other surrounded by non-host trees. Four distinct fluorescent pigment colours (selected based on the results of Objective 1) were used to distinguish the sexual maturity of flies at time of release and release site. Traps were emptied weekly for four weeks after release and captured flies inspected under a dissecting microscope with UV light to visualise fluorescent marks. Releases were carried out to cover the dry and wet season over two consecutive years for temporal replication and to encompass the range of temperatures, humidity, rainfall and host availability that can be experienced during an entire year. Temperature, humidity and rainfall were monitored during each trapping period. A survey of host fruit presence/absence, location and maturity was performed at the time of each release.

Separate generalised linear models with negative binomial distribution were used to describe recaptures of females and males in relation to the vegetation surrounding the release point, release age, time after release, placement of traps in host or non-host trees, distance from the release point, average minimum temperature, average maximum temperature, and rainfall. The data were also expressed as the mean distance travelled before recapture, which was analysed with general linear models separately for each sex, again with vegetation surrounding the release point, release age, and time after release as predictors.

Objective C: Effect of temperature on flight and mating

The effect of temperature on flight and mating behaviour was determined at CRI, Nelspruit, and the University of Pretoria. Spontaneous activity by *B. dorsalis* was determined when exposed to test temperatures of 12, 16, 20, 24, 28, 32 and 36°C at ages of 3, 10 and 21 days after adult emergence. Flight speed and distance will be determined using flight mills. Results will be important for parameterising mathematical models of fly dispersal. To determine the effect of temperature on mating behaviour, pairs of females and males of known weight were placed in temperature-controlled arenas approximately 1 hour before dusk (like some other *Bactrocera* species, *B. dorsalis* mates only at dusk). The arenas were set into an aluminium block. Channels were bored into the block, through which water of known temperature was circulated from a temperature-controlled water bath. Mating behaviour was recorded using a firewire video camera positioned above the temperature-controlled arenas. Video recordings were stored on a portable hard drive and later viewed in open source behavioural analysis software, JWatcher version 1.0, to score the frequency, bout duration and total duration of male calling (wing fanning associated with pheromone release), incidence of copulation, time elapsed before copulation, and duration of copulation. Results will be useful for refining population growth rate models.

Flight mills were built following a standard design (http://entomology.tfrec.wsu.edu/VPJ_Lab/Flight-Mill.html). At 3, 10 and 21 days of age, female and male flies were weighed using an analytical balance before being tethered to flight mills and then allowed to fly for one hour at a test temperature at one of 12, 16, 20, 24, 28, 32 and 36°C. Flight speed, distance and intermittency were recorded. After testing, flies were removed from the arm of the flight mill and stored in a freezer until a later date when their right wing was removed and affixed to a microscope slide. Wings were photographed with a digital camera mounted to a dissecting microscope, and then the digital image was used to measure their area.

Objective D: Implications for the spacing of monitoring traps and release of sterile insects

This work will be led by Prof. Roumen Anguelov. Firstly, a model will be constructed using ordinary differential equations to describe the dynamics of a *B. dorsalis* population. This model will then be applied to the context of SIT to investigate: (i) the threshold values of the parameters that determine the qualitative behaviour of model solutions, and (ii) quantifying the effect of sterile insect releases.

The first model will be extended by considering the space variable. The model for the development and spread of the *B. dorsalis* population will involve a system of partial differential equations, and advection and diffusion terms to model the dynamics of the spatial distribution. After the model has been calibrated to existing data (from Objective 2) it will be used for (i) determining optimal position of traps to ensure early detection, (ii) estimating the spread of a *B. dorsalis* population from monitoring trap data, and (iii) optimize the effect of SIT intervention in terms of location, size and frequency of releases.

Results and discussion

Objective A: Optimisation of marking with fluorescent pigments

Under laboratory conditions, pigment colour had a small but significant effect on the number of partially emerged and deformed adults; the fewest of these were observed when flies were marked with Astral Pink 1 (Figure 1; Makumbe *et al.*, 2017). Pigment concentration, on the other hand, had no effect on adult emergence, partial emergence, deformed adults and mortality on the last day of eclosion. There was no significant effect of pigment colour on adult survival under laboratory and semi-field conditions. Under laboratory conditions, however, there was an effect of pigment concentration on adult survival depending on pigment colour. Visibility under an ultraviolet light and persistence of marks was significantly affected by pigment colour and concentration when observed under laboratory conditions, but not under semi-field conditions. Regardless of colour or dose, pigments used in the study were visible for at least 14 days, but began to fade by 21 days after adult eclosion. To mark *B. dorsalis* under temperate, warm summer African conditions, all pigment colours tested in this study may be applied at 2–4 g/l pupae. Recaptures of marked and released flies maybe underestimated as the flies age.

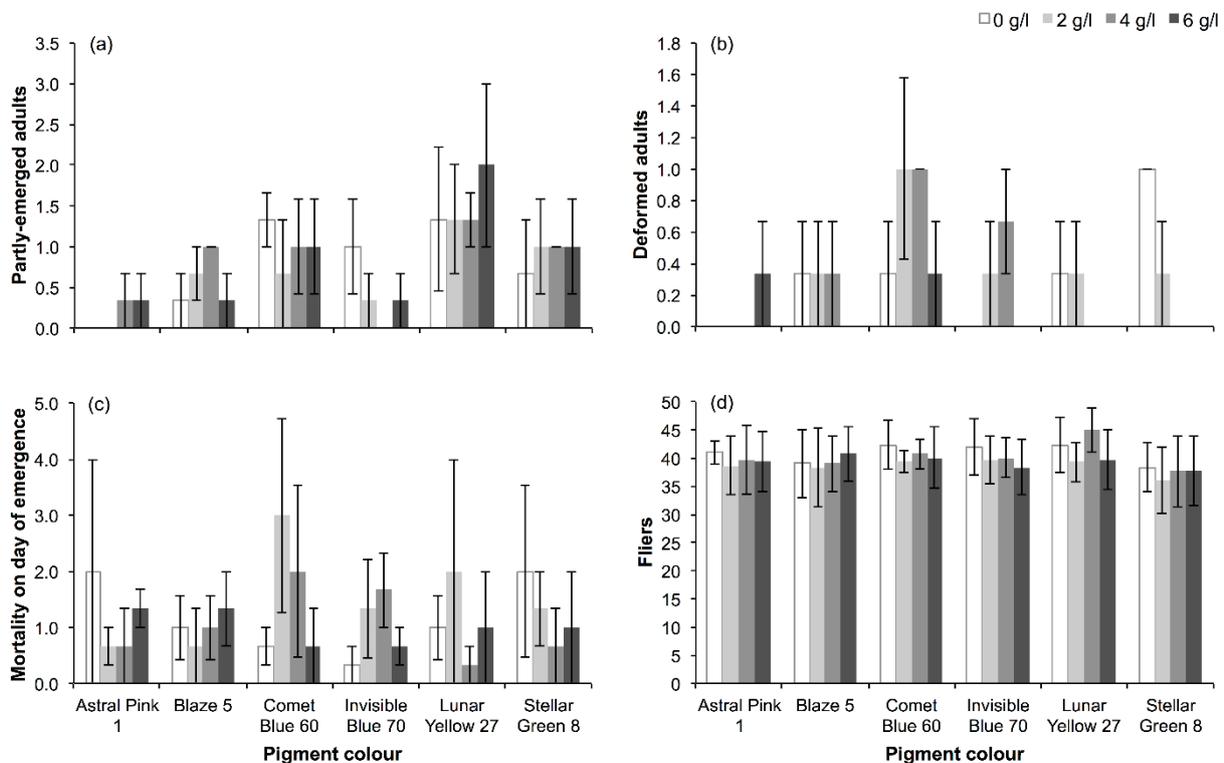


Figure 1. Effect (mean % \pm 1 S.E.) of six fluorescent pigment colours at concentrations of 0, 2, 4, and 6 g/l of *Bactrocera dorsalis* marked as pupae on partial emergence, deformed adults, mortality on day of eclosion, and flight ability. (a) Partly-emerged adults; (b) deformed adults; (c) mortality on day of eclosion; (d) fliers.

Objective B: Dispersal in relation to weather and host proximity

The complete data collected over two years indicate very different patterns of dispersal among females and males. Female recaptures in yellow bucket traps baited with BioLure three-part lure indicate a significant role for the presence of host plants at the release point on recaptures at varying distances from the release point (Figure 2). When released among host plants, female recaptures remained high around the release point, but female recaptures were detected further from the release point when they were released among non-host plants. This was the case even after only two days, with females released among non-host plants being recovered almost 3 km from their release point. Male recaptures in methyl eugenol traps were independent of where they were initially released (Figure 3). Some old males were detected approximately 3.5 km from their release point after only 2 days, and young males were detected even further away after only 7 days. No young

males were recaptured two days after release because sexually immature *B. dorsalis* do not respond to methyl eugenol. The placement of traps in host trees had no effect on female recaptures, but did affect male recaptures, with more males recaptured in traps placed in host trees. For both females and males, recaptures increased significantly as average minimum temperature increased. Rainfall also significantly affected recaptures, but they were positively affected by rainfall for females, and negatively affected by rainfall for males.

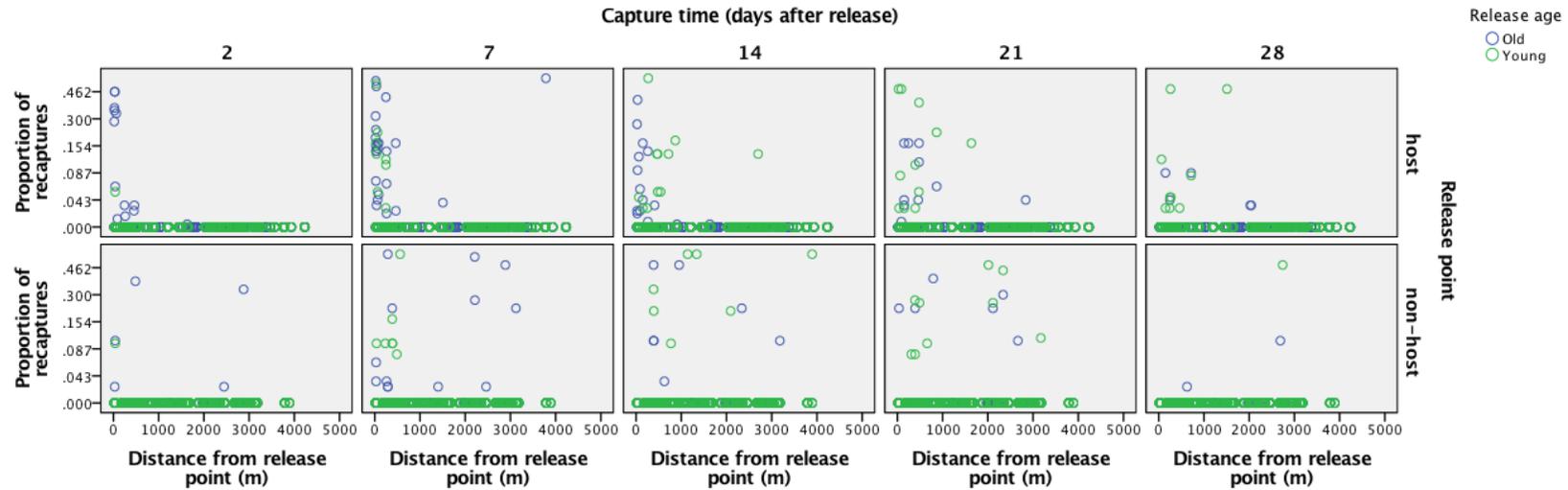


Figure 2. Effects of distance from the release point, release age (old: 11 days; young: 4 days) and location of release (among host or non-host plants) on recaptures of female *B. dorsalis* in BioLure traps. Data points represent the proportion of all females recaptured within a particular cohort.

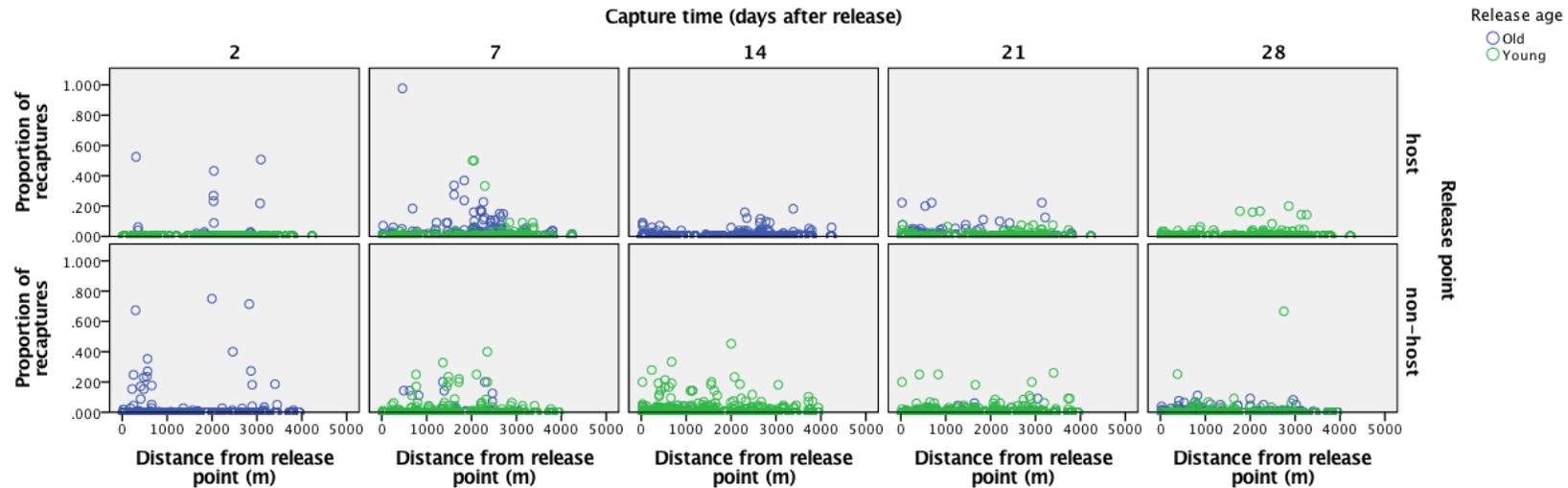


Figure 3. Effects of distance from the release point, release age (old: 11 days; young: 4 days) and location of release (among host or non-host plants) on recaptures of male *B. dorsalis* in methyl eugenol traps. Data points represent the proportion of all males recaptured within a particular cohort.

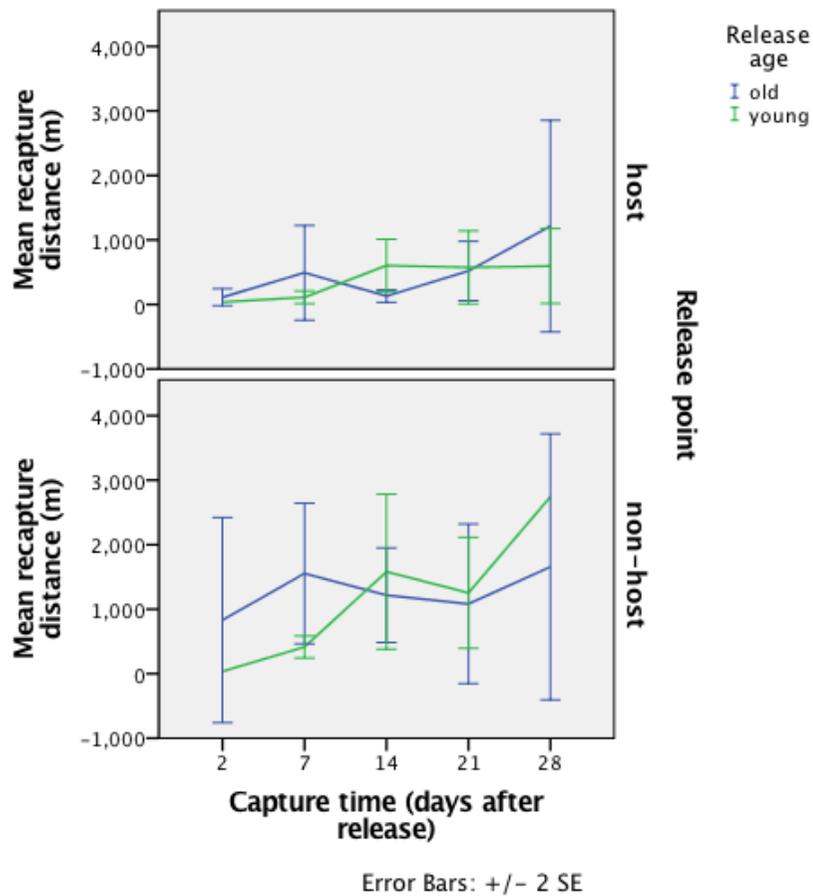


Figure 4. Mean of the average recapture distance of female *B. dorsalis* from release points surrounded by host (e.g., mango) or non-host (e.g., macadamia) trees. Females of two different ages (4 days = young; 11 days = old) were released simultaneously at each site. Females were recaptured in bucket traps baited with BioLure three part lure.

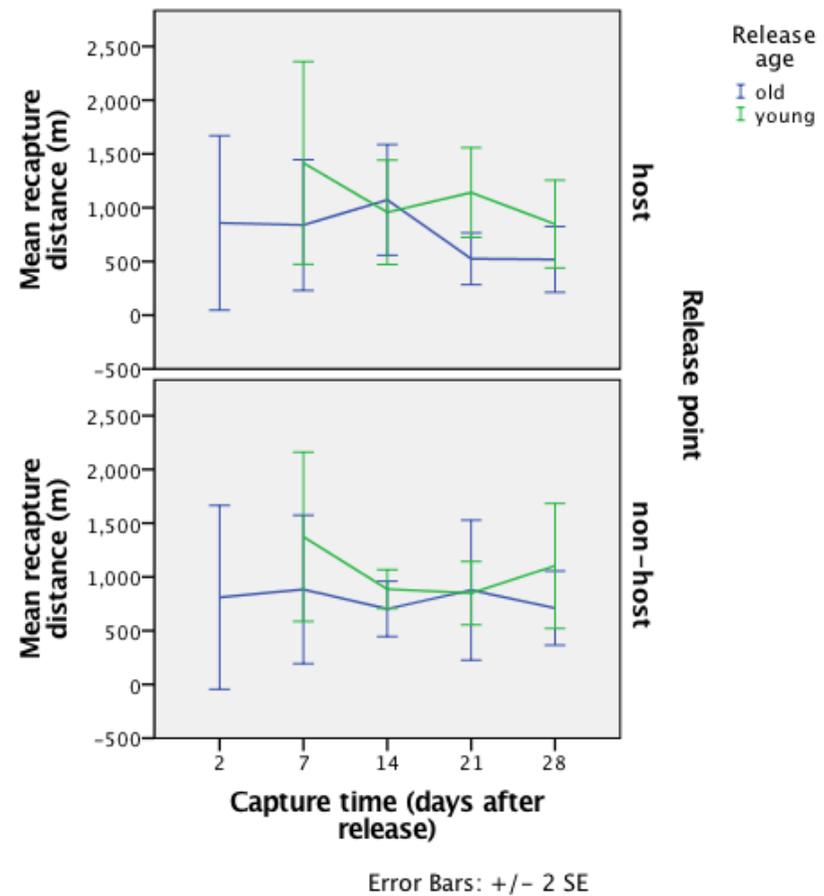


Figure 5. Mean of the average recapture distance of male *B. dorsalis* from release points surrounded by host (e.g., mango) or non-host (e.g., macadamia) trees. Males of two different ages (4 days = young; 11 days = old) were released simultaneously at each site. Males were recaptured in bucket traps baited with methyl eugenol.

The average of mean female recapture distance was affected by release among host or non-host trees (Figure 4). Females that were released among non-host trees very quickly dispersed away from the release point, whereas those released among host plants did not immediately leave the release point. Male *B. dorsalis* released at an older age did not travel as far before being recaptured in traps (Figure 5). This agrees with observations of this species in Hawaii and other *Bactrocera* species, where young flies exhibit dispersive behaviour (Weldon *et al.*, 2015). A week after release, the average recaptured old male had flown a little less than 1 km from the release point, whereas young males had flown approximately 1.5 km. Time after release was not a significant predictor for mean recapture distance of either females or males, partly because of high variability between cohorts but also because flies very rapidly dispersed.

Objective C: Effect of temperature on flight and mating

a) Spontaneous activity and mating in temperature-controlled arenas

Spontaneous activity exhibited by *B. dorsalis* varied with temperature but was also affected by fly age. Overall, walking did not occur or was very rare and of short duration at 12°C (Figure 6). At this temperature, 21-day-old females and males walked, but younger flies did not. Spontaneous flight did not occur until a temperature exceeding 20°C was reached. At high temperatures, 21-day-old females and males exhibited fewer bouts of flight, but they were of a longer duration than younger flies.

No male calling occurred at temperatures lower than 20°C or above 32°C. Male calling was greatest at 24 and 28°C. Copulation was not observed during the experiment, despite several attempts under various lighting conditions with flies of different ages.

b) Tethered flight on flight mills

Flight bouts were more frequent at low and high temperatures (Figure 7), but the distance covered (Table 1) and the duration of bouts (Figure 8) was less than at intermediate temperatures. Flight bouts were less frequent but covered greater distances and were of longer duration within the temperature range of 24-32°C. However, total flight duration was consistent across the range 20-36°C (Figure 9).

Within the test duration of one hour, the greatest distances were flown by 10 day-old flies, then 3 and 21 day-old flies (Table 1). The distance flown by 3 day-old flies did not differ between the sexes, but females flew further than males at 10 and 21 days of age. Average and maximum flight speed did not vary between the sexes, ages or test temperatures.

There was no significant difference in weight between female and male flies at 3 and 10 days of age, but female flies weighed more than males at 21 days of age. As body mass increased, mean flight distance also increased.

Objective D: Implications for the spacing of monitoring traps and release of sterile insects

To optimise spacing of traps to monitor dispersal of *B. dorsalis*, we developed a mathematical model on how interacting traps may increase recapture rates. An advection-diffusion model was used for this purpose. The model predicted that an array of five non-symmetrically arranged traps in close proximity so that their effective radius overlap would catch more flies than a single trap or even a small array of nine traps on a regular grid (Dufourd *et al.*, 2013).

A trap-insect model based on coupled partial differential equations was also developed (Anguelov *et al.*, 2017). The main predictions resulting from this model relevant to field monitoring of fruit flies were that (1) the error associated with estimates of abundance based on trap captures decreased as population size increased, and (2) emptying traps every five days reduced the error of abundance estimates in comparison with emptying traps every three days.

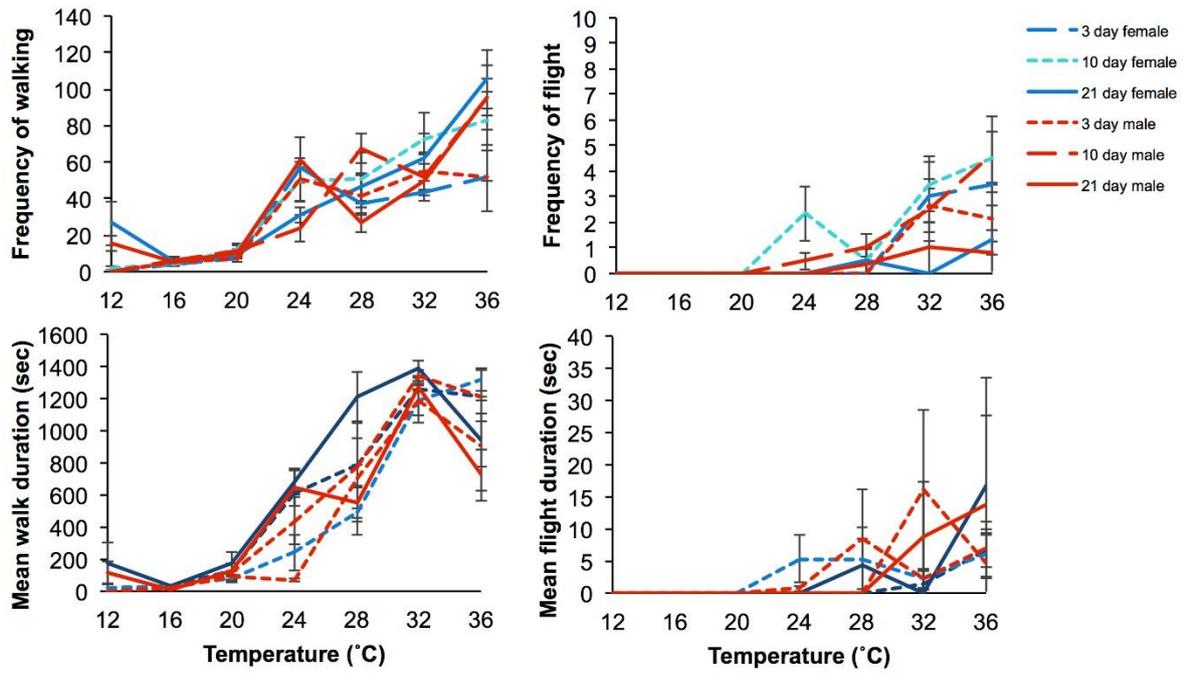


Figure 6. Frequency and duration of spontaneous walking and flight by female and male *B. dorsalis* of different ages. Error bars represent ± 1 s.e..

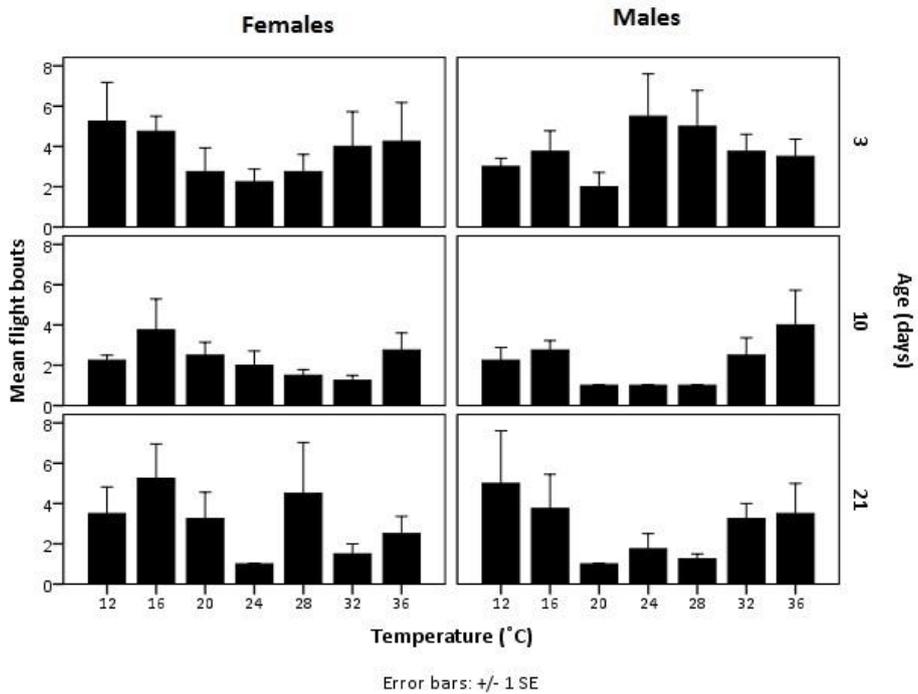


Figure 7. Number of flight bouts by *Bactrocera dorsalis* at over a range of constant temperatures.

Table 1. Mean (± 1 s.e.) distance flown over 1 hour by female and male *Bactrocera dorsalis* tethered to computerised flight mills and exposed to a range of constant temperatures. Flight was tested when flies were 3, 10 or 21 days of age.

Temperature °C	Females			Males		
	3	10	21	3	10	21
12	102.9 \pm 43.9	245.8 \pm 79.3	53.2 \pm 12.0	106.0 \pm 49.3	224.9 \pm 89.0	39.7 \pm 16.9
16	53.8 \pm 15.8	407.4 \pm 28.9	46.2 \pm 18.6	195.0 \pm 120.5	683.7 \pm 78.0	408.6 \pm 167.8
20	307.0 \pm 104.6	531.4 \pm 187.6	328.4 \pm 133.2	575.3 \pm 163.7	666.3 \pm 174.0	982.3 \pm 240.9
24	131.9 \pm 75.6	359.1 \pm 226.6	851.6 \pm 176.1	230.8 \pm 183.8	861.5 \pm 148.2	477.5 \pm 213.6
28	326.3 \pm 118.8	204.1 \pm 72.6	466.1 \pm 220.8	16.5 \pm 5.6	768.8 \pm 267.2	700.3 \pm 243.1
32	593.1 \pm 215.7	517.5 \pm 235.9	421.1 \pm 197.3	416.5 \pm 132.3	199.8 \pm 100.6	316.7 \pm 202.6
36	44.5 \pm 20.2	641.8 \pm 235.1	93.6 \pm 62.7	48.6 \pm 16.4	72.0 \pm 33.5	181.0 \pm 124.9

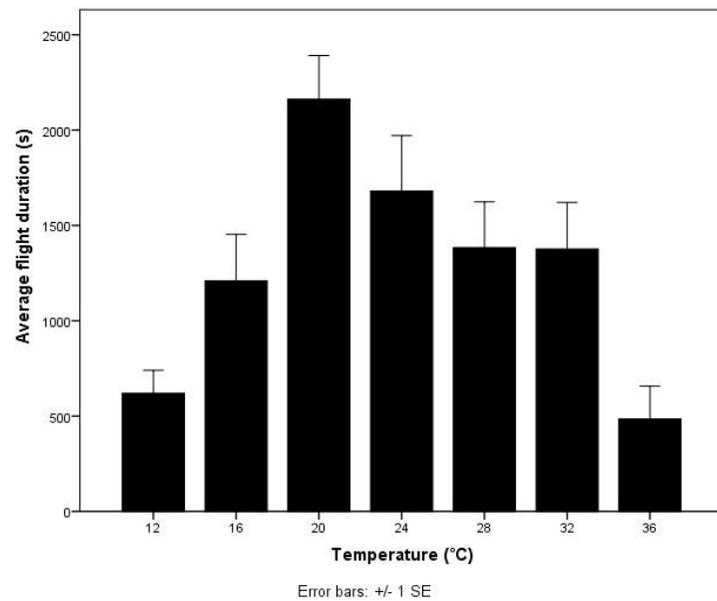


Figure 8. Mean flight bout duration (seconds) recorded for *Bactrocera dorsalis* when tethered to a computerised flight mill at a range of constant temperatures.

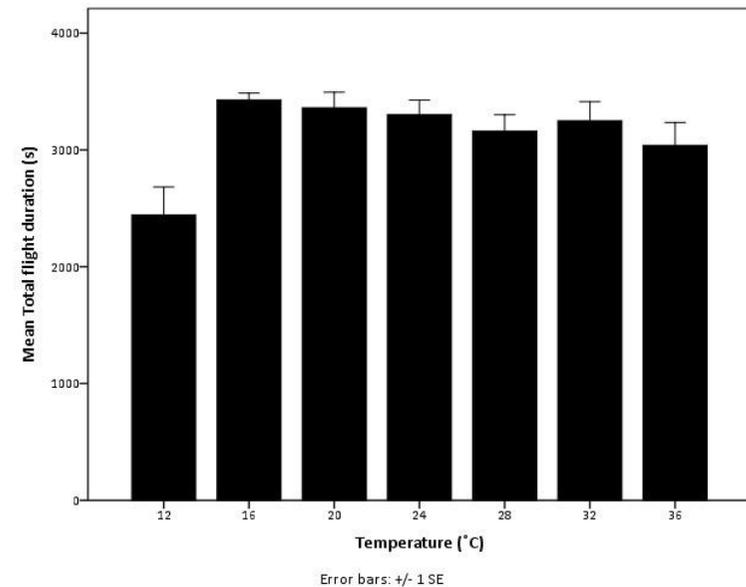


Figure 9. Mean total flight duration of *Bactrocera dorsalis* recorded for *Bactrocera dorsalis* when tethered to a computerised flight mill at a range of constant temperatures.

Complete dispersal data were only available from December 2016. For this reason, the use of these data to test mathematical models of dispersal and optimal spacing of sterile insect releases is ongoing.

Conclusion

Fly age, sex and the proximity of host plants influence the distance that *B. dorsalis* travels from a point of origin. Males, but particularly young males, quickly moved away from their site of release regardless of the presence of host trees at the release point. Even older males were caught over 3 km from the release point within 3 days of release, and by 7 days released males were caught at the furthest traps from the release point. Females also quickly moved away from the release point if released among non-host plants, but when released among host plants they did not move as far, even when host plants were not bearing fruit. These results clearly indicate that *B. dorsalis* is capable of moving large distances without human assistance. They also support observations from other species of *Bactrocera* where males disperse rapidly and over long distances at young ages. That female *B. dorsalis* released among hosts did not move as far when surrounded by host plants was not unexpected because it has been reported in Hawaii that *B. dorsalis* are often found roosting in fruit trees (McQuate & Vargas, 2007), but it raises questions regarding the cues that they use to select habitats. We propose two non-exclusive hypotheses that require further testing: (1) volatiles from host plant foliage arrest female movement, leading to reduced dispersal from their natal orchard, and (2) the local microclimate of preferred host plants is highly suitable for the fitness of female fruit flies, due to an abundance of food, water, and a canopy structure that provides shelter.

Observations of spontaneous activity and tethered flight in the laboratory provide a mechanistic basis for some of the observations from the field. The dispersal ability of younger male flies may result from more short flights when temperatures are suitable. Despite this, it is clear that female *B. dorsalis* are stronger fliers than males. It is important to note that tethered female flight in the laboratory was studied in the absence of any cues from host plants, so it will be valuable in future to use flight mill experiments to test the effect of exposure to volatiles produced by fruit and foliage of host and non-host plants to assess their effect on flight in *B. dorsalis*.

For both females and males, recaptures in the field increased significantly as average minimum temperature increased. Based on the results of the laboratory experiments, it is clear that voluntary flight by *B. dorsalis* is limited by temperature, with a lower threshold of approximately 20°C. At temperatures lower than this, flies are still capable of movement within a tree canopy, but will be less likely to respond to traps. In addition, at temperatures favourable for *B. dorsalis* flight, higher temperatures may be associated with higher release rates of lures and greater fly attraction, as reported recently for trimedlure and the Mediterranean fruit fly (Flores *et al.*, 2017). It was also interesting to note that rainfall affected female recaptures in BioLure traps and male recaptures in methyl eugenol traps differently. This raises the possibility that associated changes in humidity also affect the efficiency of each type of trap, perhaps by influencing the carrying capacity of air for volatilised lure components. This is another area of research that needs to be pursued to improve interpretation of fruit fly trap captures, but has received little attention to date.

The results that we have obtained unfortunately indicate that *B. dorsalis* can rapidly cover large distances under lowveld environmental conditions. This suggests that any accidental human introduction of *B. dorsalis* into regions currently unaffected but with a suitable range of temperatures for movement should be prevented. Area freedom should be protected by the placement of large, clear signs in multiple languages on roads approaching areas free of *B. dorsalis* to ask travellers to eat or dispose of fruit in roadside bins that are regularly emptied and their contents destroyed. Signage and disposal bins should be located far away from fruit production areas, but could be located close to fuel depots and truck stops where travellers frequently stop. The approaches into the Western Cape from the N1 and N7 are ideal for implementing this approach due to the arid regions that form a buffer between other production areas. Community awareness should also be prioritised for the N2 approach into the Western Cape due to the relatively milder climate, which although potentially unsuitable for longer-term establishment due to low winter temperatures (Pieterse *et al.*, 2017), may represent a pathway for summer invasion that could affect summer fruit production and high pest numbers going into the early citrus season. Information campaigns should also be implemented, comprising television,

radio and print media advertisements and news stories, to inform residents of affected and pest-free areas to not transport fruit into pest-free areas.

Future research

As noted above, there is a need for several studies to explain observed patterns of dispersal or response to traps in the field by *B. dorsalis*:

1. Determine the cues used by female *B. dorsalis* to select habitats. This could involve a combination of behavioural, chemical and ecological studies in the field, semi-natural conditions in field cages, and the laboratory. Methods such as the use of tethered flight described in this report could represent part of this work.
2. Establish the role of temperature and relative humidity on the efficiency of fruit fly lures. Results of this research, which would require both field and laboratory studies, will lead to improved estimates of fruit fly population size. Other research funded by CRI (Theron et al., 2017) has found a correlation between fruit infestation and relative abundance of adult male *B. dorsalis* caught in methyl eugenol traps, so improved understanding of this relationship represents an important component in calculating the risk of fruit infestation by fruit flies.

Technology transfer

a) Publications (popular, press releases, semi-scientific, scientific)

Anguelov R, Dufourd C, Dumont Y. (2017) Simulations and parameter estimation of a trap-insect model using a finite element approach. *Mathematics and Computers in Simulation* 133, 47-75.

Dufourd C, Weldon C, Anguelov R, Dumont Y. (2013) Parameter identification in population models for insects using trap data. *Biomath* 2, 1312061.

Makumbe LDM, Manrakhan A, Weldon CW. (2017) Optimisation of fluorescent pigment marking for *Bactrocera dorsalis* (Diptera: Tephritidae). *African Entomology* 25, 220-234.

Weldon CW. (2018) Colour run for fruit flies. *South African Fruit Journal* 17, 20-21.

Weldon CW, Schutze MK, Karsten M. (2014) Trapping to monitor tephritid movement: results, best practice and assessment of alternatives. Chapter 6 in: Shelly TE, Epsky N, Jang E, Reyes J, Vargas R (eds.), *Trapping Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications*. Springer Dordrecht, Heidelberg. pp. 175-217.

b) Presentations/papers delivered

Makumbe LDM, Manrakhan A, Weldon CW. (2015) Optimisation of fluorescent pigment marking for the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), under African climatic conditions. Presentation at XIX Entomological Society of Southern Africa Congress, Rhodes University, Grahamstown, 12-15 July 2015.

Makumbe LDM, Manrakhan A, Weldon CW. (2016) Fly on a hot tin roof: Optimal temperature for the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) to move. Presentation at 3rd International Symposium of the Tephritid Workers of Europe, Africa and the Middle East. Stellenbosch, South Africa. 11-14 April 2016.

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Makumbe LDM, Manrakhan A, Weldon CW. (2018) Effects of sex, age and morphological traits on tethered flight performance of the oriental fruit fly, *Bactrocera dorsalis*, at constant temperatures. Presentation at 10th International Symposium on Fruit Flies of Economic Importance, Tapachula, Mexico. 23-27 April 2018.

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

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

Parts of the report were adapted from two peer-reviewed publications:

Manrakhan A, Daneel J-H, Beck RB, Virgilio M, Meganck K & De Meyer M (2017a) Efficacy of trapping systems for monitoring Afrotropical fruit flies. *Journal of Applied Entomology* 141:825-840. doi:10.1111/jen.12373.

Manrakhan A, Daneel JH, Virgilio M & De Meyer M (2017b) Sensitivity of an enriched ginger oil based trapping system for *Ceratitidis* fruit fly pests (Diptera: Tephritidae). *Crop Protection* 99: 26-32. doi:<https://doi.org/10.1016/j.cropro.2017.05.002>.

Summary

The aim of the ERAfrica_NI-027 'FRUIT FLY' project was to develop effective and accurate detection methods for fruit fly pests in the Afrotropical region. This project assembled a team of researchers from South Africa, Côte D'Ivoire, Belgium and France to address different aspects of fruit fly detection systems. The project was funded by designated institutions in the partnering countries and was co-ordinated by Citrus Research International (CRI). Studies undertaken directly by CRI aimed at determining (1) efficacy of different trap and attractant combinations for fruit fly monitoring, (2) sensitivity of key *Ceratitidis* pests to an Enriched Ginger Oil (EGO) lure based trapping system and (3) efficacy of various trap types and attractant dispensers for monitoring of fruit fly pests. The efficacy of different trap and attractant combinations were determined over one year in natural areas and commercial citrus orchards in Limpopo and Mpumalanga provinces. In these studies, the three component Biolure was found to be the most effective attractant for monitoring females of key fruit fly pests on citrus in South Africa. A wide range of *Ceratitidis* species was found to be attracted to EGO lure based traps. The distance-dependent responses of laboratory-reared *C. capitata*, *C. rosa* and *C. cosyra* males to an EGO Pherolure baited trap was determined using mark-release-recapture methods. There were no significant differences in recapture rates of the three *Ceratitidis* species in the EGO baited trap. Most of the recaptures of all species occurred within a 50 m distance from the EGO baited trap. Efficacy of different trap

types and dispensers in trimedlure and methyl eugenol trapping systems were determined on natural *C. capitata* and *Bactrocera dorsalis* populations in commercial orchards. Both trap types and lure dispensers had significant effects on catches of *C. capitata* and *B. dorsalis* in trimedlure and methyl eugenol based trapping systems respectively.

Opsomming

Die doel van die ERAfrica_NI-027 'FRUIT FLY' projek was om effektiewe en akkurate opsporingsmetodes vir vrugtevliegplae in die Afro-tropiese area te ontwikkel. Hierdie projek het 'n span van navorsers uit Suid-Afrika, Ivoorkus, België en Frankryk laat vergader ten einde verskillende aspekte van vrugtevlieg opsporingsstelsels aan te spreek. Die projek is deur aangewese institute in die deelnemende lande befonds en is deur *Citrus Research International* (CRI) gekoördineer. Studies wat direk deur CRI onderneem is, het ten doel gehad om die volgende te bepaal: (1) effektiwiteit van verskillende lokval en lokmiddel kombinasies vir vrugtevliegmonitering, (2) sensitiwiteit van sleutel *Ceratitidis* plae vir *Enriched Ginger Oil* (EGO) lokaas-gebaseerde lokvalstelsel en (3) effektiwiteit van verskeie lokvaltipes en lokmiddelvrystellers vir monitering van vrugtevliegplae. Die effektiwiteit van verskillende lokval en lokmiddel kombinasies is oor een jaar in natuurlike areas en kommersiële sitrusboorde in Limpopo en Mpumalanga provinsies bepaal. In hierdie studies is gevind dat die drie-komponent Biolure die effektiwiefste lokmiddel vir die monitering van wyfies van sleutel vrugtevliegplae op sitrus in Suid-Afrika was. 'n Wye reeks *Ceratitidis* spesies is deur EGO lokaas-gebaseerde lokvalle aangetrek. Die afstand-afhanklike reaksies van laboratorium-geteelde *C. capitata*, *C. rosa* en *C. cosyra* mannetjies op 'n EGO Pherolure aas-lokval is bepaal deur gebruik te maak van merk-vrylaat-hervang metodes. Daar was geen betekenisvolle verskille in hervangkoers van die drie *Ceratitidis* spesies in die EGO aas-lokval nie. Die meeste van die hervang van al die spesies het binne 'n 50 m afstand vanaf die EGO aas-lokval plaasgevind. Effektiwiteit van verskillende lokvaltipes en -vrystellers in trimedlure en metiel eugenol lokvalstelsels, is op natuurlike *C. capitata* en *Bactrocera dorsalis* populasies in kommersiële boorde bepaal. Beide lokvaltipes en lokmiddelvrystellers het betekenisvolle effekte op vangste van onderskeidelik *C. capitata* en *B. dorsalis* in trimedlure en metiel eugenol-gebaseerde lokvalstelsels gehad.

Introduction

Fruit production in South Africa is affected by a number of fruit fly pest species. Pest fruit flies lay eggs in commercial fruit. The eggs hatch into larvae which feed on the pulp of fruit leading to fruit decay. Pest fruit flies cause direct yield losses and can also restrict fruit export due to quarantine restrictions imposed by importing countries to limit the spread of these pests. The use of insecticides for fruit fly control is limited due to low tolerance of residues in fruit. An integrated management approach which combines accurate detection and control should be followed for fruit fly pests in order to achieve timely and effective control and at the same time limit insecticide residues in fruit.

The aim of the ERAfrica fruit fly project was to develop effective and accurate detection methods for fruit fly pests in Africa and the Indian Ocean region. The project assembled a team of fruit fly experts from South Africa (Citrus Research International), Côte D'Ivoire (Centre National de Recherche Agronomique), Belgium (Royal Museum for Central Africa) and France (CIRAD- Reunion) to address different aspects of fruit fly detection systems. The project was funded by designated institutions in the partnering countries and was co-ordinated by Citrus Research International (CRI).

The objectives of the ERAfrica fruit fly project were to (1) determine the efficacy and sensitivity of different trapping systems for Afrotropical fruit fly pests, (2) analyse the population genetic structure of key indigenous and exotic fruit fly pests in the Afrotropical region for a better understanding of their geographic ranges within the region, (3) develop identification tools for Afrotropical fruit flies and (4) set up a standardised fruit fly detection system in Africa and the Indian Ocean region through the development of a regional fruit fly detection protocol.

Studies undertaken directly by CRI aimed at determining (A) efficacy of different trap and attractant combinations for fruit fly monitoring, (B) sensitivity of key *Ceratitidis* pests to Enriched Ginger Oil (EGO) lure based trapping system and (C) efficacy of various trap types and attractant dispensers for monitoring of fruit fly pests.

Materials and methods

Objective A. Efficacy of different trap and attractant combinations for fruit fly monitoring

Study sites

Studies were carried out in four selected commercial citrus orchards and two natural areas in Limpopo and Mpumalanga provinces. In each province, two commercial citrus orchards and one natural area were selected. The study was carried out for one year between September 2014 and October 2015

Attractants

Three categories of attractants were evaluated: male attractants targeting *Ceratitis* species, male attractants targeting predominantly Dacine species and food-based attractants. Male attractants targeting *Ceratitis* species were: (1) EGO Pherolure, which consisted of enriched ginger root oil and (2) trimedlure liquid. Male attractants targeting Dacine flies were: (1) methyl eugenol (ME) liquid, (2) zingerone or vanillylacetone and (3) cue lure liquid. EGO Pherolure was dispensed from a bulb like septum with each septum containing 2 ml of lure. Zingerone was available in a crystalline form, which was then melted at 40°C in a glass beaker on a hot plate. Two ml of liquid trimedlure, ME, cue lure and zingerone were then each applied separately onto a dental roll (3.6 cm in length and 1 cm diameter). The following food-based attractants were evaluated: (1) 3-component Biolure, which consisted of three separate dispensers, each containing ammonium acetate, trimethylamine and putrescine; (2) a combination of ammonium acetate and trimethylamine in separate dispensers referred to herein as AA+TMA; (3) a combination of ammonium acetate and putrescine in separate dispensers referred to herein as AA+P; (4) torula yeast available as solid pellets and used at the rate of 3 pellets per litre of water according to the product label and (5) Questlure containing protein hydrolysate and alpha-cypermethrin in a sponge fitted inside a plastic capsule.

Trap types

Each trap contained a single attractant. Different trap types were used for the three categories of attractants. EGO Pherolure and trimedlure were evaluated in bucket type Sensus traps. ME and zingerone were evaluated in bucket type Moroccan traps. All food-based attractants were evaluated in Chempac Bucket traps. Traps baited with torula yeast contained 300 ml of the attractant solution. The retention system of the torula yeast baited trap was by drowning. Traps with all other attractants contained a 3g Dichlorvos strip to kill any attracted flies.

Experimental layout

In each commercial orchard, the 10 attractant/trap combinations were allocated at random in each of three randomly selected rows within the orchard which represented three sampling units of each attractant trap combination. In natural areas, traps were placed along three transect lines. Distances between transect lines and between traps in a transect line were kept at approximately 30 m.

Traps were checked for fruit flies and emptied on a fortnightly basis until February 2015 and on a monthly basis thereafter. Specimens collected in traps were emptied in vials which were labelled by site, trap/attractant type, sampling unit and date. Traps were rotated within each row or line after every check. All attractants except for EGO Pherolure were replaced after 4 weeks. EGO Pherolure was replaced after six months as per product label. All Dichlorvos strips were replaced after 4 weeks. For traps baited with Torula Yeast, water was added when necessary to compensate for evaporation. Precautions were taken during lure preparation in the laboratory and during trap placement and servicing in the field to prevent cross contamination of attractant dispensers and traps. These precautions included the use of gloves during lure preparation and placement in traps and the use of a separate set of devices (brushes, forceps, wood sticks) for collection of specimens from different trap types

Fly identification

Flies collected were identified to species and sex.

Data analysis

Differences between attractant trap combinations were determined for *C. capitata*, *C. rosa* and *C. quilicii*, *C. cosyra* and *B. dorsalis*. Data on males and females of each species were averaged for each attractant/trap combination at each site and 4 weeks interval. Females of *C. rosa* and *C. quilicii* could not be distinguished morphologically, consequently data were pooled over the two species. The average catch per trap was then converted to average catch per trap per day. In order to deal with the non-normality of data, differences in average daily catches of male and female of the above species were evaluated with generalized linear mixed models (GLMMs).

Objective B. Sensitivity of key Ceratitis pests to EGO based trapping system

Mark-release-recapture studies were carried out to compare the sensitivity of males of three fruit fly pest species: *C. capitata*, *C. rosa* and *C. cosyra* to EGO. Results from the mark-release-recapture trials were used to estimate the detection sensitivity of an EGO Pherolure trapping system for *C. capitata*, *C. rosa* and *C. cosyra*, using probability models.

Study sites

Releases and recaptures were conducted in three commercial orchards in Mpumalanga province. Each orchard was about 2 ha in size. The orchards were on three different farms and were between 7 and 20 km apart. Two of the commercial orchards were *Citrus sinensis* (Valencia orange) orchards (Crocodile Valley Estates: S25° 28' 26.18" E31° 00' 49.53"; Sterkspruit: S25° 26' 11.77" E30° 53' 08.51") and one of the commercial orchards was a *Persea americana* (avocado) orchard (Oewersig: S25° 25' 53.58" E30° 48' 59.46"). The study was carried out between December 2015 and February 2016, which was outside of the citrus and avocado ripening season. No fruit fly control actions were carried out in the orchards at the time of the study. The mean maximum temperature and mean minimum temperature during the study were 31.74°C ± 0.29°C and 19.38°C ± 0.10°C, respectively. The wind direction was mainly from the east-south east or east-north east. On the day of the first release at all sites, the wind speed varied between 0.94 and 1.14 metres per second. On the day of the second release at all sites, the wind speed varied between 0.54 and 0.83 meters per second.

Insect materials and marking

Ceratitis capitata, *C. rosa* and *C. cosyra* used in the study were obtained from colonies maintained at CRI, Nelspruit, South Africa for over 200 generations. Colonies were refreshed with wild males reared from fruit every two years.

Each puparial batch of each fruit fly species was divided into four equal lots. Each lot was between 19.5 and 49 ml in volume. Each lot was dyed with a selected fluorescent pigment powder. Fluorescent pigments adhere to the puparia and are then retained in the ptilinum suture during adult emergence. These pigments can be readily identified on the adults under ultra-violet light. The same fluorescent pigment powder was used for all fruit fly species released at a particular distance in a particular site. Flies dyed with the two most contrasting colours were released closest and furthest from the trap. Four colours were used for four release distances: 25, 50, 100 and 200 m, at each site. A different set of four pigments was used between releases at a particular site in order to reduce effect of pigment colour on recapture of flies from a specific release distance and to have more contrasting colours between release distances. The fluorescent pigment powders used in the study were: Lunar Yellow 27, Astral Pink 1, Magenta 10, Stellar Green 8 and Blaze 5 of the Swada HMP series, as well as Invisible Blue 70 of the Swada T series (all Swada, Cheshire, UK). In the first release at all sites, flies of all species dyed with Astral Pink 1, Magenta 10, Stellar Green 8 and Lunar Yellow 27 were released at 25 m, 50 m, 100 m and 200 m respectively from the trap. In the second release at all sites, flies of all species dyed with Lunar Yellow 27, Stellar Green 8, Invisible Blue 70 and Blaze 5 were released at 25 m, 50 m, 100 m and 200 m respectively from the trap. Pigments were added directly onto the pupae at the rate of 2 g/L and were evenly distributed by gently swirling the containers with the pupae and pigments. Flies were dyed at about two days before adult emergence. Pupae and emerged flies were kept in aerated cages (54.5 cm x 39.0 cm x 31.0 cm) at a temperature of 27.7 ± 0.0°C until the release day. Emerged flies were provided with water

and a mixture of sugar and yeast extract at a rate of 3 parts sugar to 1-part enzymatic yeast hydrolysate. One day before release, males of each species and each fluorescent pigment were then separated from the females and placed in smaller aerated containers (diameter: 12.0 cm, height: 7 cm) for release. Males were provided with water and a mixture of sugar and enzymatic yeast hydrolysate until release. For all species, males of 9-14 days old were used in the releases.

EGO trapping system

The EGO Pherolure dispenser was used in the EGO trapping system evaluated. The EGO Pherolure is a polyethylene bulb that contains 2 ml of the EGO Pherolure liquid. The EGO Pherolure dispenser was placed inside a plastic basket and fitted in the middle of a white Delta trap (11 cm x 28 cm x 20 cm) over a sticky liner, which was placed on the floor of the trap. Traps were hung at 1.5 m above the ground inside the tree canopy on the south-eastern side of the trees.

Releases and recaptures

The experimental layout for the release-recapture trials consisted of a centrally placed EGO Pherolure baited white Delta trap in the middle of each orchard and release points that were at the four distances: 25, 50, 100 and 200 m from the trap along four cardinal directions (north, south, east and west).

Two releases of each species were carried out in each orchard with a month interval between the releases. One-month interval was deemed sufficient to ensure that flies in the first release would not continue to be captured in the second release.

Equal numbers of males of each species were released at each release point for a particular distance. For each release point in an orchard, 50 males of each species were collected for release such that there would be 200 males of each species released per distance and per site. However, during the second release at Sterkspruit only 25 males of *C. capitata* could be collected for release at each of the four points located at a distance of 200 m from the trap. Also, during the second release at Oewersig, only 35, 30 and 25 *C. capitata* males could be collected for release at each of the four points located at 25 m, 100 m and 200 m from the trap respectively. For the 50 m release distance at Oewersig during the second release, 50 males of *C. capitata* could be collected for release at each of the release points for that distance. Releases of marked flies at each release point were carried out by gently opening the aerated plastic container and allowing the flies to fly out. Those remaining at the bottom of the cage were gently tapped to initiate flight. Flies found dead in the release containers were brought back to the laboratory for identification and counting. The exact number of males of each species released at each release point was calculated as the number of males collected for release minus the number of dead males at the particular release point.

For each release, a new EGO Pherolure dispenser was used inside the white Delta trap. Traps were placed just before release in the middle of the orchard and were serviced the following day, one week, two weeks and a month after release at each site. During each trap service, the sticky liner was removed and replaced with a new liner. The liner was placed inside a plastic container such that the liner remained flat. The plastic containers with the liners were brought back to the laboratory for processing and analysis.

In the release-recapture trials, the sites and release times were considered as replicates. There were therefore six replicates.

Identification of recaptured flies

Each fly captured on the sticky liner was removed using a pair of weakly-sprung soft forceps. The fly was identified to species and sexed. Males of *Ceratitis capitata*, *C. rosa* and *C. cosyra* captured in the trap in a particular orchard on a particular date were placed in separate Petri dishes, which were then labelled according to species, site and recapture date. The thorax and abdomen of each male of each species were cut out from the insect body using a sharp micro-scalpel, so that only the head remained on the Petri dish. Each head was then dissected anteriorly so that the dye contained in the ptilium suture would be exposed. All forceps were cleaned with paper towel dipped in ethanol after the handling of each fly. The heads of the flies captured were then checked under UV light, peak wavelength of 365 nm, and through a stereo microscope at 20x

magnification to determine the presence of fluorescent pigment on the ptilinum. The presence of pigment and the pigment colour on each fly species was identified and recorded.

Data analysis

The overall effects of species, release distance and recapture period were tested using a log linear regression analysis. Data were first summarised as percentage recapture of males of each fly species for each site, distance released and recapture date owing to the different numbers of males released at the different distances, sites and release periods. Since there were no captures of marked flies one month after release, the data for this recapture date were omitted from statistical analysis. The percentage recapture data were then converted to count data by multiplying by 100. The count data were log (x+1) transformed. A log-linear model assuming a Poisson data distribution was used to determine the effect of release distance, species and recapture date on recaptures.

Objective C. Efficacy of various trap types and attractant dispensers for monitoring of fruit fly pests

The focus of this study was on trimedlure and ME based trapping systems. Effects of trap type and dispenser type and the combination thereof on efficacy of trimedlure and ME based trapping systems were determined on natural *C. capitata* and *B. dorsalis* populations in commercial orchards in a series of three experiments. Each experimental series ran for 4 consecutive weeks.

Study sites

Experiments were carried out in commercial farms in each of two provinces: Mpumalanga and Western Cape. Experiments on trimedlure trapping systems targeting *C. capitata* were carried out in both Mpumalanga and Western Cape provinces whilst those on ME trapping systems targeting *B. dorsalis* were carried out only in Mpumalanga province. In all experiments except the first experiment, three commercial orchards were used per province. In the first experiment, three commercial orchards were used for trimedlure and ME traps in Mpumalanga province while only one commercial orchard was used for trimedlure traps in Western Cape province. Experiments were carried out between November 2016 and April 2017. Experiments in Mpumalanga province were carried out in citrus farms whilst those in Western Cape province were carried out in deciduous fruit farms.

Experiment 1: Effects of trap types on efficacy of trimedlure and ME based trapping systems

Trimedlure was evaluated in two main trap types used in South Africa: yellow Delta trap and Sensus bucket trap. The yellow Delta trap contained a sticky insert to retain flies in the trap whilst the Sensus bucket trap contained a dichlorvos strip to kill attracted flies. Trimedlure was dispensed as 2 ml of liquid onto a dental roll. One dental roll containing trimedlure was placed in each trap. There was a blank trap for each trap type (trap with no trimedlure) in order to determine the effect of the trap itself on fly response. ME was evaluated in two main trap types used in South Africa: Lynfield trap and Chempac Bucket trap. A dichlorvos strip was placed inside each trap to kill attracted flies. ME was dispensed as 2 ml of liquid onto a dental roll. There was a blank trap for each trap type in order to determine the effect of the trap itself on fly response.

Experiments on traps with trimedlure and ME followed similar layouts in the three farms. In each farm there were six replicates of each treatment, with each replicate being set in a separate orchard block with at least 200 m between blocks. Within each block, one treatment was placed per tree with a distance interval of 50 m between treatments. The treatments were allocated using a randomized complete block design.

Experiment 2: Effects of dispenser types on efficacy of trimedlure and ME based trapping systems

Four trimedlure dispensers were evaluated in yellow Delta traps: (1) Trimedlure capsule containing 1g Trimedlure per dispenser, (2) Trimedlure liquid containing 2 ml of trimedlure liquid absorbed onto a dental roll, (3) Capilure Capsule containing 1.8 g active Trimedlure and (4) Capilure liquid containing 2 ml of liquid absorbed onto a dental roll.

Four commercial ME dispensers currently available in South Africa were evaluated in Chempac Bucket trap: (1) Chempac ME lure containing 4 g of ME per dispenser, (2) ME Pherolure containing 2 ml of ME per dispenser, (3) Invader Lure containing 15 g of ME per dispenser and (4) ME liquid containing 2 ml of ME liquid absorbed onto a dental roll

Experiments on trimedlure and ME dispensers followed similar layouts in the three farms. In each farm there were six replicates of each treatment, with each replicate being set in a separate orchard block with at least 200 m between blocks. Within each block, one treatment was placed per tree with a distance interval of 50 m between treatments. The treatments were allocated using a randomized complete block design.

Experiment 3: Efficacy of combinations of traps and dispensers in trimedlure and ME based trapping systems

Two commercially available combinations of traps and trimedlure dispensers were compared concurrently: Trimedlure capsule (containing 1g Trimedlure per dispenser) in Yellow Delta trap and Capilure capsule (containing 1.8 g active Trimedlure) in Sensus trap

Three commercially available combinations of traps and ME dispensers were compared concurrently: (1) Chempac ME lure (containing 4 g of ME per dispenser) contained in Chempac bucket trap, (2) ME Pherolure (2 ml of ME per dispenser) contained in Chempac bucket trap and (3) Invader Lure (15 g of ME per dispenser) contained in Lynfield trap.

Each yellow Delta traps contained a sticky insert to retain flies in the trap whilst each Sensus bucket, Chempac bucket and Lynfield trap contained a dichlorvos strip to kill attracted flies.

Experiments on trimedlure and ME trapping systems followed similar layouts in the three farms. In each farm there were six replicates of each treatment, with each replicate being set in a separate orchard block with at least 200 m between blocks. Within each block, one treatment was placed per tree with a distance interval of 50 m between treatments. The treatments were allocated using a randomized complete block design.

Trap servicing, fruit fly collection and identification

In each experiment, each trap was checked on a weekly basis for four weeks. The traps were rotated across a particular block every week. The dispensers and dichlorvos strips were not replaced with fresh ones for the four weeks trapping period. The sticky inserts were changed only when high numbers of flies were trapped. In cases where few fruit fly specimens were trapped, individual flies were simply removed from the inserts and the inserts were placed back inside the traps. Fruit flies collected each week were removed from the traps, placed in a vial and brought back to the laboratory for identification to species and sex.

Data analysis

Trapping data in each experiment were summarised as mean numbers of males of the target fruit fly pest species per treatment per day. Trap catches were log (x+1) transformed before analysis. Since there were no fruit fly catches in blank traps in the first experiment, the blank trapping data were filtered from the data sets. Some traps in the first and third experiments were damaged. These missing data were also filtered from the data set. Mixed models with week as a repeated factor were used to analyse the effects of trap type, dispenser type and traps and lure combinations in experiments 1, 2 and 3 respectively. The effect of site on fruit fly catches was also analysed for each experiment. Interactions between site and trap were determined only for experiment 2 which had balanced data sets (no missing data).

Results and discussion

Efficacy of different trap and attractant combinations for fruit fly monitoring

The relative responses of fruit fly pest species of phytosanitary concern on citrus: *C. capitata*, *C. rosa*, *C. quilicii*, and *B. dorsalis*, to 10 different attractant trap combinations are presented in Figures 1-2.

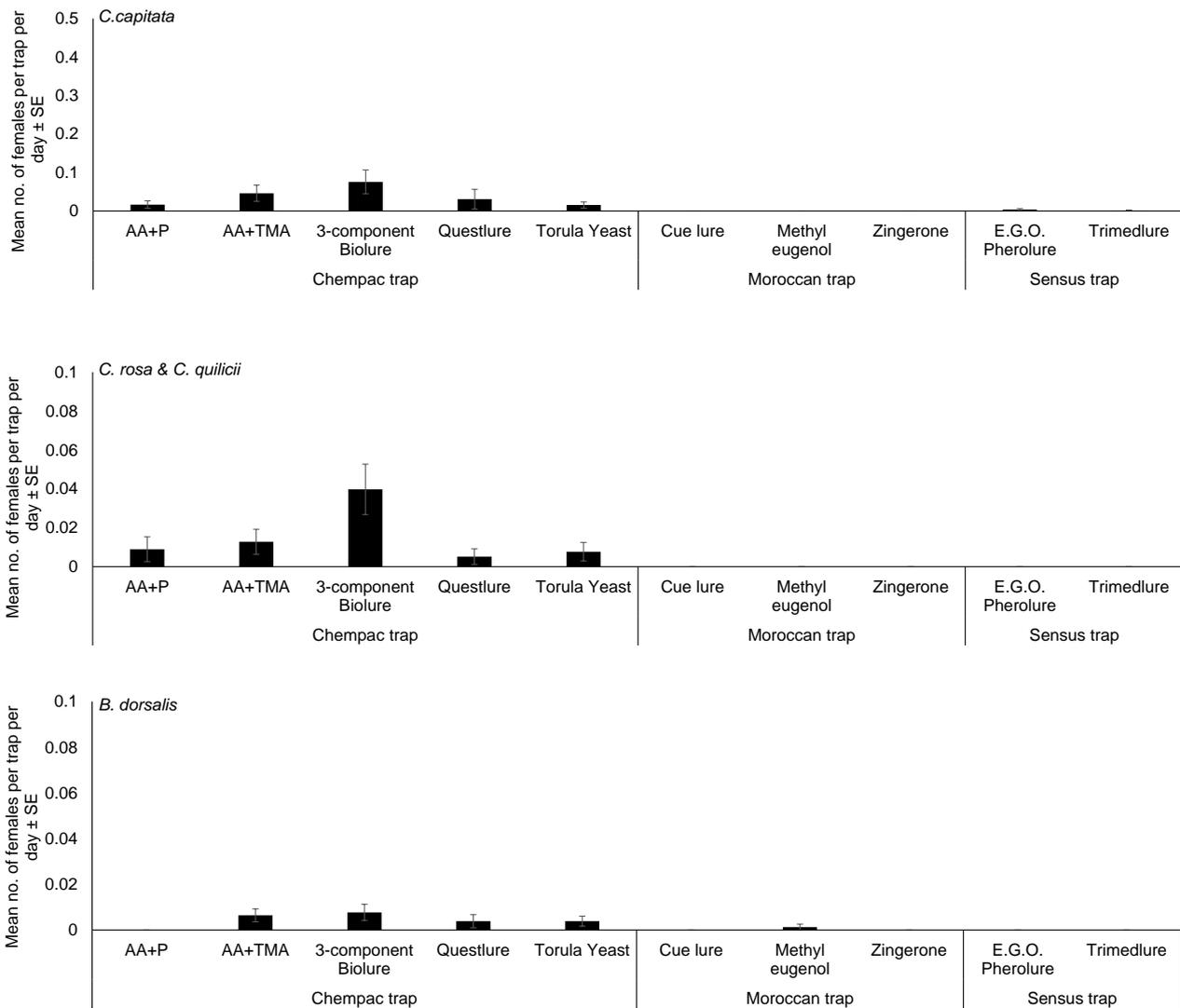


Figure 1. Relative responses of females of *C. capitata*, *C. rosa* (*C. rosa* and *C. quilicii*) and *B. dorsalis* to five food-based attractants, ammonium acetate + putrescine (AA+P), ammonium acetate + trimethylamine (AA+TMA), 3-component Biolure, Questlure and torula yeast, and five male attractants, cue lure, ME, zingerone, EGO Pherolure and trimedlure, baited in three trap types, Chempac Bucket trap, Moroccan trap and Sensus trap, in commercial citrus orchards and natural areas in Limpopo and Mpumalanga Provinces, South Africa, between September 2014 and October 2015

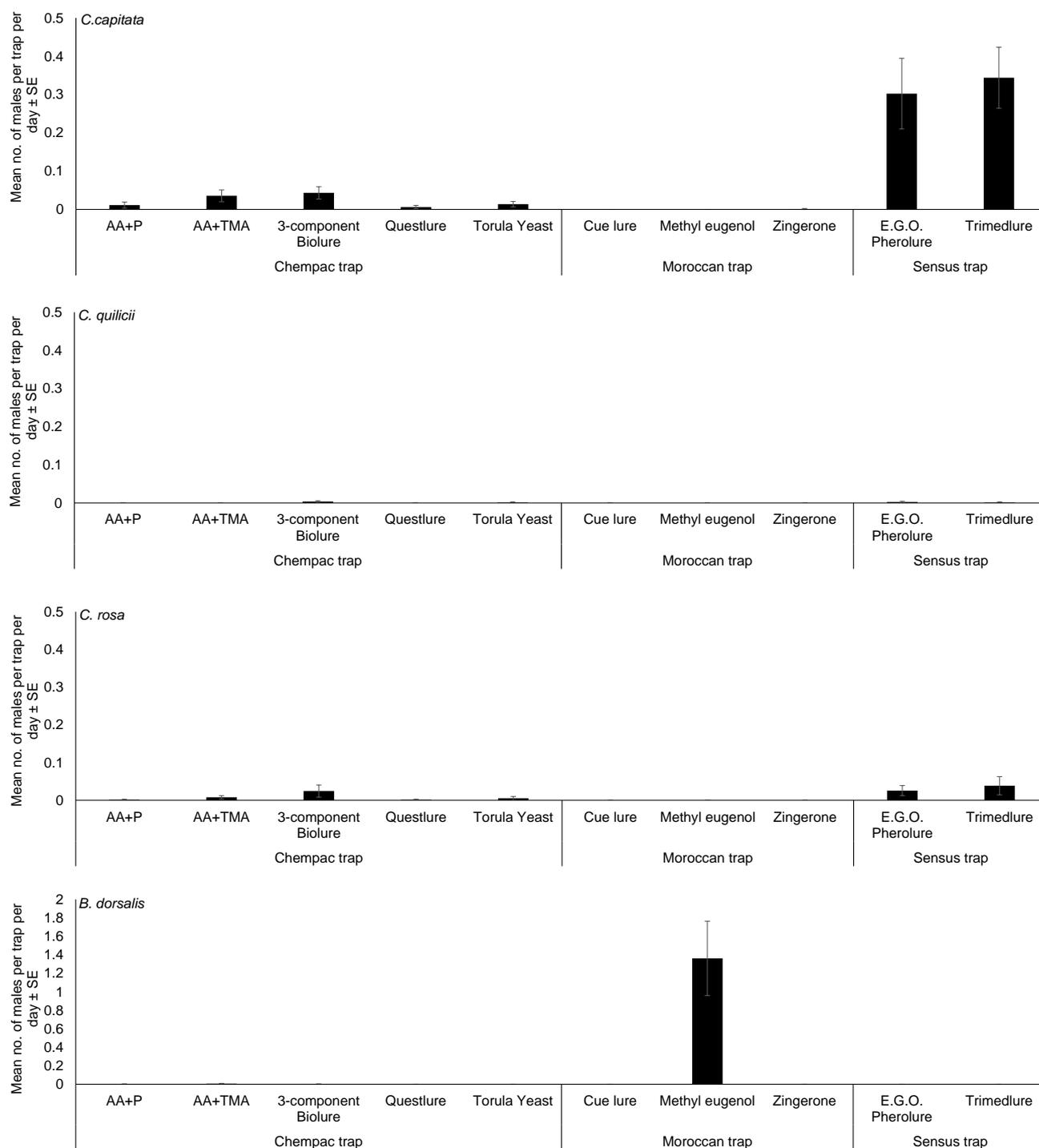


Figure 2. Relative responses of males of *C. capitata*, *C. rosa*, *C. quilicii* and *B. dorsalis* to five food-based attractants, ammonium acetate + putrescine (AA+P), ammonium acetate + trimethylamine (AA+TMA), 3-component Biolure, Questlure and torula yeast, and five male attractants, cue lure, ME, zingerone, EGO Pherolure and trimedlure baited in three trap types, Chempac Bucket trap, Moroccan trap and Sensus trap, in commercial citrus orchards and natural areas in Limpopo and Mpumalanga Provinces, South Africa, between September 2014 and October 2015. Species were plotted on different scales on the y-axes.

Significantly more *C. capitata* females were trapped in 3-component Biolure baited Chempac Bucket trap than in other trapping systems ($t=2.843$, $P=0.005$). Females of *C. rosa* occurred in higher abundances in natural areas than in commercial areas ($t=2.559$, $P=0.011$) and within natural areas, they were significantly more attracted to the 3-component Biolure baited Chempac Bucket trap than other trapping systems ($t=4.653$,

$P < 0.005$). *Bactrocera dorsalis* females responded significantly more to 3-component Biolure and the combination of ammonium acetate and trimethylamine than other attractant trap combinations (3-component Biolure: $t = 2.960$, $P = 0.003$, ammonium acetate and trimethylamine: $t = 2.467$, $P = 0.014$).

Results on responses of *Ceratitidis* species to food-based attractants concur with those of Grout et al. (2011), who also found, under South African conditions, higher responses of the same *Ceratitidis* species to 3-component Biolure and 2-component Biolure (ammonium acetate and trimethylamine) compared to other food attractants, including Questlure, which was also tested in this study. Various field studies conducted across different geographical regions also showed the superior performance of 3-component Biolure in attracting *C. capitata* compared to other more traditional food-based attractants, like liquid protein hydrolysates (Gazit et al., 1998; Heath et al., 1997; Katsoyannos et al., 1999; Miranda et al., 2001).

Here though, unlike previous studies (Ekesi et al., 2014; Leblanc et al., 2010; Mwatawala et al., 2006), we found the 3-component Biolure to be among the most effective food-based attractants for monitoring *B. dorsalis* females. In some earlier trapping studies of *B. dorsalis* in Africa (Tanzania), Mwatawala et al. (2006) recorded higher numbers of *B. dorsalis* in traps containing liquid protein bait (bait unspecified) than in traps containing 3-component Biolure. Leblanc et al. (2010) and Ekesi et al. (2014) found higher catches of *B. dorsalis* females in torula yeast traps than in 3-component Biolure traps. In the latter two studies, the 3-component Biolure traps had water as a retention system, whilst in this study an insecticidal strip was used to retain flies that entered the 3-component Biolure traps. It is likely that the differences in retention systems used in these previous studies and this study led to the contrasting results. In a study on McPhail type traps baited with synthetic food-based attractants, Katsoyannos et al. (1999) inferred the possibility of flies escaping from McPhail type traps, especially when no insecticidal strips are used. Practically, though, the 3-component Biolure used in dry traps would be a better option for monitoring of *B. dorsalis* females. There are practical problems in the use of liquid food-based attractants, which are cumbersome to carry to the field and difficult to handle during trap placement and servicing. Putrefaction of liquid protein baits inside traps is problematic as it leads to decomposition of the fruit flies captured, rendering identification impossible.

The male lure based trapping systems using either EGO Pherolure or trimedlure were more effective than the other trapping systems for *C. capitata* males (EGO Pherolure: $t = 6.304$, $P < 0.001$; trimedlure: $t = 6.866$, $P < 0.001$). However, the effect of these lures for *C. capitata* were significantly lower in the natural sites (EGO Pherolure and natural: $t = -3.161$, $P = 0.002$; trimedlure and natural: $t = -3.383$, $P = 0.001$). Males of *C. rosa* and *C. quilicii* showed different responses to the different trapping systems evaluated depending on site type (Fig. 3). In natural sites, males of *C. rosa* had a significantly higher attraction to Sensus traps baited with either EGO Pherolure or trimedlure and 3-component Biolure baited Chempac Bucket traps (EGO Pherolure and natural: $t = 2.322$, $P = 0.021$; trimedlure and natural: $t = 3.831$, $P < 0.001$; 3-component Biolure and natural: $t = 2.388$, $P = 0.017$). Males of *C. quilicii* responded significantly more to the 3-component Biolure baited Chempac Bucket trap than to other trapping systems (3-component Biolure: $t = 2.575$, $P = 0.010$). The ME baited Moroccan trap was the most effective trapping system for *B. dorsalis* males (ME: $t = 2.008$, $P = 0.045$). The effect of this trapping system was significantly higher in natural sites (ME and natural: $t = 8.292$, $P < 0.001$).

EGO Pherolure was found to be a promising attractant for *Ceratitidis* species. In this study, we also found that *C. capitata* males responded equally well to EGO Pherolure and trimedlure. Enriched ginger root oil in the EGO Pherolure contains α -copaene (Shelly & Pahio, 2002), which was found to be attractive to *C. capitata* (Flath et al., 1994). Mwatawala et al. (2013) also found that *C. capitata* responded equally well to trimedlure and EGO Pherolure in citrus orchards in Morogoro, Tanzania. In a later study conducted in other host environments (mango, peach and others), Mwatawala et al. (2015) found that *C. capitata* males were significantly more attracted to EGO Pherolure than to trimedlure. In that study, EGO Pherolure and trimedlure were replaced on a weekly basis unlike in our study, where trimedlure was changed after 4 weeks and EGO Pherolure was only replaced after 6 months (as per product label). Shelly (2013) found changes in the relative responses of *C. capitata* to EGO Pherolure and trimedlure as the lures aged in the field. Fresh trimedlure and fresh EGO Pherolure were equally attractive to *C. capitata* males, but as these lures aged, trimedlure was more attractive than EGO Pherolure (Shelly, 2013). Here we also found time-dependent changes in the relative responses of *C. capitata* to the two male lures within commercial citrus areas, with trimedlure being more attractive at certain times of the year and EGO Pherolure being attractive at other times of the year. It is likely

that the aging of EGO Pherolure might have compromised its efficacy on *C. capitata* males in our study and evened out the relative responses of *C. capitata* to EGO Pherolure and trimedlure.

For both *C. rosa* and *C. quilicii*, we found no difference in their responses to EGO Pherolure and trimedlure. This is in stark contrast to findings by Mwatawala *et al.* (2013; 2015). In the latter studies, EGO Pherolure was a more effective lure than trimedlure for *C. rosa* (type unspecified). As discussed above, the differences in the results between the previous studies and this study might have been as a result of differences in experimental methods in particular with regards to lure replacement and hence lure aging. Moreover, the population densities of *C. rosa* and *C. quilicii* were low in our study (below 1 fly per trap per day throughout the year), and this might have concealed differences that might exist between the lures.

Sensitivity of key *Ceratitis* pests to EGO based trapping system

The mean overall recapture rates (all releases combined) with the EGO baited trap for *C. capitata*, *C. rosa* and *C. cosyra* were 5.70% ± 0.81%, 8.51% ± 1.30% and 9.49% ± 1.36%, respectively. Despite overall numerical differences in recapture rates of the three species in an EGO baited trap, the differences between the species were not statistically significant (Table 1 and Fig. 3). Males of the three *Ceratitis* species released at a distance of 200 m from the EGO baited trap could be captured within one week. For all species, however, male responses to EGO were significantly higher when they were within 50 m from the male lure than when they were further away (Table 1 and Fig. 3). The distance dependent responses of *Ceratitis* males to EGO did not follow an exponential relationship. For all species, there were numerically higher recaptures of flies when released at 50 m compared to 25 m from the EGO trap, although recaptures of flies when released from these two distances were not statistically significant ($\chi^2=0.71$, $P=0.40$). Recapture rates of all species were significantly higher on the day after release than a week or 2 weeks post release (One week after release: $\chi^2=12.66$, $P=0.00$; Two weeks after release: $\chi^2=12.55$, $P=0.00$) (Table 1 and Fig. 4). There were no recaptures of any of the released flies a month after their release (Fig. 4).

Table 1. Log-linear regression results showing effects of species, release distance and recapture times on recaptures of *C. capitata*, *C. rosa* and *C. cosyra* in an EGO Pherolure baited white Delta trap. The data followed a Poisson distribution (Overdispersion test: $P= 0.84$)

Regression tests	Regression parameters	d.f	χ^2	$P > \chi^2$
Statistic	-2 Log(Likelihood)	7, 208	96.89	<0.0001
	Score	7, 208	85.13	<0.0001
	Wald	7, 208	49.40	<0.0001
Source	Species	2, 208	0.24	0.89
	Release distance	3, 208	43.22	<0.0001
	Recapture date	2, 208	53.42	<0.0001

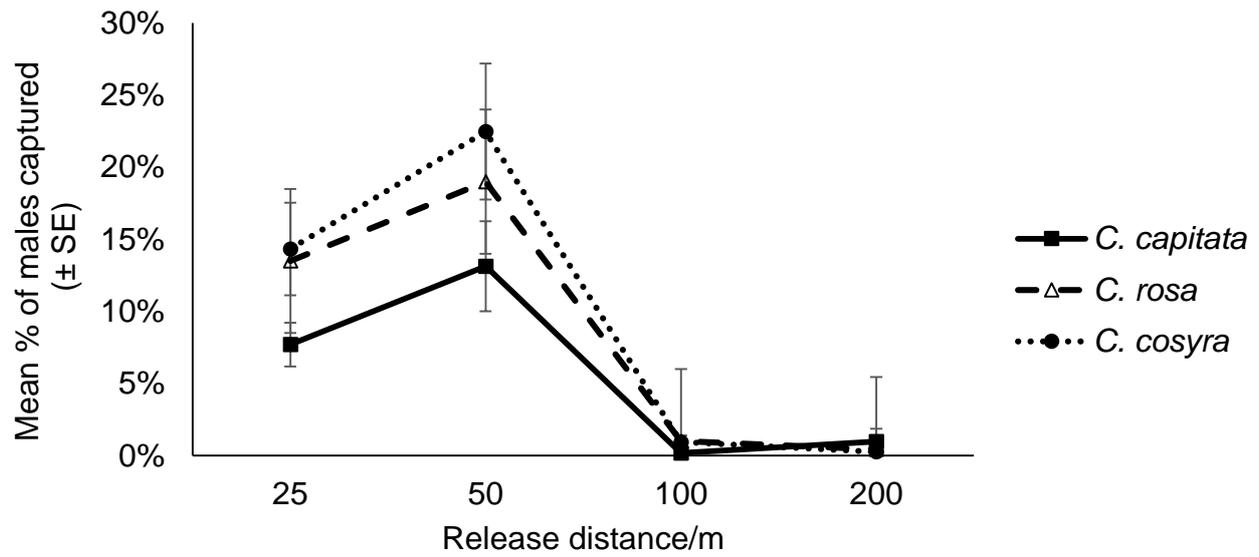


Figure 3. Mean percentage of males of *C. capitata*, *C. rosa* and *C. cosyra* captured when released at four distances: 25 m, 50 m, 100 m and 200 m from a centrally placed EGO baited white Delta trap in commercial orchards in Mpumalanga, South Africa. Recapture data were pooled from all trap services (recapture dates and sites).

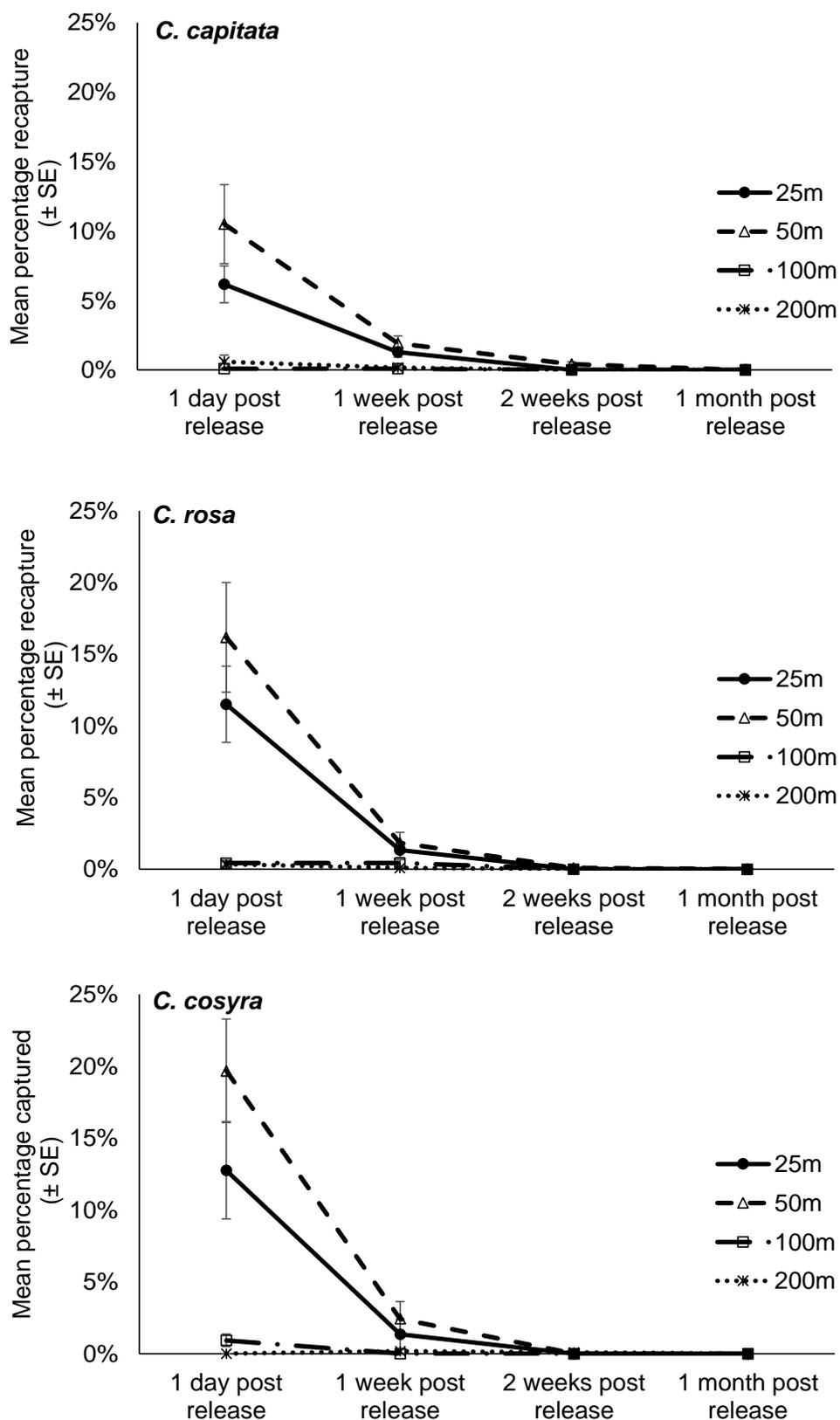


Figure 4. Captures of males of *C. capitata*, *C. rosa* and *C. cosyra* across time when released at four distances: 25 m, 50 m, 100 m and 200 m from a centrally placed EGO baited white Delta trap in commercial orchards in Mpumalanga, South Africa.

Ceratitis capitata, *C. rosa* and *C. cosyra* were found to be equally attracted to the male lure EGO in this study. This is in contrast to responses of these congeneric species to the existing male lure trimedlure to which *C. cosyra* does not respond and *C. capitata* has a higher response than *C. rosa* (Grout et al., 2011). The release-

recapture results of this study support the findings of catches of wild *C. cosyra* and *C. rosa* in EGO baited traps in both Tanzania and South Africa (Manrakhan et al., 2017; Mwatawala et al., 2015; Mwatawala et al., 2013). Our results here also support the conclusion of other studies that EGO targets a wider taxonomic spectrum than trimedlure (Mwatawala et al., 2015; Mwatawala et al., 2013). Trimedlure, unlike EGO containing α -copaene, is an anthropogenic male lure (Tan et al., 2014). Trimedlure was developed mainly as an attractant for *C. capitata*, and its development followed the screening of a series of esters of carboxylic acid (Cunningham, 1989). Exposure of *C. capitata* males to trimedlure was found to confer a mating advantage (Shelly, 1999) to these males, possibly through increases in calling (Shelly et al., 1996) although the mechanism behind this has not been fully elucidated. Alpha-copaene is a component of a number of plants and has been extracted from leaves of various citrus species (Nishida et al., 2000). Alpha-copaene, similar to trimedlure, has been shown to confer a mating advantage to *C. capitata*, possibly through elevated calling levels (Shelly, 2001). Ginger root oil, containing α -copaene, was also found to confer a mating advantage to *C. rosa* (Quilici et al., 2013). The similar levels of responses of *C. capitata*, *C. rosa* and *C. cosyra* to EGO could therefore result from similar advantages gained in all these species with regards to their mating behaviour.

When comparing our study with a similar mark-release-recapture study by Cunningham and Couey (1986) on *C. capitata* in a trimedlure baited trap in a *Macadamia* sp. F. Muell. orchard, we found that the overall recapture rate of *C. capitata* in the EGO baited trap (5.7%) was much lower than the overall recapture rate of *C. capitata* in the trimedlure baited trap (an estimated 28.3%). The recapture rate of *C. capitata* in the EGO baited trap in this study was, however, higher than recapture rates of *C. capitata* in trimedlure baited traps in similar studies by Lance and Gates (1994) and Shelly et al. (2014) in residential areas (recaptures ranging between below 1% and 3%). Lance and Gates (1994) also used sterile *C. capitata* males which could have different responses to male lures compared to non-sterile laboratory reared flies and wild flies (Wong et al., 1982). In this study, we used uniform orchard environments and non-sterile flies. Recapture rates of either wild or sterile *C. capitata* males in EGO baited traps in heterogeneous environments could possibly have been different to recapture rates obtained in this study.

Since we did not directly compare responses of *C. capitata* to EGO and trimedlure, it is difficult to suggest which would be a more effective attractant for *C. capitata*. In a recent study using fresh EGO in a similar dispenser as used in this study, Mwatawala et al. (2015) found that *C. capitata* had a higher response to EGO than to trimedlure. However, results from other studies using the same EGO dispenser showed that when EGO and trimedlure were evaluated over a longer period (with aging of lures factored in), similar levels of catches of *C. capitata* were obtained in EGO and trimedlure baited traps (Manrakhan et al., 2017; Mwatawala et al., 2013). Shelly's (2013) results on the effect of lure age on responses of *C. capitata* males to EGO and trimedlure showed that as the lures aged, *C. capitata* males responded more to trimedlure traps than to EGO traps. Lengthening the field efficacy of EGO would greatly increase its effectiveness for *C. capitata*.

The results of this study indicated that *C. capitata*, *C. rosa* and *C. cosyra* effectively responded to the EGO lure from a distance of 50 m, although responses of the three fruit fly species could still be recorded when they were 200 m from an EGO trap. The EGO lure sampling range falls within the sampling range of trimedlure for *C. capitata* found in other studies (with most recaptures of *C. capitata* occurring between 46 m and 60 m from a trimedlure baited trap) (Cunningham & Couey, 1986; Lance & Gates, 1994; Shelly et al., 2014).

An EGO lure based trapping system could serve as an alternate attractant for area-wide detection programmes targeting *Ceratitis* pests. Thresholds of catches of different *Ceratitis* pest species should be developed for this trapping system as a guidance for control actions in areas of low pest prevalence.

Efficacy of various trap types and attractant dispensers for monitoring of fruit fly pests

There was a significant effect of trap type on catches on *C. capitata* for traps baited with trimedlure ($F_{1,39}=17.71$, $p=0.00$). The yellow Delta trap was generally more effective than the Sensus trap (Fig. 5). There was a significant difference in catches between sites (across provinces), with higher catches of *C. capitata* in two of the farms in Mpumalanga province ($F_{3,39}=19.67$, $p<0.0001$). Trap type was also previously found to have a significant effect on catches of *C. capitata* in trimedlure based trapping systems. The Jackson trap (which is a type of Delta trap) was found to be more effective than Steiner and bucket traps when baited with trimedlure for monitoring of *C. capitata* (Uchida et al., 1996). Katsoyannos (1994) compared different trap types baited

with trimedlure and found the International Pheromone Mc Phail trap to be more effective than Delta trap in capturing *C. capitata* males.

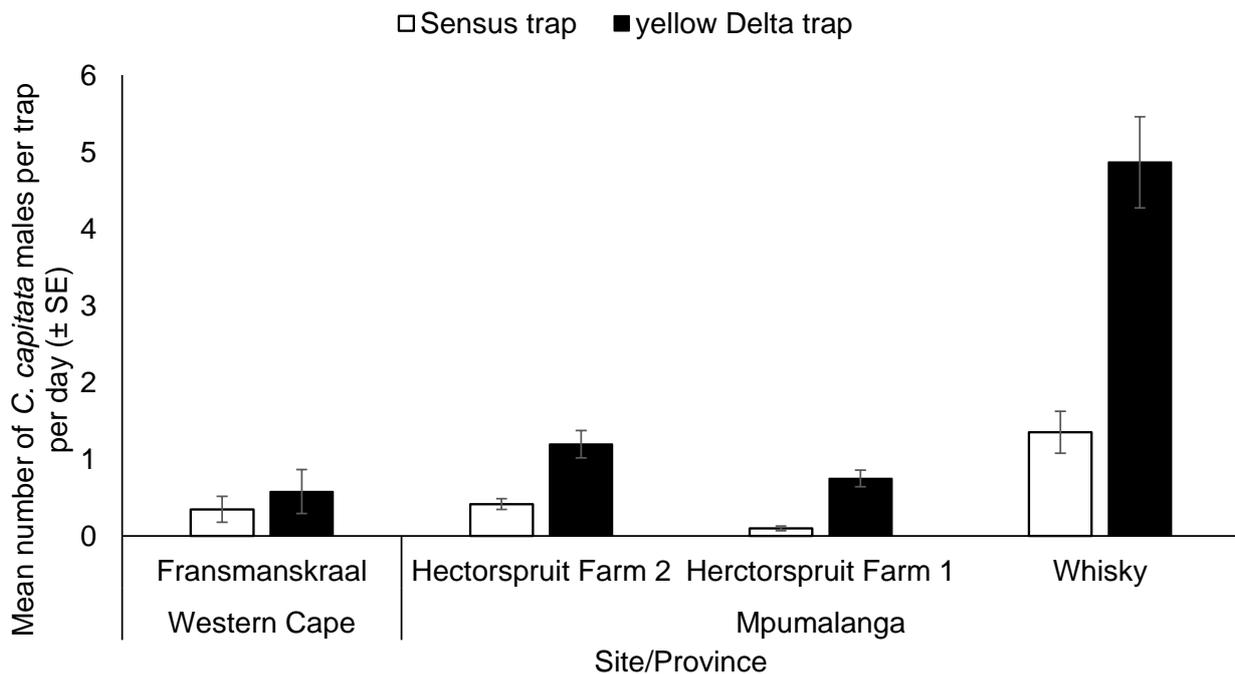


Figure 5. Captures of *C. capitata* males in Sensus trap and yellow Delta trap baited with trimedlure liquid (2 ml) in four study sites in Western Cape province and Mpumalanga province in South Africa.

Although there were higher catches of *B. dorsalis* in ME baited Lynfield traps compared to ME baited Chempac bucket trap in two of the sites, differences between the two traps were not statistically significant ($F_{1,32}=0.67$, $p=0.42$) (Fig. 6). Catches of *B. dorsalis* were however influenced by site ($F_{3,32}=3.98$, $p=0.03$). Vargas et al. (2009) compared the Jackson trap and bucket trap both baited with the same ME dispenser and found no differences in catches of *B. dorsalis* between the traps. In ME trapping systems, trap type may not have an important influence on catches of *B. dorsalis* as is the case in trimedlure based trapping systems for *C. capitata*.

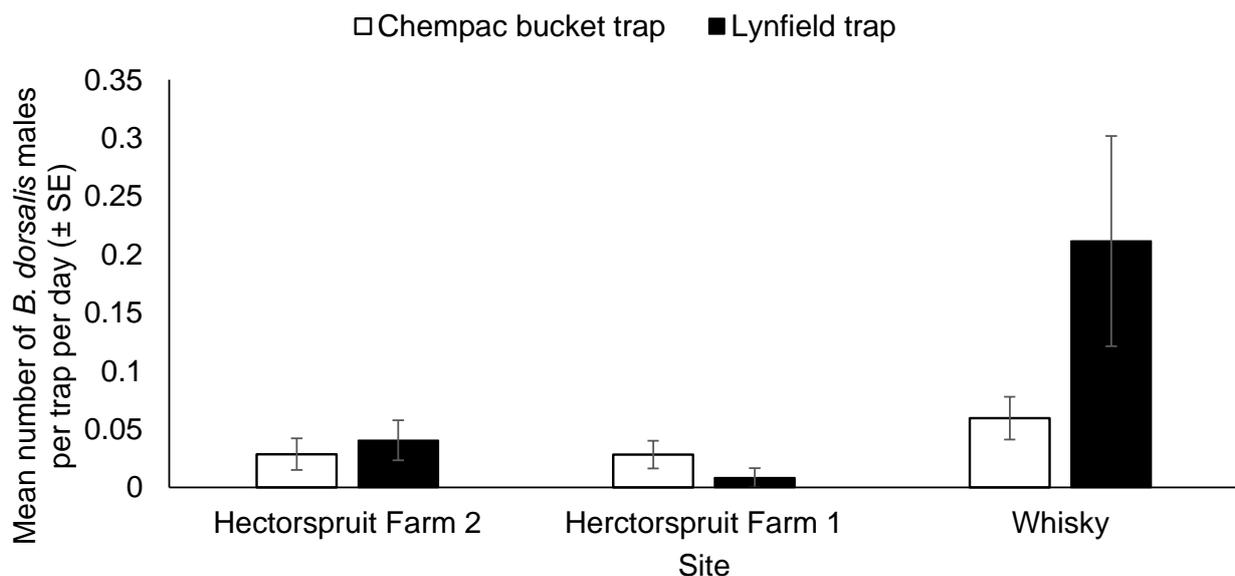


Figure 6. Captures of *B. dorsalis* males in Chempac bucket and Lynfield traps baited with ME liquid (2 ml) in three study sites in Mpumalanga province in South Africa.

The type of dispenser in a trimedlure based trapping system had a significant effect on catches of *C. capitata* males ($F_{3,120}=5.660$, $P=0.001$). There was also a significant effect of site on catches of *C. capitata* ($F_{5,120}=21.792$, $P<0.0001$). There were higher catches of *C. capitata* males in sites in Mpumalanga province compared to sites in Western Cape province at the time of the study. There was however no significant interaction between site and trap on *C. capitata* captures ($F_{15,120}=0.323$, $P=0.992$). In all sites, higher catches of *C. capitata* were recorded in traps baited with liquid trimedlure on a dental roll (Fig. 7). Differences in catches of *C. capitata* between trimedlure dispensers could be a function of trimedlure release rates. Leonhardt et al. (1984) found higher initial release rates of trimedlure when exposed as liquid on a dental roll compared to controlled release dispensers of trimedlure. The authors however found that the controlled release dispensers had a higher longevity than liquid trimedlure on a dental roll (Leonhardt et al. 1984).

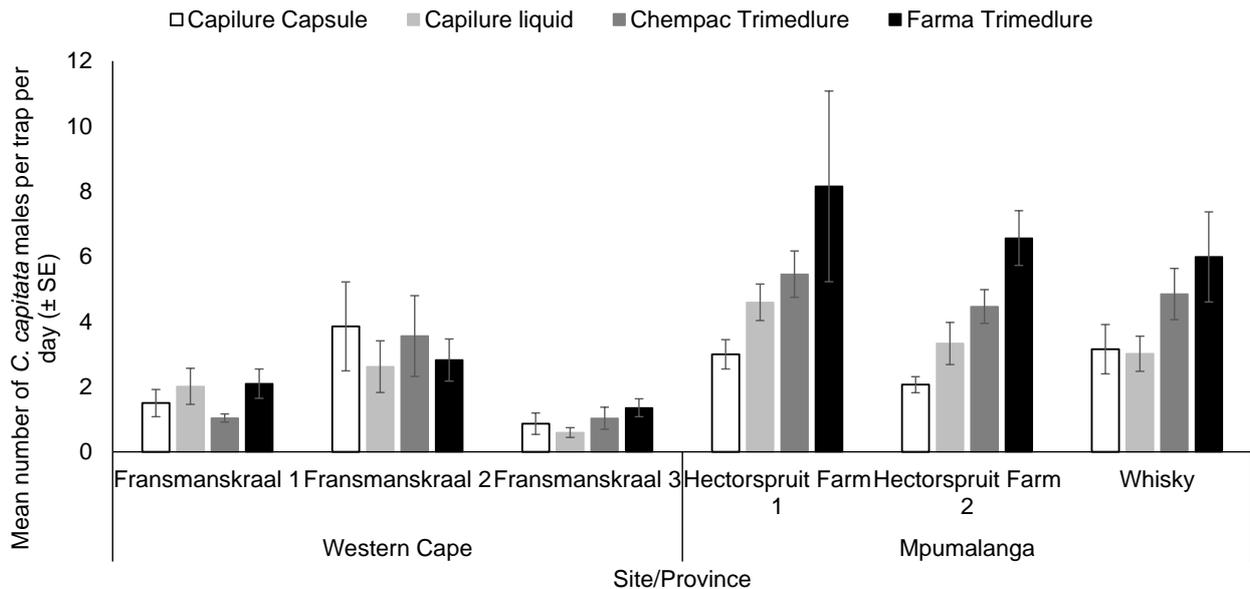


Figure 7. Captures of *C. capitata* males in yellow Delta traps baited four types of trimedlure dispensers: Capilure capsule, Capilure liquid, Chempac trimedlure and Farmatech trimedlure in six study sites in Western Cape and Mpumalanga provinces in South Africa.

The type of ME dispenser had a significant effect on catches of *B. dorsalis* males with significantly higher catches in traps baited with Invader lure compared to other dispensers ($F_{3,60}=12.297$, $P<0.0001$) (Fig. 8). There was no significant effect of site and no significant effect of the interaction between site and lure on catches of *B. dorsalis* males (Site: $F_{2,60}=0.851$, $P=0.432$, Lure x site: $F_{6,60}=0.197$, $P=0.976$). Invader lure had a higher amount of ME per dispenser (15g) compared to other dispensers which had between 2ml or 4 g of ME. Howarth and Howarth (2000) determined effect of ME dose in a dispenser on catches of *B. dorsalis* and found higher catches of the pest in traps with higher doses of ME. So Invader lure could possibly be more sensitive for early detection of *B. dorsalis* males in areas which are currently free of this pest in South Africa.

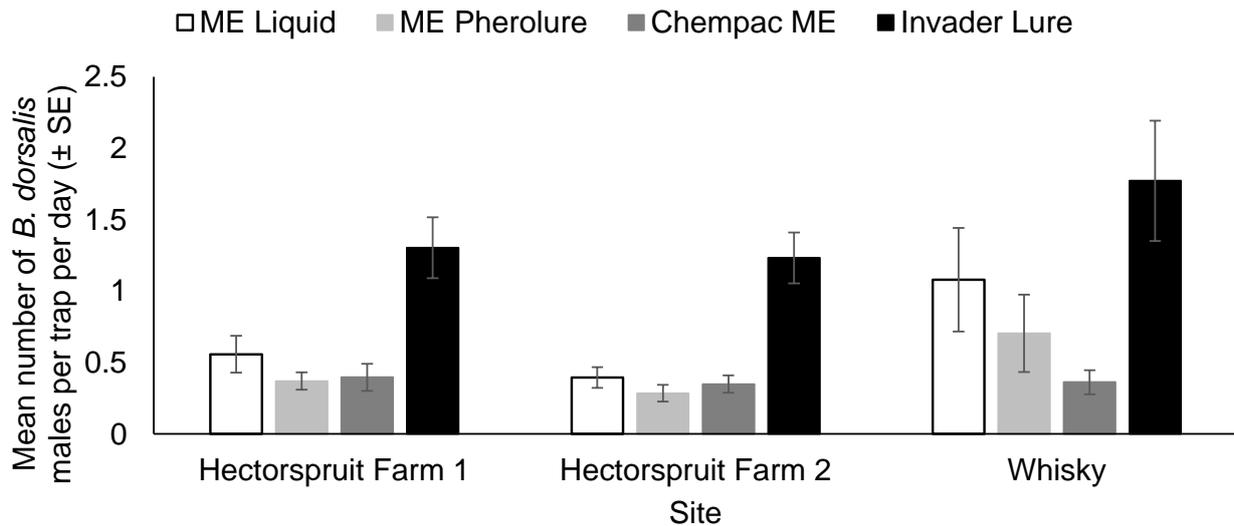


Figure 8. Captures of *B. dorsalis* males in Chempac bucket traps baited four types of ME dispensers: ME liquid on a dental roll, ME pherolure, Chempac ME plug and Invader lure in three study sites in Mpumalanga province in South Africa.

When trimedlure dispensers and trap types were combined, the trimedlure capsule baited yellow Delta trap was found to be more effective in catching *C. capitata* males than the Capilure baited Sensus bucket trap ($F_{1,65}=6.44$, $P=0.01$) (Fig. 9). There was a significant effect of site ($F_{5,65}=8.69$, $P<0.0001$) on catches of *C. capitata* males. Differences between sites were not specific to provinces. When overall *C. capitata* captures (all sites and all weeks combined) in trimedlure capsule baited yellow Delta trap were compared with Capilure baited Sensus bucket trap (the reference trap), the equivalence was at 2.5. In the citrus industry in southern Africa, Capilure baited Sensus trap is recommended for monitoring of *C. capitata* mainly because there is an existing threshold associated with this trapping system. Based on the results of this study, the threshold for *C. capitata* males in trimedlure capsule baited yellow Delta trap could be 2.5 times higher than its current threshold in Capilure baited Sensus trap.

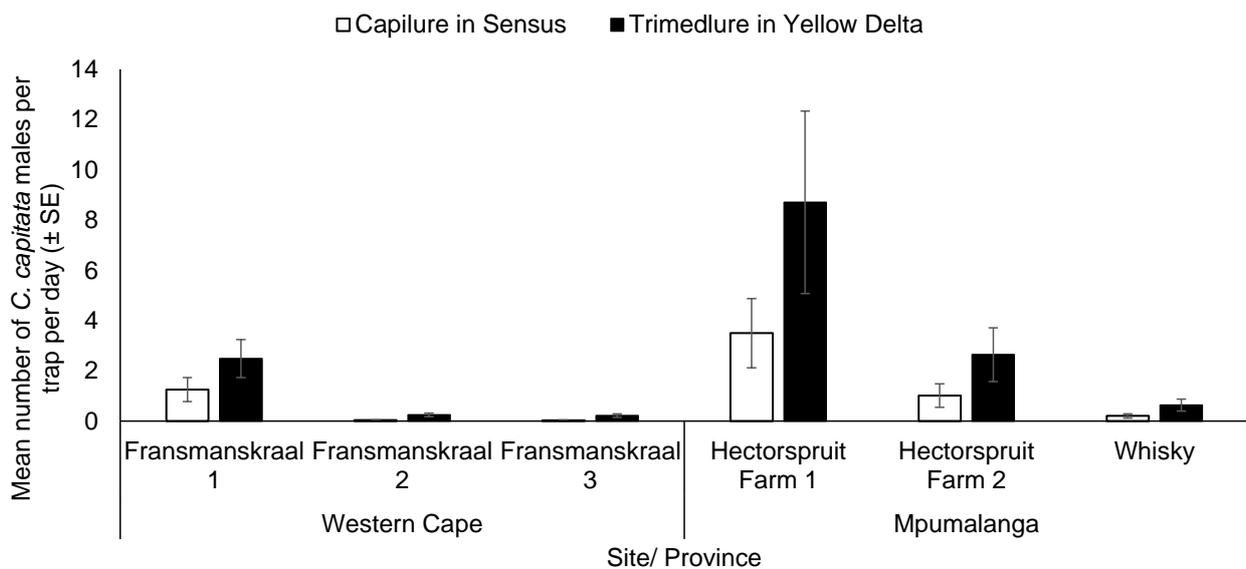


Figure 9. Captures of *C. capitata* males in two combinations of trimedlure dispensers and trap types: Capilure capsule in Sensus bucket trap and Trimedlure capsule in yellow Delta trap in six study sites in Western Cape and Mpumalanga provinces in South Africa.

When ME dispensers and trap types were combined, the Invader lure baited Lynfield trap was found to be more effective in catching *B. dorsalis* males than the other ME trapping systems ($F_{2,51}=14.81$, $P<0.0001$) (Fig. 10). The higher catches in an Invader lure baited lynfield trap were more likely to be due to the higher efficacy of the lure dispenser than the trap itself.

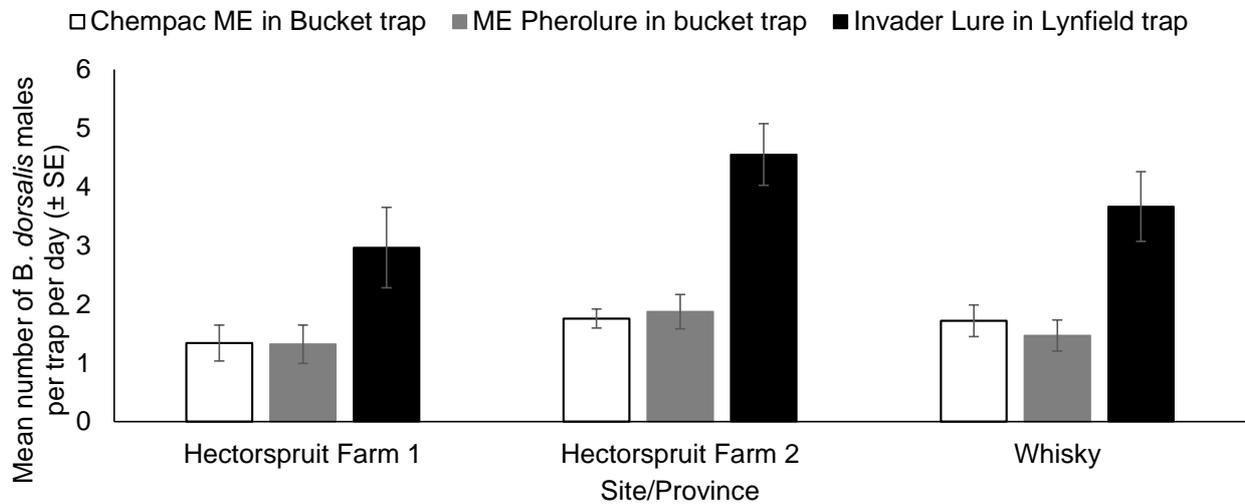


Figure 10. Captures of *B. dorsalis* males in three combinations of ME dispensers and trap types: Chempac ME lure in Chempac bucket trap, ME Pherolure in Chempac bucket trap, Invader lure in Lynfield trap in three study sites in Mpumalanga province in South Africa.

Conclusion

Results from this study would be useful in optimizing monitoring tools for fruit fly pests which are problematic to citrus. For the new invasive fruit fly pest: *B. dorsalis*, the three-component Biolure would be a more effective attractant for monitoring of females of the pest. EGO Pherolure could be an alternative male attractant to trimedlure for *Ceratitis* pests. The sampling range of EGO Pherolure seems to be similar to that of trimedlure. As such densities of EGO lure baited traps could be similar to those of trimedlure/capilure baited traps for monitoring of *Ceratitis* males.

In both trimedlure and ME based trapping systems, the type of lure dispenser influenced catches of males of *C. capitata* and *B. dorsalis* respectively. The type of traps only played a significant role in trimedlure based trapping systems and not in ME based trapping systems.

Finally, trimedlure capsule baited yellow Delta trap and Invader lure baited Lynfield trap were found to be the most effective trapping systems for *C. capitata* and *B. dorsalis* respectively. In case growers use the trimedlure capsule baited yellow Delta trap, a threshold of 10 *C. capitata* males per trap per week could be considered for decision on intensity and methods of fruit fly control in orchards.

Future research

- Similar studies on efficacy of lure dispensers and trap types should be conducted for *C. rosa* and *C. quilicii* such that relative captures to the standard Capilure baited Sensus traps could be derived for these pests.

Technology transfer

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2.3.4 **FINAL REPORT: Determination of non-host status of lemon to Natal fly, Medfly and Oriental fruit fly**

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

This report was adapted from the publication: Manrakhan A, Daneel JH, Beck R, Theron CD, Weldon CW, Moore SD & Hattingh V (2018) Non-Host Status of Commercial Export Grade Lemon Fruit (*Citrus limon* (L.) Burman f. cv. Eureka) for *Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis quilicii* and *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. *African Entomology* 26: 202-214. doi:10.4001/003.026.0202.

Summary

No fruit fly infestation has ever been recorded on lemons exported from South Africa. In this study, the host status of commercial export grade Eureka lemons for pest fruit flies: *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal fly), *Ceratitis quilicii* (Cape fly), and *Bactrocera dorsalis* (oriental fruit fly) was determined. Trapping was conducted in 10 Eureka lemon orchards in two major citrus production regions over two citrus seasons between 2016 and 2017 to determine the level of fruit fly abundance in the sampled orchards. Lemons were collected at harvest over the two seasons in the same orchards where trapping was conducted. Lemons collected were dissected to determine fruit fly infestation. Additionally, infestation of lemons was determined under forced exposure to mature mated females of *C. capitata* and *B. dorsalis*. Trapping data showed the presence of adults of all four fruit fly species in the sampled lemon orchards. No fruit fly infestation was detected in 43 222 Eureka lemons sampled at harvest. There was also no infestation of lemons under forced exposure conditions. The results of this study provide evidence that South African commercial export grade Eureka lemon fruit is not a host for *C. capitata*, *C. rosa*, *C. quilicii* or *B. dorsalis*.

Opsomming

Geen vrugtevlieg infestasië is al ooit aangeteken op suurlemoene wat vanaf Suid-Afrika uitgevoer is nie. In hierdie studie is die gasheerstatus van kommersiële uitvoergraad 'Eureka' suurlemoene vir die volgende vrugtevliegplae bepaal: suurlemoene vir vrugtevliegplae: *Ceratitis capitata* (Mediterreense vrugtevlieg), *Ceratitis rosa* (Nataalse vlieg), *Ceratitis quilicii* (Kaapse vlieg), en *Bactrocera dorsalis* (Oosterse vrugtevlieg). Lokvalle is in 10 'Eureka' suurlemoenboorde in twee groot sitrus produksie-areas oor twee sitrusseisoene tussen 2016 en 2017 gestel ten einde die vlak van vrugtevlieg volopheid in die boorde wat gemonster is, te bepaal. Suurlemoene is tydens oes oor die twee seisoene versamel, in dieselfde boorde waar die lokvalle gestel is. Suurlemoene wat versamel is, is gedissekteer ten einde vrugtevlieg infestasië te bepaal. Infestasië van suurlemoene is bykomend onder gedwonge blootstelling aan volwasse gepaarde wyfies van *C. capitata* en *Bactrocera dorsalis* bepaal. Lokval data het die teenwoordigheid van volwassenes van al vier vrugtevliegspesies in die suurlemoenboorde wat gemonster is, aangetoon. Geen vrugtevlieg infestasië is waargeneem in 43 222 'Eureka' suurlemoene wat tydens oes versamel is nie. Daar was ook geen infestasië van suurlemoene onder gedwonge blootstellingstoestande nie. Die resultate van hierdie studie verskaf bewyse dat Suid-Afrikaanse kommersiële uitvoergraad 'Eureka' suurlemoene nie 'n gasheer vir *C. capitata*, *C. rosa*, *C. quilicii* of *B. dorsalis* is nie.

Introduction

For some citrus types such as lemon, post-harvest cold treatment as a phytosanitary risk mitigation measure is not feasible due to their sensitivity to cold damage. For those citrus types such as lemon that are sensitive to cold damage and are known to be conditional hosts for some fruit fly species, non-host status testing could be an alternative to post-harvest cold treatment (Follett and Hennessey 2007).

Lemon is listed as a host for Medfly and Oriental fruit fly based largely on field records (Eskafi 1988, Liquido et al. 1990, Katsoyannos et al. 1998, De Meyer et al. 2002, Clarke et al. 2005, Rwomushana et al. 2008). However, in those field records no details on the condition of lemon from which the flies were reared were provided. Damaged and ripe lemons are conditions that could favour Medfly infestation (Sproul 1976, Staub et al. 2008). Laboratory assays determining susceptibility and suitability of lemon to Medfly have shown that lemon is a poor host for Medfly. In a review done by an expert team from USDA-APHIS and USDA-ARS on the host status of lemon, the conclusion drawn was that green lemons are not hosts of Medfly.

Historical data on inspections carried out on lemons at pack houses within South Africa between 2011 and 2015 have shown a very low rejection rate (0.0006%) due to fruit fly infestation (Perishable Products Export Control Board (PPECB), unpublished). For the lemons rejected however, there was no confirmation of the presence of live immature stages of fruit flies and therefore cannot be taken as confirmed fruit fly infestation. Furthermore, there have been no reported interceptions of fruit flies in lemons imported into the European Union (EU) and Switzerland from South Africa between 2011 and 2014 (Europhyt 2011, 2012, 2013, 2014). Considering that the EU does not require cold treatment for citrus fruit imported from South Africa and no such cold treatment can be applied to lemons due to their sensitivity to chilling injury, this provides strong support to the assertion that export grade lemons from South Africa are free of fruit flies.

In a recent study by Moore et al. (2015), the authors found no false codling moth and fruit fly infestation out of 30 346 export grade Eureka lemon inspected from four communal packhouses in the Sundays River Valley, Eastern Cape. In that study, the presence of false codling moth in lemon orchards where the inspected fruit originated from was demonstrated by trapping. The lack of false codling moth infestation despite the presence of the pest in lemon orchards provided strong support for non-host status of lemon for false codling moth. Moore et al. (2015) recommended the exclusion of Eureka lemons from mandatory cold treatment as a false codling moth phytosanitary risk mitigation measure in citrus fruit export protocols. Although there are published records of both Medfly and Natal fly in the Sundays River Valley where the non-host status of lemon for false codling moth was demonstrated, the prevalence of fruit flies in traps in lemon orchards was not determined during the study by Moore et al. (2015).

Fruit fly monitoring data in lemon orchards in South Africa coupled with sampling of lemon, in a similar way as done by Moore et al. (2015) would be needed for the non-host testing of export grade lemon for fruit fly pests of citrus in South Africa.

For Medfly and Oriental fruit fly which have been previously reared from lemon in previous host surveys, additional semi field tests determining susceptibility of attached lemon to the two fruit fly species would be required as per the International Standards of Phytosanitary Measures (ISPM No. 37) Determination of host status of fruit to fruit flies (Tephritidae).

Stated objectives

A. To determine the host status of export grade Eureka lemons for fruit fly pests of citrus in South Africa: Mediterranean fruit fly, Natal fly and Oriental fruit fly through natural field surveillance.

B. To determine the susceptibility of Eureka lemon to infestation by Medfly and Oriental fruit fly under no choice semi-field tests

Materials and methods

Objective A. Natural field surveillance

Study sites

Surveys were conducted between February 2016 and May 2017 in 10 Eureka lemon orchards from 10 farms in the Mpumalanga and Limpopo Provinces, South Africa. The surveys covered two citrus production seasons: 2016 and 2017.

Five attractant-based traps were used for monitoring of adult female and male fruit fly pest populations in each Eureka lemon orchard. Two of these attractant-based traps: Questlure baited Sensus trap and three-component Biolure baited Chempac Bucket trap were used for monitoring of adult female fruit flies.

Three of the attractant-based traps were used for monitoring of adult male fruit fly pests. Two of these traps targeted male *Ceratitis* species: (1) EGO Pherolure, which consisted of enriched ginger root oil, and (2) Capilure, which contains the male attractant trimedlure. EGO Pherolure and Capilure were each placed inside a Sensus trap. One yellow Lynfield trap baited with methyl eugenol (ME) was used to monitor *B. dorsalis* males. Each attractant-based trap contained a 3 g dichlorvos strip to kill any attracted flies. In each lemon orchard, each trap was suspended on a tree in the shade at about 1.5 m above ground. The five attractant-based traps were placed along one selected row with a distance of approximately 30 m between the traps. Trapping was initiated from the beginning of February 2016 and traps were maintained and serviced monthly until May 2017. All traps were checked and emptied monthly. Dichlorvos and all attractants except EGO Pherolure were changed on a monthly basis. EGO Pherolure was changed every six months as per the recommendation of the manufacturer. Flies collected from the traps were identified and categorised according to species and sex.

Fruit sampling

At harvest between February and June of each citrus production season, Eureka lemons were picked from trees in the same orchards where the traps were placed. The lemons were taken to Citrus Research International (CRI) laboratories in Nelspruit. The collected fruit was first graded according to rind colour using Set Number 37 of the Colour Prints for Blemish and Appearance Standards (1997), published by CRI, endorsed by the Department of Agriculture Forestry and Fisheries, South Africa. The colour grades in Set Number 37 range from completely yellow (colour grade 1) to completely green (colour grade 8), with grades 2 to 4 being predominantly yellow and grades 5 to 7 predominantly green.

The fruit were then dipped for 1 minute in a fungicidal mixture to prevent fungal growth during storage before examination. The fungicide solution was a combination of Sporekill at 1 ml/L and Citricure at 48ml/L. The fungicidal mixture is regularly used in studies on post-harvest disinfestation treatments for fruit flies. The fruit

were then covered with a fine mesh material (3 mm mesh size) in order to prevent fruit fly infestation during storage and kept in a room at $25.4^{\circ}\text{C} \pm 0.0^{\circ}\text{C}$ between 1 and 34 days before examination for fruit fly eggs and larvae. During fruit examination, the fruit were first peeled to determine presence of fruit fly eggs in the flavedo and albedo. Thereafter the fruit were cut to determine presence of larvae in the pulp. A headband magnifier was used for detection of eggs and larvae in the different fruit regions.

Data analysis

Adult field population data for males and females of *B. dorsalis*, *C. capitata*, *C. rosa*, *C. quilicii* were summarized as flies per trap per day (FTD) and flies per trap per month (FTM). The FTD data were $\log(x+1)$ transformed. A log-linear model assuming a Poisson distribution of the response variable was used to analyse the effect of farm and sampling time on catches of the different fruit fly groups. Analysis of female fruit fly catches was only done for the three-component Biolure trapping system due to very low catches obtained with the Questlure traps. Analysis of male *C. quilicii* and *C. rosa* catches was only done for the EGO Pherolure trapping system due to very low catches of these fruit fly groups obtained in the Capilure trapping system. Level of infestation in Eureka lemons sampled and examined at harvest was summarized as percentage fruit fly infestation.

Objective B. Susceptibility of Eureka lemon to infestation by Medfly and Oriental fruit fly in a no choice semi field test

Study sites

One of the Eureka lemon orchards used in the natural field surveillance, Ryton Estates, Mpumalanga Province, was used for tests under semi-natural field conditions. Tests were carried out between 8 and 12 May 2017. During the tests the mean daily maximum temperature was $23^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and the mean daily minimum temperature was $6.6^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$. There was no rain during the tests.

Insect materials

Ceratitidis capitata and *B. dorsalis* originating from colonies maintained at CRI, Nelspruit, were used in the tests. Colonies of *C. capitata* were maintained for over 200 generations. Colonies of *B. dorsalis* were maintained for over 15 generations. Colonies were refreshed with wild males reared from fruit every two years. *Ceratitidis capitata* was reared on a bran-based diet and *B. dorsalis* was reared on a carrot-based diet. For *C. capitata*, 9-12 day old mated females were used. For *B. dorsalis*, 21-24 day old mated females were used. Before the test, males and females were kept together and fed with a mixture of sugar and yeast hydrolysate mixture in a ratio of 3:1 plus water *ad libitum*. Mating was observed during the day for *C. capitata* between 09:00 and 15:00 as from the 3rd day after adult emergence. For *B. dorsalis*, mating was observed at dusk between 17:30 and 18:00 at the end of the second week after adult emergence. Mating couples found were gently captured in a vial and transferred to a different cage to ensure that all females used were mated. Flies were kept in the laboratory with natural light conditions at $20.4^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ until use.

No choice semi-field tests

For each fruit fly species, five Eureka lemon trees bearing fruit at picking ripeness were selected in one row in the study orchard. On each selected tree, two separate branches, each with five unharvested and undamaged Eureka lemon, were selected on two opposite sides (northern and southern sides) of the tree. Each branch was covered with a cylindrical wire frame mesh cage of 43.5 cm in diameter and 52.5 cm in height. Two control cages for each fly species were also set up on each tree, on opposite sides. Each control cage consisted of a branch on which five harvested undamaged Golden Delicious apples were suspended. For each fruit fly species, there were therefore 10 cages with lemons and 10 cages with the control fruit, representing 10 replicates for each treatment. All cages were covered on top with a transparent plastic material to protect the cages from rain. The cages were secured from ants by smearing petroleum jelly on the branches just outside of the sleeve cages. Leaves from other branches touching the sleeve cages were trimmed.

For *C. capitata*, 25 mated females were released into each cage. For *B. dorsalis*, seven mated females were released into each cage. A lower number of *B. dorsalis* were used in the tests, due to fewer mated females being available. In each cage, flies were provided with water, granulated sugar and yeast hydrolysate *ad libitum*. Flies were left exposed to the fruit for 4 days. Mortality was recorded daily from each cage and dead mated females were replaced by mated females of the same ages in order to maintain a constant fruit fly pressure in each cage.

Four days after exposure, Eureka lemon fruit and control fruit from each branch were brought back to the laboratory at CRI, Nelspruit, weighed individually and incubated individually in aerated plastic containers over a layer of sterilised sand. A sample of 10 Golden Delicious apples used for the control cages were also incubated individually in the lab to determine natural infestation of the control fruit. The containers were maintained in a room at $25.9^{\circ}\text{C} \pm 0.0^{\circ}\text{C}$ for 10 weeks to ensure larval development. Fruit samples were checked daily for pupal and adult emergence. Pupae found were placed in plastic Petri dishes on a moist filter paper. Emerged flies were kept for 3 days to allow for full colour development before being killed and identified. Fruit were dissected to determine presence of fruit fly larvae before being discarded.

During the trial, three samples of 12 Eureka lemon fruit from the test site were brought back to the laboratory at CRI, Nelspruit, on three separate dates (8 May 2017, 10 May 2017 and 12 May 2017) and were analysed for physical and chemical characteristics. Each fruit was weighed and the equatorial diameter was measured. The peel thickness was measured using a Vernier calliper. The juice was extracted from each sample of 12 fruit. The Brix degrees, acidity and pH of the juice were measured by a refractometer, titration with NaOH and a pH meter.

Data analysis

Levels of infestation of lemon fruit and control fruit in the no choice semi field tests were determined as pupae per fruit and adults per fruit.

Results

Adult fruit fly populations in lemon orchards

The presence of *B. dorsalis*, *C. capitata*, *C. rosa* and *C. quilicii* in Eureka lemon orchards was confirmed by trapping (Fig. 1 and Fig. 2). All target fruit fly species except males of *C. rosa* were trapped in all lemon orchards. *Ceratitis rosa* males were not captured in two of the lemon orchards in Limpopo Province (Schoeman Boerdery and Schoonbee Boerdery). The two fruit fly species which dominated the overall catches in most lemon orchards were *B. dorsalis* and *C. capitata*.

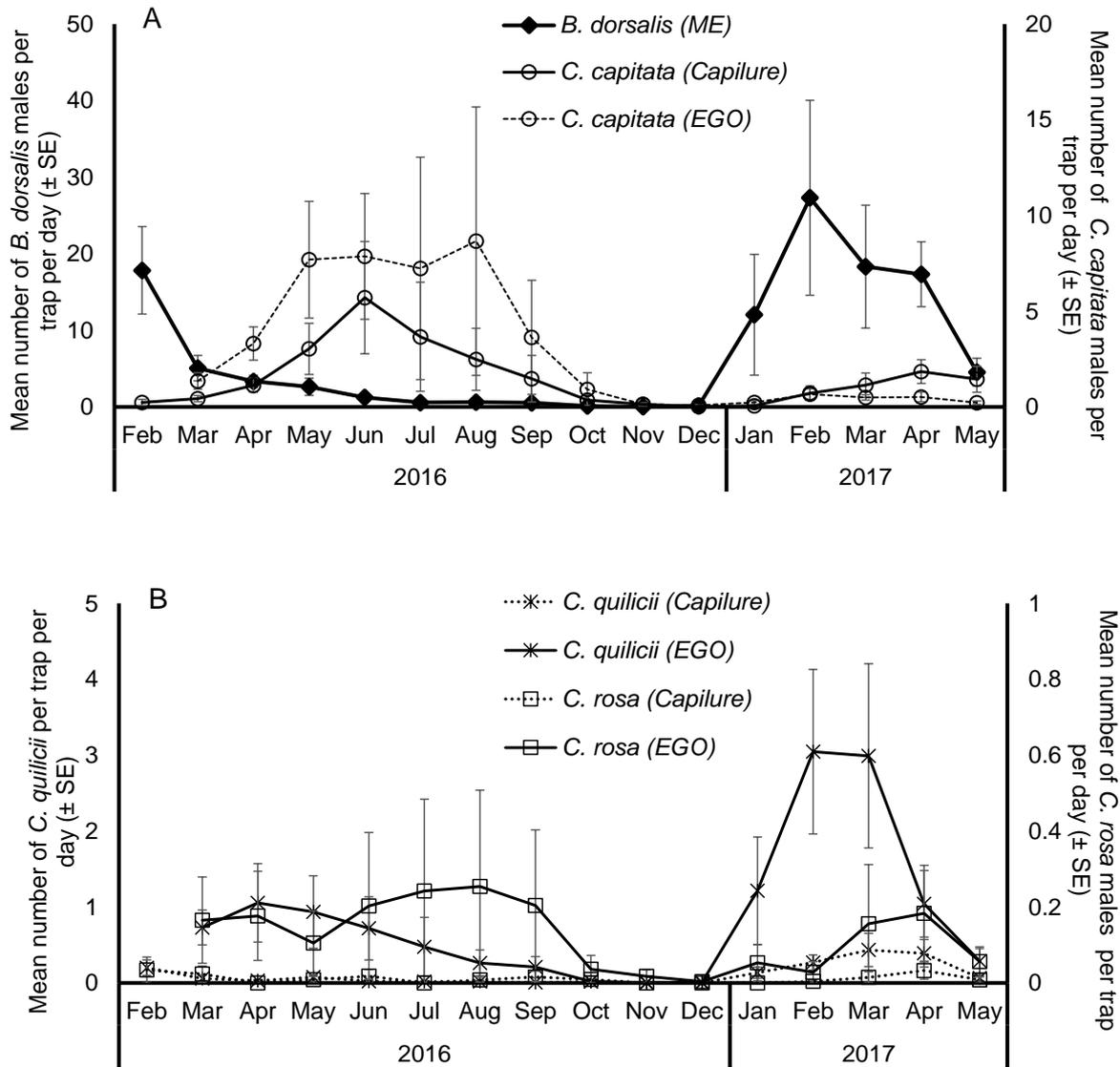


Figure 1. Daily mean catches of male (A) *B. dorsalis* and *C. capitata*; and (B) *C. quilicii* and *C. rosa* in male attractant-based traps in Eureka lemon orchards in Limpopo and Mpumalanga Provinces, South Africa, between February 2016 and May 2017. *Bactrocera dorsalis* males were targeted using methyl eugenol (ME) baited Lynfield traps. *Ceratitidis capitata*, *C. quilicii* and *C. rosa* were targeted using Capilure baited and EGO Pherolure baited Sensus traps.

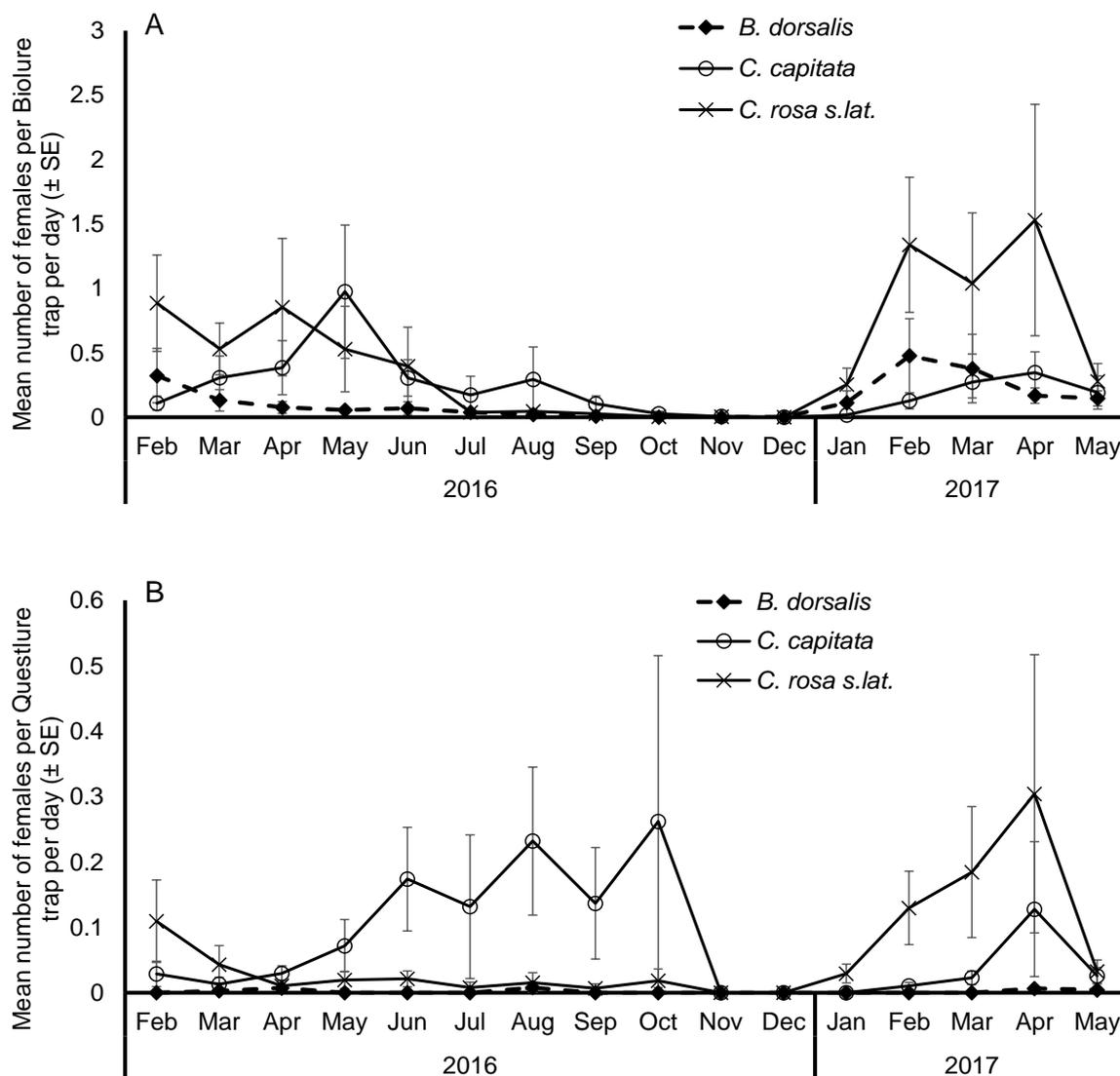


Figure 2. Daily mean catches of female *B. dorsalis*, *C. capitata* and *C. rosa s. lat.* in (A) three-Component Biolure and (B) Queslure baited traps in Eureka lemon orchards in Limpopo and Mpumalanga Provinces of South Africa between February 2016 and May 2017.

Peaks of *B. dorsalis* and *C. capitata* male catches occurred during the harvesting period of Eureka lemon (between February and June) (Fig. 1). Catches of *B. dorsalis* males in ME baited traps were not significantly different between lemon orchards ($\chi^2=12.00$, $df=9$, $P=0.21$) but were significantly different across time ($\chi^2=53.17$, $df=15$, $P<0.0001$) and peaked between February and April. Catches of *C. capitata* males in EGO Pherolure baited traps were significantly different between orchards ($\chi^2=19.83$, $df=9$, $P=0.02$) and across time ($\chi^2=24.46$, $df=14$, $P=0.04$), peaking between April and August in the first study year. In Capilure baited traps, there were also significant differences in catches of *C. capitata* males between orchards ($\chi^2=19.79$, $df=9$, $P=0.02$) but no significant differences in catches across time ($\chi^2=17.11$, $df=15$, $P=0.31$). For *C. quilicii* and *C. rosa* males there were significant differences in catches between orchards (*C. quilicii*: $\chi^2=25.01$, $df=9$, $P=0.00$; *C. rosa*: $\chi^2=51.99$, $df=9$, $P<0.05$) but not across time (*C. quilicii*: $\chi^2=16.94$, $df=14$, $P=0.26$; *C. rosa*: $\chi^2=9.76$, $df=14$, $P=0.78$).

There were no significant differences in catches of female *B. dorsalis*, *C. capitata* or *C. rosa s. lat.* in Biolure baited traps between orchards (*B. dorsalis*: $\chi^2=7.60$, $df=9$, $P=0.58$; *C. capitata*: $\chi^2=7.36$, $df=9$, $P=0.60$; *C. rosa s. lat.*: $\chi^2=15.71$, $df=9$, $P=0.07$). There were also no significant differences in catches of female fruit flies over

time (Fig. 2) (*B. dorsalis*: $\chi^2=5.59$, $df=15$, $P=0.99$; *C. capitata*: $\chi^2=6.81$, $df=15$, $P=0.96$; *C. rosa* s.l.: $\chi^2=15.15$, $df=15$, $P=0.44$).

Sampling of export grade lemons

No live or dead fruit fly larvae were found in 43,222 Eureka lemons inspected (Table 1), despite the presence, and in some cases high abundance of female fruit flies in the orchards during the harvesting period (February to May/June) of each year (Table 1). Eureka lemons obtained from the orchards at harvest in both years were mostly between colour grades 5 and 6 (predominantly green).

Table 1. Fruit fly infestation in export-grade Eureka lemons at harvest and trap catches of female fruit flies during the harvesting period (February to May/June) in 10 commercial orchards on 10 farms in Mpumalanga and Limpopo Provinces, South Africa, in 2016 and 2017.

Farm name	Sampling date	Number of Eureka lemon fruit examined	% fruit fly infestation	Mean catch of female fruit flies per trap per month (combined Biolure and Questlure catches) during Eureka lemon harvesting period (February-May/June)		
				<i>B. dorsalis</i>	<i>C. capitata</i>	<i>C. rosa s.lat.</i>
Ambrosia Citrus Estate	30 March 2016	1732	0	0.1 ± 0.1	2.4 ± 1.5	0.5 ± 0.3
	7 February 2017	3419	0	0.5 ± 0.2	0.4 ± 0.4	0.0 ± 0.0
Schoeman Boerdery	16 May 2016	1636	0	0.3 ± 0.2	2.6 ± 0.8	0.3 ± 0.2
	19 April 2017	2185	0	0.3 ± 0.3	9.7 ± 3.7	0.6 ± 0.4
Schoonbee Boerdery	5 April 2016	1750	0	0.5 ± 0.3	1.1 ± 0.6	0.0 ± 0.0
	19 April 2017	1996	0	0.3 ± 0.2	1.9 ± 0.9	0.2 ± 0.1
Unifrutti	3 April 2016	1497	0	0.1 ± 0.1	0.9 ± 0.5	0.0 ± 0.0
	6 February 2017	2636	0	0.3 ± 0.3	0.6 ± 0.4	0.0 ± 0.0
Van Veijeren Boerdery	30 March 2016	2210	0	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0
	27 March 2017	2119	0	0.1 ± 0.1	1.5 ± 0.6	0.3 ± 0.2
Bakgat Boerdery	29 April 2016	1588	0	3.0 ± 1.4	20.9 ± 14.0	3.5 ± 2.3
	8 March 2017	2647	0	2.7 ± 2.1	7.8 ± 4.5	4.4 ± 3.8
Daarbo Boerdery	11 April 2016	2192	0	8.8 ± 4.1	19.2 ± 5.9	2.4 ± 0.8
	2 May 2017	2601	0	23.0 ± 9.6	11.6 ± 3.9	0.9 ± 0.4
Fountains	30 March 2016	1741	0	0.7 ± 0.4	3.0 ± 1.9	0.5 ± 0.5
	1 March 2017	2674	0	4.5 ± 0.5	0.5 ± 0.3	0.2 ± 0.2
Ryton Estates	7 June 2016	1625	0	0.9 ± 0.4	14.0 ± 7.0	1.2 ± 0.8
	18 April 2017	2466	0	2.2 ± 1.6	0.2 ± 0.2	0.0 ± 0.0
Siyalima Boerdery	8 March 2016	2135	0	2.8 ± 1.7	0.4 ± 0.1	1.1 ± 0.9
	8 March 2017	2373	0	10.1 ± 5.4	0.8 ± 0.5	0.4 ± 0.1

Tests under semi-natural field conditions for infestation by *C. capitata* and *B. dorsalis*

There was no infestation of Eureka lemons by *B. dorsalis* and *C. capitata* in the additional trials under semi-natural field conditions (Table 2). The Eureka lemons tested were between colour grades 4 and 6 (based on Set Number 37 of the Colour Prints for Blemish and Appearance Standards 1997 published by CRI and endorsed by the Department of Agriculture Forestry and Fisheries, South Africa). Based on samples of Eureka lemons collected within the same colour grade range in the test orchard, the mean weight of Eureka lemon fruit at this ripeness condition was 121.51 ± 4.4 g. The mean equatorial diameter was 61.44 ± 0.80 mm. The peel thickness was 5.37 ± 0.13 mm. The mean Brix degree values, percentage acidity and pH were 8.07 ± 1.36 , 6.76 ± 0.31 and 2.36 ± 0.02 respectively.

In the trial on *C. capitata*, the percentage infestation of the control fruit (Golden Delicious apples) was 42% whilst in the trial on *B. dorsalis* the percentage infestation of the control fruit was only 8%. The percentage infestation of a sample of Golden Delicious apples that was not exposed to fruit flies was 0%. The mean pupal developmental time (days from incubation to first pupal formation) of *C. capitata* in the control fruit was 18.95 ± 0.87 days. For *B. dorsalis*, the mean pupal developmental time on the control fruit was 10.00 ± 0.00 days. During the trial, zero or very low mortality was observed in cages with *C. capitata*. In cages with *B. dorsalis*, fly mortality was observed in five out of 10 cages with the control fruit on the second day of the trial. In each of these cages, except for one, there was one dead female which was then replaced. In one cage, there were three dead *B. dorsalis* females on the second day of the trial, which were then replaced.

Table 4. Mean number of pupae and adults per fruit in attached undamaged Eureka lemons and detached undamaged Golden Delicious apples exposed separately to 25 mated *C. capitata* or seven mated *B. dorsalis* females over four days under semi-natural field conditions.

Fruit fly species	Mean number of pupae per fruit		Mean number of adults per fruit	
	Eureka lemon	Apple	Eureka lemon	Apple
<i>C. capitata</i>	0.00 ± 0.00	8.00 ± 2.51	0.00 ± 0.00	6.04 ± 1.81
<i>B. dorsalis</i>	0.00 ± 0.00	0.94 ± 0.49	0.00 ± 0.00	0.46 ± 0.31

Discussion

The absence of fruit fly infestation in 43,222 Eureka lemons sampled in commercial orchards in this study clearly demonstrated that commercial, export grade, Eureka lemon is not a host for *B. dorsalis*, *C. capitata*, *C. quilicii* or *C. rosa*. In order to demonstrate a 99.99% non-host level with 95% confidence, 30 000 fruit should be tested (Follett & Hennessey 2007). Based on the number of Eureka lemon tested in this study, we demonstrated a 99.99% non-host status of Eureka lemon to fruit fly pests in South Africa at a 99% confidence level (Couey & Chew 1986; Follett & Hennessey 2007). The non-host status of many other crop pest combinations have similarly been established on the basis of such data and the analysis thereof (Armstrong 1991; Hennessey *et al.* 1992; Moore *et al.* 2015; Pringle *et al.* 2015).

The resistance of Eureka lemon to fruit fly infestation occurred despite the presence of *B. dorsalis*, *C. capitata*, *C. quilicii* and *C. rosa* in the area as demonstrated by catches of female and male flies of these species in traps placed in the same Eureka lemon orchards. The occurrence of such catches of fruit flies in non-host Eureka lemon orchards can be attributed to adult fruit flies using habitats to not only search for egg laying sites but to also forage for food, water and mates (Prokopy & Roitberg 1984). In studies on *C. capitata* in a heterogeneous region in central Israel, Israely *et al.* (1997) trapped significant numbers of the pest in the non-host English walnut, *Juglans regia* L. The trapping of *C. capitata* in English walnut was related to the presence of honeydew deposits on these trees by aphids (Israely *et al.* 1997), with honeydew representing a natural protein and sugar source for adult flies (Neilson & Wood 1966). It is likely that the Eureka lemon orchards were used as habitats for other resources, like food and shelter, by these fruit fly species.

Following the steps in determination of host status of fruit to fruit flies in ISPM 37 (FAO 2016), for *C. rosa* and *C. quilicii* there was no need to conduct field surveys of fruit infestation given that there are no existing records of these pests on lemons, and as such lemons would immediately qualify as a non-host for these two species. The results from this study nonetheless reinforce the non-host status of lemon for *C. rosa* and *C. quilicii*.

For *C. capitata* and *B. dorsalis*, species where there are some historical records of having been reared from lemons, albeit without evidence of any naturally occurring infestation of commercial export grade lemons, the absence of infestation of commercially produced export grade Eureka lemons in the larval field survey establishes the non-host status of this cultivar for these pests, based on ISPM 37 (FAO 2016). Nonetheless, trials under semi-natural conditions for *B. dorsalis* and *C. capitata* were conducted as confirmation of the non-host status of Eureka lemon for these two species. Absence of infestation of Eureka lemon by *C. capitata* and *B. dorsalis* was confirmed in these semi-natural tests. The control fruit used in these tests, Golden Delicious apple, has been recorded as a host for both *B. dorsalis* and *C. capitata* (Clarke *et al.* 2005; De Meyer *et al.* 2002), and was one of the few host fruit common to these two species which was in season at the time of the study (in May). Infestation of the control fruit by the two species was recorded in the semi-natural trials, albeit at a low level. The rate of infestation of *C. capitata* in the control fruit compared well to natural infestation rates of the same cultivar by *C. capitata* in Greece (between 4.2 and 18.8 pupae per fruit) (Papadopoulos *et al.* 2002). Apple was reported to be a less preferred host for *B. dorsalis* in southwestern China (Ye & Liu 2005), which could explain the low infestation rates of apple by *B. dorsalis* in this study.

The resistance of lemons to fruit fly infestation has been attributed to chemicals in the peel (Back & Pemberton 1915; Greany *et al.* 1983; Salvatore *et al.* 2004). Oil from the rind of oranges and lemons was found to cause high mortality of *C. capitata* eggs (Back & Pemberton 1915). Citral, coumarins and linalool found in lemon peel extracts were found to have significant larvicidal activity when tested on *C. capitata* (Salvatore *et al.* 2004). Concentrations of citral and coumarins were, however, found to decrease in the peel after harvest (Salvatore *et al.* 2004). The flavedo of 'Eureka' and 'Lisbon' lemons were found to be thicker than those of other citrus varieties and lemons were found to have more oil per unit area of peel than other citrus varieties (Greany *et al.* 1983). The latter authors suggested that the flavedo thickness and amount of oil in the peel could render lemons resistant to fruit fly infestation.

The categorization of lemons as a non-host for fruit flies is relevant to international phytosanitary trade regulation. In 2008, the United States Department of Agriculture (USDA) convened an expert panel to assess the host status of lemons for fruit flies and the panel concluded that green lemons were not a host for *C. capitata* (USDA 2008). This conclusion was based on an evaluation of over 90 publications and reports with regard to host status of lemons for *C. capitata* (USDA 2008). The panel considered the potential susceptibility of lemons to *C. capitata* to be associated with over-ripeness and high *C. capitata* population pressure (USDA 2008). The Animal and Plant Health Inspection Service (APHIS) of USDA subsequently applied this recognition of non-host status of lemons to new lemon fruit import regulations, as is evident in a pest risk analysis conducted on the import of citrus from Uruguay (USDA-APHIS 2012). In 2013, USDA promulgated a final rule which included authorizing the importation of lemon fruit from Uruguay (Federal Register 2013). The final rule in the USA Federal Register (Volume 78, No. 132), specifies that lemons from Uruguay, may be imported into continental USA if harvested when green and do not require disinfestation treatment for *C. capitata* and the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Federal Register 2013). USDA APHIS also conducted a pest risk analysis on the import of lemons from Argentina (USDA-APHIS 2015). In 2016, USDA promulgated a final rule in the US Federal register (Volume 81, No. 247) to authorise import of lemons from Argentina (Federal Register 2016). In the final rule, it was stipulated that lemons harvested green and within a specified time period, can be imported into USA without the need for application of a disinfestation treatment (81 Federal Register 2016).

Conclusion

The findings in this study provide (1) experimental evidence, compliant with international standards for phytosanitary measures, that commercially produced export standard Eureka lemon fruit, qualifies as a non-host for *B. dorsalis*, *C. capitata*, *C. quilicii* and *C. rosa* and (2) technical justification for exempting South African commercially produced export grade Eureka lemons from post-harvest fruit fly disinfestation treatments.

Future research

- Similar future field surveys and susceptibility tests should be carried out on other lemon cultivars (such as Lisbon lemon)

Technology transfer

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2.3.5 PROGRESS REPORT: Fruit fly rearing

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

Summary

Five fruit fly species: *Ceratitidis capitata* (Mediterranean fruit fly or Medfly), *Ceratitidis rosa* (Natal fly), *Ceratitidis quilicii* (Cape fly), *Ceratitidis cosyra* (Marula fly) and *Bactrocera dorsalis* (oriental fruit fly), are being reared at Citrus Research International (CRI), Nelspruit. The laboratory-reared flies were used in CRI-funded projects (915, 1107, 1170, 1171 and 1213) and were also provided on request to external institutions for experimental use. Fruit fly colonies have to be regularly refreshed with wild flies in order to prevent loss of traits. The Medfly colony was refreshed in September 2017 by crossing 305 males reared from coffee, *Coffea canophora*, collected at Burgershall with 386 females from the CRI laboratory colony. Between August 2017 and February 2018, two Cape fly colonies sourced from two areas (Elands Valley and Pretoria) were established. From

Elands Valley, 331 Cape fly adults caught in 3-component Biolure traps were used as founder flies. From Pretoria, 410 Cape fly adults reared from pineapple guava, *Acca sellowiana*, were used as founder flies. In March 2018, a colony of oriental fruit fly was re-established using 395 adults reared from mangoes, *Mangifera indica*, collected in Nelspruit. Mean (\pm SE) weekly pupal volumes (ml) of Medfly, Natal fly, marula fly and oriental fruit fly produced were 417.2 ± 16.1 , 269.2 ± 10.2 , 128.3 ± 6.1 and 215.0 ± 11.3 respectively. One ml of pupae contains approximately 50 pupae. Very few pupae were produced weekly for the two Cape fly colonies. Mean (\pm SE) egg hatch rates for Medfly, Natal fly, Cape fly, marula fly and oriental fruit fly were $85.0 \pm 3.5\%$, $92.1 \pm 1.4\%$, $75.9 \pm 1.7\%$, $95.5 \pm 2.1\%$ and $78.2 \pm 4.6\%$. Mean (\pm SE) adult emergence rates for Medfly, Natal fly, Cape fly, marula fly and oriental fruit fly were $95.8 \pm 1.9\%$, $96.6 \pm 1.4\%$, $42.8 \pm 7.1\%$, $93.7 \pm 1.6\%$ and $94.4 \pm 1.5\%$.

Opsomming

Vyf vrugtevliespesies word by Citrus Research International (CRI), Nelspruit geteel: *Ceratitis capitata* (Mediterreense vrugtevlies of "Medfly"), *Ceratitis rosa* (Nataalse vlieg), *Ceratitis quilicii* (Kaapse vlieg), *Ceratitis cosyra* (Marula vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlies). Die laboratorium-geteelde vrugtevlies is in CRI-befondsde projekte (915, 1107, 1170, 1171 en 1213) gebruik, en is ook op versoek aan eksterne institute vir eksperimentele gebruik verskaf. Vrugtevlieskolonies moet gereeld met wilde vlieë versterk word ten einde verlies aan kenmerke te voorkom. Die Mediterreense kolonie is in September 2017 versterk deur 305 mannetjies wat vanaf koffie, *Coffea canophora*, geteel is en by Burgershall versamel is, met 386 wyfies vanaf die CRI-laboratorium kolonie te kruis. Tussen Augustus 2017 en Februarie 2018, is twee Kaapse vlieg kolonies afkomstig vanaf twee areas (Elandsvallei en Pretoria) gevestig. Vanaf Elandsvlei is 331 Kaapse vlieg volwassenes wat in 3-komponent Biolure lokvalle gevang is, as stigtersvlies gebruik. Vanaf Pretoria is 410 Kaapse vlieg volwassenes wat vanaf pynappel koejawel, *Acca sellowiana*, geteel is, as stigtersvlies gebruik. In Maart 2018 is 'n kolonie Oosterse vrugtevlies hervestig deur 395 volwassenes wat vanaf mango's, *Mangifera indica*, geteel en in Nelspruit versamel is, te gebruik. Gemiddelde (\pm SE) weeklikse papiestadium volume (ml) Mediterreense vrugtevlies, Nataalse vlieg, Marula vlieg en Oosterse vrugtevlies geproduseer, was onderskeidelik 417.2 ± 16.1 , 269.2 ± 10.2 , 128.3 ± 6.1 en 215.0 ± 11.3 . Een ml van die papie volume bevat ongeveer 50 papies. Baie min papies is weekliks vir die twee Kaapse vlieg kolonies geproduseer. Gemiddelde (\pm SE) eier uitbroeitempo's vir die Mediterreense vrugtevlies, Nataalse vlieg, Kaapse vlieg, Marula vlieg en Oosterse vrugtevlies was $85.0 \pm 3.5\%$, $92.1 \pm 1.4\%$, $75.9 \pm 1.7\%$, $95.5 \pm 2.1\%$ en $78.2 \pm 4.6\%$. Gemiddelde (\pm SE) volwasse verskyningstempo's vir Mediterreense vrugtevlies, Nataalse vlieg, Kaapse vlieg, Marula vlieg en Oosterse vrugtevlies was $95.8 \pm 1.9\%$, $96.6 \pm 1.4\%$, $42.8 \pm 7.1\%$, $93.7 \pm 1.6\%$ en $94.4 \pm 1.5\%$.

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

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

Summary

The key outcomes of this project were to determine the fruit species and varieties used by the Oriental fruit fly, *Bactrocera dorsalis*, with a particular emphasis on citrus, and the properties of citrus varieties that make them more or less susceptible to attack. *Bactrocera dorsalis* were reared from seven plant species: *Mangifera indica* cv. [Tommy Atkins, Sensation], *Citrus sinensis* cv. [Valencia], *Psidium guajava*, *Anacardium occidentale*, *Solanum mauritianum*, *Xylothea kraussiana* and *Vangueria infausta*. Fruit utilised by *B. dorsalis* was also infested or damaged by other species, which may indicate opportunism by the pest. Studies were then performed using Star Ruby grapefruit, Eureka lemons, Glen Ora Late navels, Delta Valencia, and Nadorcott mandarins to assess their susceptibility and suitability under laboratory and semi-field conditions. Female *B. dorsalis* did not oviposit on undamaged Star Ruby, Glen Ora Late, Delta Valencia or Nadorcott in the laboratory. When eggs were artificially deposited into the flavedo and albedo of the five citrus types, no pupae were recovered. Larval development occurred only when eggs were placed in the pulp of the five citrus types. Larval development time in the pulp increased with higher pupal recovery, which may indicate resource competition among larvae. Under semi-field conditions where *B. dorsalis* females were forced to encounter fruit of the five tested citrus types, eggs were laid into intact Star Ruby, Eureka, and Nadorcott, but

none supported the development of *B. dorsalis*. Overall, these results suggest that citrus types grown for export are not at high risk of infestation by *B. dorsalis* due to the protection afforded by the peel. Successful development only occurred when eggs were deposited in the pulp of damaged fruit. This highlights the importance of sanitation, packhouse sorting, and the integration of *B. dorsalis* management with other citrus pests.

Opsomming

Die hoof doelwitte van die projek is om die vrugspesies en -variëteite te bepaal wat deur die Oosterse vrugtevlieg, *Bactrocera dorsalis*, benut word, met spesifieke klem op sitrus en die eienskappe van sitrusvariëteite wat die vrugte meer of minder vatbaar vir aanvalle maak. *Bactrocera dorsalis* is op sewe plantspesies geteel: *Mangifera indica* cv. [Tommy Atkins, Sensation], *Citrus sinensis* cv. [Valencia], *Psidium guajava*, *Anacardium occidentale*, *Solanum mauritianum*, *Xylothea kraussiana* en *Vangueria infausta*. Vrugte wat deur *B. dorsalis* benut word, is ook deur ander spesies geïnfesteer of beskadig, wat opportuniste deur die plaag kan aandui. Studies is toe uitgevoer deur gebruik te maak van 'Star Ruby' pomelo, 'Eureka' suurlemoene, 'Glen Ora Late' navels, 'Delta Valencia' en 'Nadorcott' mandaryne om hul vatbaarheid en geskiktheid onder laboratorium en semi-veldtoestande vas te stel. Geen eierlegging deur wyfie *B. dorsalis* het op onbeskadigde 'Star Ruby', 'Glen Ora Late', 'Delta Valencia' of 'Nadorcott' in die laboratorium plaasgevind nie. Geen papies is verkry ná kunsmatige plasing van eiers in die 'flavedo' en 'albedo' van die vyf sitrustipes nie. Ontwikkeling van larwes het slegs plaasgevind wanneer eiers binne die pulp van die vyf sitrustipes geplaas is. Die larwe ontwikkelingsyklusperk in die pulp het toegeneem met hoër papie verhalings, wat op hulpbron kompetisie tussen larwes kan dui. Onder semi-veldtoestande waar *B. dorsalis* wyfies geforseer is om met vrugte van die vyf getoetste sitrustipes kontak te maak, is eiers in onbeskadigde 'Star Ruby', 'Eureka' en 'Nadorcott' gelê, maar geen van hulle het die ontwikkeling van *B. dorsalis* ondersteun nie. Oor die algemeen dui hierdie resultate daarop dat sitrustipes wat vir uitvoer verbou word, nie 'n hoë risiko toon vir infestasië deur *B. dorsalis* nie, weens die beskerming verskaf deur die skil. Suksesvolle ontwikkeling het slegs plaasgevind wanneer eiers in die pulp van beskadigde vrugte gelê is. Dit beklemtoon die belang van sanitasie, pakhuis sortering en die integrasie van *B. dorsalis* bestuur met ander sitrusplae.

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

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

Summary

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

Opsomming

Proteïen lokaas-toediening is een van die belangrikste beheerhulpmiddels in 'n vrugtevlug bestuursprogram in 'n kommersiële sitrusboord in suidelike Afrika. Vrugtevlugbeheer met proteïen lokaasmiddels werk op 'n " aantrek en doodmaak " beginsel, waar volwasse vlieë na proteïenagtige reuke in 'n lokaasmiddel aangetrek word, en daaropvolgend deur 'n insekdoder binne die lokaasmiddel-omgewing gedood word. Proteïen lokaasmiddels word as spuite of stasies toegedien. Hierdie projek het drie hoof doelwitte gehad: (1) Evaluasie van alternatiewe vrugtevlug lokmiddels, (2) ondersoek van alternatiewe gifstowwe in lokaasmiddels en (3) ondersoek van 'n nuwe vrystellingstegnologie vir lokaasmiddels. Reaksies van drie *Ceratitis* plaagspesies: *C. capitata*, *C. rosa* en *C. cosyra* op huidig gebruikte lokaasmiddels, HymLure, GF-120 en M3 lokaasmiddel, is eerstens in veldhokke geëvalueer. *Ceratitis capitata* was die spesie wat die meeste op al die lokaasmiddels wat getoets is, gereageer het. *Ceratitis capitata* en *C. rosa* het goed gereageer op al die lokaasmiddels wat getoets is. *Ceratitis cosyra*, aan die ander kant, het swak op HymLure gereageer. In 'n tweede reeks veldhok- en veldproewe, is twee nuwe belowende lokmiddels gevind, ProLure en Mazoferm, wat aanloklik vir al drie *Ceratitis* spesies was. Effektiviteit van verskillende insekdoders, in kombinasies met verskillende konsentrasies HymLure, is in laboratoriumproewe op *C. capitata* wyfies geëvalueer. Daar is gevind dat Spinosad die effektiëste alternatiewe gifstof vir malathion was vir gebruik saam met HymLure teen 2% en 10% (verdunding met water). 'n Papier-gebaseerde stasie, bevattende 'n malathion-gebaseerde lokaasmiddel (nou verwys na as *Tephri pyramid*) is ontwikkel. In veldproewe wat oor twee jaar (2016 en 2017) in Midnight Valencia boorde in Mpumalanga, Oos-Kaap en Wes-Kaap uitgevoer is, was die papier-gebaseerde lokaasstasie, bevattende malathion teen 200 dele per ha, net so effektië in die beheer van vrugtevlugplaagpopulasies, as M3 lokaasstasie teen 300 dele per ha.

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

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

Summary

There have been previous claims of phytotoxicity on citrus fruit when copper sprays and fruit fly baits are combined. In this study, incompatibility between applications of copper products and fruit fly baits on Late Valencia fruit was investigated in an orchard at Crocodile Valley Citrus, Nelspruit, Mpumalanga in 2017 and 2018. Three copper products were evaluated: cuprous oxide, copper hydroxide and copper oxychloride each applied at different rates (up to four consecutive monthly applications). A treatment of no copper was included whereby fruit were either bagged or shielded during application of copper sprays. Two types of fruit fly baits: GF-120 and a mixture of HymLure and malathion were applied on fruit which were either previously exposed to a copper treatment or to no copper. In the first year, baits were applied only once on fruit as a 2 ml cover spray. In the second year, baits were applied only once as 4 2 µl droplets on 4 marked areas at the stylar end of each tested fruit at three selected times of the year (February, March and April). Different fruit were used for the different fruit fly bait application times. Phytotoxicity on fruit due to treatments applied were assessed on naturally ripened fruit. In the first year, the major damage symptom observed on tested fruit was discoloration (lack of full colour). However in that trial year, there were no significant differences in percentages of fruit showing discoloration between treatments (combinations of copper treatments and fruit fly baits) and control (combinations of no copper and fruit fly baits).

Opsomming

Daar was al vorige bewerings van fitotoksiteit op sitrusvrugte wanneer koperspuite en vrugtevlug lokaasmiddels gekombineer is. In hierdie studie is onverenigbaarheid tussen toedienings van koperprodukte en vrugtevlug lokaasmiddels op 'Late Valencia' vrugte in 'n boord by Crocodile Valley Citrus, Nelspruit, Mpumalanga, in 2017 en 2018 ondersoek. Drie koperprodukte is geëvalueer: Koper-I-oksied, koperhidroksied en koperoksichloried, elk teen verskillende tempo's toegedien (tot vier opeenvolgende maandelikse toedienings). 'n Behandeling van geen koper is ingesluit waar vrugte gedurende toediening van koperspuite óf in sakkies toegemaak is, óf beskerm is. Twee tipes vrugtevlug lokaasmiddels, GF-120 en 'n mengsel van HymLure en malathion, is op vrugte toegedien wat óf voorheen aan 'n koperbehandeling blootgestel was, óf aan geen koper nie. In die eerste jaar is lokaasmiddels slegs een keer aan vrugte as 'n 2 ml bedekkingspuit

toegedien. In die tweede jaar is lokaasmiddels slegs een keer as 4 2 µl druppels op 4 gemerkte areas by die styl-ent van elke getoetste vrug by drie geselekteerde tye toegedien (Februarie, Maart en April). Verskillende vrugte is vir die verskillende vrugtevlieg lokaasmiddel toedieningstye gebruik. Fitotoksisiteit op vrugte weens behandelings wat toegedien is, is op natuurlik rypwordende vrugte bepaal. In die eerste jaar was verkleuring (tekort aan vol kleur) die grootste skade simptome wat op die getoetste vrugte waargeneem is. Gedurende daardie proefjaar was daar egter geen betekenisvolle verskille in persentasies van vrugte wat verkleuring getoon het tussen behandelings (kombinasies van koperbehandelings en vrugtevlieg lokaasmiddels) en kontrole (kombinasies van geen koper en vrugtevlieg lokaasmiddels) nie.

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

Project 1170 (April 2016 – March 2019) by J Daneel and A Manrakhan (CRI)

Summary

The Natal fly, *Ceratitis rosa*, was recently split into two species: *C. rosa* and *Ceratitis quilicii*. The aim of the project was to quantify similarities and differences between *C. rosa* and *C. quilicii* in citrus. The relative abundance of the two species were determined using attractant-based traps in nine citrus farms in Limpopo and Mpumalanga provinces. The larval development of the two species was determined in four citrus types (sweet orange, soft citrus, grapefruit and lemon) by artificial inoculation of eggs in the pulp and dissection of fruit daily thereafter for 15 days. In the relative abundance study, *C. quilicii* was found to be the most widely distributed of the two species among the study sites. *Ceratitis rosa* was more dominant in the low altitude hot-wet regions. In the field, neither of the two species were reared from citrus collected from the trees and from the ground. In artificial conditions in the laboratory, however, fruit fly development of both species was more optimal in oranges compared to the other citrus types. The peaks in development of each larval instar were the same for both species. Each instar of both species can be exposed to a cold treatment on the same day to determine the least susceptible instar.

Opsomming

Die Natalse vrugtevlieg, *Ceratitis rosa*, is onlangs verdeel in twee spesies: *C. rosa* en *Ceratitis quilicii*. Die doel van hierdie projek was om die ooreenkomste en verskille tussen *C. rosa* en *C. quilicii* in sitrus te kwantifiseer. Die relatiewe rykdom van die twee spesies was bepaal deur lokaas gebaseerde lokvalle te gebruik wat oor nege sitrusplase in die Limpopo and Mpumalanga provinsies versprei was. Die larwes van die twee spesies se ontwikkeling was in vier sitrussoorte (soet lemoene, sagte sitrus, pomelos en suurlemoene) bepaal, deur die pulp van die vrugte kunsmatig met eiers te inokkuleer en daarna vrugte vir 15 dae lank, daaglik te dissekteer. In die relatiewe rykdom studie was bevind dat van die twee spesies, *C. quilicii* die wydste oor al die studieterreine versprei was. *Ceratitis rosa* was meer dominant op die lae hoogtes in die warm-klam streke. Nie een van die twee spesies kon vanuit vrugte van bome of opgetelde vrugte wat in sitrusboorde versamel was, geteel word nie. In kunsmatige laboratorium omstandighede was vrugtevlieg ontwikkeling egter vir beide spesies meer optimaal in lemoene, in vergelyking met die ander sitrustipes. Die ontwikkelingspieke vir elke larwe instar was dieselfde vir beide spesies. Elke instar vir beide spesies kan op dieselfde dag aan 'n koue behandeling blootgestel word om die minste vatbare instar te bepaal.

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

Project 1171 (April 2017- March 2019) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Glorious Shongwe, Sean Moore, Vaughan Hattingh (CRI)

Summary

A partial cold treatment at 4°C for 22-26 days for False Codling Moth (FCM), *Thaumatotibia leucotreta*, is currently being investigated as part of a systems approach for risk management of the pest. The cold treatment at 4°C falls outside of the approved range of cold treatment temperatures for pest fruit flies of phytosanitary concern on citrus from southern Africa. These pest fruit flies are *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal

fly), *Ceratitis quilicii* (Cape fly) and *Bactrocera dorsalis* (oriental fruit fly). The efficacy of a disinfestation treatment of 4°C for 22-26 days for fruit fly pests should as such be investigated. The objectives of this study were to determine the relative cold tolerances of third instar larvae of the four fruit fly pests and the efficacy of the 4°C treatment for 22-26 days on the most cold tolerant fruit fly species. So far, tests could only be carried out on three fruit fly species: Medfly, Natal fly and oriental fruit fly. *In vitro* development of the three fruit fly species was first determined in order to establish incubation times required for the third larval stage. *In vitro* cold tolerances of the third instar larvae of the fruit fly species were then determined at 4°C for 4, 6, 8, 12, 16, 18, 20 and 22 days. At a constant temperature of 26°C, all three species were at their third instar larval stage at 6 days after inoculation of eggs on artificial diet. Only two replicates of the cold tolerance study could be conducted. Mean corrected mortality of Medfly larvae at 4, 6 and 8 days were 35.4% ± 17.7%, 58.7% ± 19.6% and 91.1% ± 3.3% respectively. Mean corrected mortality of Natal fly larvae at 4, 6 and 8 days were 90.1% ± 5.5%, 98.2% ± 1.8% and 99.7% ± 0.3%. Mean corrected mortality for oriental fruit fly larvae at 4, 6 and 8 days were 55.3% ± 13.8%, 91.5% ± 7.0% and 99.7% ± 0.3%. There were no survivors of any species beyond 8 days of cold exposure at 4°C. Results so far indicate that Medfly is more cold tolerant than Natal fly and oriental fruit fly.

Opsomming

’n Gedeeltelike koue-behandeling teen 4°C vir 22-26 dae vir Valskodlingmot (FCM), *Thaumatotibia leucotreta*, word tans as deel van ’n sisteembenadering vir risikobestuur van die plaag ondersoek. Die koue-behandeling teen 4°C val buite die goedgekeurde reeks van koue-behandeling temperature vir vrugtevliegplae van fitosanitêre belang op sitrus vanaf suidelike Afrika. Hierdie vrugtevliegplae is *Ceratitis capitata* (Mediterreense vrugtevlieg), *Ceratitis rosa* (Natalse vlieg), *Ceratitis quilicii* (Kaapse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg). Die effektiwiteit van ’n disinfestasië behandeling van 4°C vir 22-26 dae vir vrugtevliegplae moet as sodanig ondersoek word. Die doelwitte van hierdie studie was om die relatiewe koue-toleransies van derde instar larwes van die vier vrugtevliegplae te bepaal, en die effektiwiteit van die 4°C behandeling vir 22-26 dae op die mees koue-bestande vrugtevliegspesies. Sover kon toetse nog net op drie vrugtevliegspesies uitgevoer word: Mediterreense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg. *In vitro* ontwikkeling van die drie vrugtevliegspesies is eerste bepaal ten einde inkubasietye benodig vir die derde larwe stadium vas te stel. *In vitro* koue-toleransies van die derde instar larwe van die vrugtevliegspesies is dan teen 4°C vir 4, 6, 8, 12, 16, 18, 20 en 22 dae vasgestel. By ’n konstante temperatuur van 26°C, was al drie spesies by hul derde instar larwe stadium by 6 dae ná inokulasie van eiers op kunsmatige voeding. Slegs twee herhalings van die koue-toleransie studie kon uitgevoer word. Gemiddelde mortaliteit van Mediterreense vrugtevlieg larwes by 4, 6 en 8 dae was onderskeidelik 35.4% ± 17.7%, 58.7% ± 19.6% en 91.1% ± 3.3%.

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

Project 1177 (2017-2020) by Martin Gilbert (CRI)

Summary

A trial site has been identified near Porterville on a farm where grapes and citrus are grown close together. Previous results from project 1081 have shown how large numbers of fruit fly persist in grape plantings after harvest and pose a danger to nearby citrus, particularly early maturing soft citrus. Unfortunately a suitable trial site could not be identified before the soft citrus harvest period. Nevertheless, at the present time, the value or otherwise of extra investment of M3s being hung in lemon orchards is being investigated as these are traditionally not sprayed for fruit fly control in this area. Lemon blocks may therefore also serve as a reservoir for fruit fly. Initial results show that lemon orchards host large numbers of fruit flies even though damage to the fruit does not occur. The experiment will be expanded to include soft citrus and grapes during the 2018/19 season.

Opsomming

'n Proefperseel is naby Porterville op 'n plaas geïdentifiseer waar druiwe en sitrus naby mekaar verbou word. Vorige resultate vanuit projek 1081 het aangedui hoe groot getalle vrugtevlieë in druiwe-aanplantings bly ná oes, en daardeur gevaar inhou vir nabygeleë sitrus, veral vroeg-rypwordende sagte sitrus. 'n Geskikte proefperseel kon ongelukkig nie vóór die sagte sitrus oesperiode geïdentifiseer word nie. Tans word die waarde of andersins die ekstra belegging van M3's wat in suurlemoenboorde gehang word, ondersoek aangesien dit nie tradisioneel vir vrugtevliegbeheer in hierdie area gespuit word nie. Suurlemoenblokke kan dus ook as 'n reservoir vir vrugtevlieë dien. Aanvanklike resultate dui daarop dat suurlemoenboorde groot getalle vrugtevlieë huisves, selfs al kom skade aan die vrugte nie voor nie. Die proef gaan uitgebrei word ten einde sagte sitrus en druiwe gedurende die 2018/19 seisoen in te sluit.

2.3.12 **PROGRESS REPORT: Cold tolerance of immature stages of *Ceratitis capitata* (Wiedemann) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in artificial diet**

Project 1213 (January 2018) by Aruna Manrakhan, John-Henry Daneel, Peter Stephen & Vaughan Hattingh (CRI)

Summary

The cold tolerances of four immature stages of *Ceratitis capitata* (Medfly) and *Bactrocera dorsalis* (oriental fruit fly) were compared concurrently at $-0.59^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ in a carrot-based medium. Mortality rates of immature stages of the two fruit fly species were determined at six exposure periods over 11 consecutive days. Medfly was more cold tolerant than oriental fruit fly for all life stages tested. These results demonstrate that current cold treatment schedules for citrus fruit exports from South Africa for disinfestation of *C. capitata* would also be efficacious against *B. dorsalis*.

Opsomming

Die koue-toleransies van vier onvolwasse stadiums van *Ceratitis capitata* (Mediterreense vrugtevlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg) is gelyktydig teen $-0.59^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ in 'n geelwortel-gebaseerde medium vergelyk. Mortaliteitstempo's van onvolwasse stadiums van die twee vrugtevliegspesies is by ses blootstellingsperiodes oor 11 opeenvolgende dae bepaal. Mediterreense vrugtevlieg was meer koue-bestand as Oosterse vrugtevlieg vir alle lewensstadiums wat getoets is. Hierdie resultate demonstreer dat huidige koue-behandelingskedules vir sitrus vruguitvoere vanaf Suid-Afrika vir disinfestasië van *C. capitata* ook effektief teen *B. dorsalis* sal wees.

2.4 **PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS**

Programme coordinator: Sean D Moore (CRI)

2.4.1 **Programme summary**

Although the two main phytosanitary pests for citrus in southern Africa are considered to be FCM and fruit flies, certain of the mealybug species known to occur on citrus also have phytosanitary status for certain export markets. One of these species is *Delotococcus aberiae*, of which conspicuous outbreaks were reported in Letsitele a few years ago. A project was initiated to survey for parasitoids attacking this species (2.4.4). However, *D. aberiae* infestation has been a lot lower during the the last couple of seasons and consequently no parasitoids were found and it was not possible to establish a laboratory culture. Additionally, another such study is being conducted by a Spanish student, from whom results will be obtained once completed. Another project on mealybug investigated a range of biological traits of selected entomopathogenic fungi, as previously only the virulence of these isolates has been examined (2.4.5). This included humidity requirements, temperature tolerance, UV sensitivity and endophytic potential. The lower and upper thermal thresholds for all isolates were 6 and 35°C, respectively, with optimal growth between 25 and 29°C. Humidity did not seem to influence virulence much. However, conidia of all isolates were extremely sensitive to UV radiation, with complete inactivation after only 2 h exposure. Another project on entomopathogenic fungi looked at the performance of commercially available products against key citrus pests in the field (2.4.6). Unfortunately, no significant efficacy was recorded against red scale, mealybug and thrips, even after four monthly sprays. A

project on the development of GRAS postharvest fumigation treatments for phytosanitary pests came to an end. The project consisted of two components: the first was the evaluation of Vapormate (2.4.2) and the second was the evaluation of a combination of CO₂ and cold (2.4.3). A 4 h fumigation with Vapormate effectively controlled grain chinch bug, oribatulid mites, armoured scale insects and motile mealybugs, but was not effective against internal pests. However, CO₂ fumigation, followed by a short cold treatment was effective against FCM and fruit fly larvae in fruit. This combination is now being optimised for commercial use under a new project within the FCM programme.

Programopsomming

Alhoewel VKM en vrugtevlieë beskou word as die twee hoof fitosanitêre plae op sitrus in suidelike Afrika, het sekere van die witluis spesies wat op sitrus voorkom ook fitosanitêre status vir sekere markte. Een van hierdie spesies is *Delotococcus aberiae*, waarvan oplettende uitbrake op Letsitele 'n paar jaar gelede gerapporteer is. 'n Projek is geïnisieer om 'n opname te doen vir parasiete van hierdie spesie (2.4.4), maar *D. aberiae* besmetting is die laaste paar seisoene heelwat laer en gevolglik is geen parasitisme gekry nie en 'n laboratorium kultuur kon ook nie gevestig word nie. Daarbenewens word nog so 'n studie deur 'n Spaanse student gedoen en ons sal sy resultate van hom kry sodra die studie voltooi is. Nog 'n projek op witluis, onder andere, het 'n reeks biologiese eienskappe van geselekteerde entomopatogeniese swamme ondersoek, omrede net virulensie van hierdie isolate is voorheen ondersoek (2.4.5). Hierdie het humiditeits vereistes, temperatuur toleransie, UV sensitiwiteit en endofitiese vermoë ingesluit. Die boonste en onderste termiese drempelwaardes vir alle isolate was onderskeidelik 6 en 35°C, met optimale groei tussen 25 en 29°C. Blykbaar het humiditeit nie virulensie veel beïnvloed nie, maar konidia van alle isolate is baie gevoelig vir UV bestraling, met volledige inaktivering na net 2 ure se blootstelling. Nog 'n projek op entomopathogeniese swamme het die optrede van kommersieel beskikbare produkte teen sleutel sitrusplae in die veld ondersoek (2.4.6). Ongelukkig is geen betekenisvolle werking teen rooidopluis, witluis en blaaspootjie ondersoek nie, selfs na vier maandelikse bespuitings. 'n Projek op die ontwikkeling van GRAS (algemeen beskou as veilig) na-oes berokings behandelings vir fitosanitêre plae het tot 'n einde gekom. Die projek het twee dele besit: die eerste was evaluasie van Vapormate (2.4.2) en die tweede was evaluasie van 'n kombinasie van CO₂ en koue (2.4.3). 'n 4 uur berokking met Vapormate het graanstinkbesie, oribatulied myte, harde dopluis insekte en beweeglike witluis doeltreffend beheer, maar was nie teen interne plae doeltreffend nie. CO₂ berokking, gevolg deur 'n kort koue behandeling was teen VKM en vrugtevlieë larwes in vrugte doeltreffend. Hierdie kombinasie word nou onder 'n nuwe projek binne die VKM program geoptimaliseer vir kommersiële gebruik.

2.4.2 FINAL REPORT: Evaluating GRAS post-harvest fumigants for phytosanitary pests Project 913 (2011/2 – 2016/7) by T G Grout, P R Stephen and K C Stoltz (CRI)

Summary

The objectives of this research were to develop specific Vapormate treatments that will guarantee freedom from external phytosanitary pests and to develop the most effective combination of fumigation with Vapormate and a short cold treatment to control the internal phytosanitary pests, fruit fly and false codling moth. Successful control of grain chinch bug and oribatulid mites was achieved with Vapormate and published, resulting in registration on citrus in South Africa. The same treatment for 4 h controlled two different species of armoured scale insects and motile mealybugs. However, results with Vapormate against internal pests were erratic and more variable than those achieved with carbon dioxide fumigation so research with Vapormate against these pests was curtailed and emphasis placed on investigating the sequential use of carbon dioxide fumigation followed by a short cold treatment.

Opsomming

Die doelwitte van hierdie navorsing was om spesifieke Vapormate behandelings te ontwikkel wat vryheid van eksterne fitosanitêre plae sal waarborg, en om die mees effektiewe kombinasie van berokking met Vapormate en 'n kort koue-behandeling te ontwikkel, ten einde die interne fitosanitêre plae, vrugtevlieë en valskodlingmot, te beheer. Suksesvolle beheer van graanstinkluis en 'oribatulid' myte is met Vapormate behaal en gepubliseer,

wat tot die registrasie op sitrus in Suid-Afrika gelei het. Dieselfde behandeling vir 4 h het twee verskillende spesies van gepantserde dopluise en beweeglike witluise beheer. Resultate met Vapormate teen interne plaë was egter wisselvallig en meer variërend as dié verkry met koolstofdiksiedberoking, so navorsing met Vapormate teen hierdie plaë is beperk, en klem is geplaas op die ondersoek van gebruik van koolstofdiksiedberoking, gevolg deur 'n kort koue-behandeling.

Introduction

The only post-harvest disinfestation treatment currently in commercial use for false codling moth (FCM) *Thaumatotibia leucotreta* (Meyrick) 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 Clementines 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. Additionally, phytosanitary, or quarantine pests result in rejection of export consignments of citrus at extremely low levels of infestation. Growers therefore have to control pests at levels in the orchards that are not sustainable for their natural enemies. Some pests such as the grain chinch bug, *Macchiademus diplopterus* (Distant), are not even pests of citrus but are hitch-hikers looking for somewhere to hide (Myburgh and Kriegler 1967, Johnson and Addison 2008, Johnson and Neven 2011). If these pests could be controlled by using a relatively safe fumigant before the fruit is packed, higher levels of pests could be tolerated in the orchard that would be more sustainable for Integrated Pest Management and biological control.

Vapormate is a GRAS (generally recognised as safe) fumigant containing ethyl formate and carbon dioxide that has been commercially used for the fumigation of pests of dried fruit in Australia for several years (Ryan et al. 2004). Although it is not very effective against insect eggs (Soderstrom et al. 1991, Simpson et al. 2007), it has shown great promise for *Thrips obscuratus* (Crawford) (Chhagan et al. 2013), adult western flower thrips *Frankliniella occidentalis* (Pergande) and third instar California red scale *Aonidiella aurantii* (Maskell) (Pupin et al. 2013) as well as motile life stages of grape mealybug *Pseudococcus maritimus* (Ehrhorn) (Simpson et al. 2007). It has also been evaluated for the control of cereal pests in silos where it is effective against beetles. The fumigant is usually effective after exposure periods of 24 h or less and is more effective at ambient temperatures than cold temperatures. On the other hand, Phosphine, which is sometimes recommended as an alternative to methyl bromide, may require exposure periods of 3 days or longer (Liu et al. 2018) and is not a GRAS chemical. Recently there has been research with Vapormate on bananas, kiwis, grapes and other fruit, but the only use on citrus has been in combination with a cold treatment (De Lima 2006).

With the increasing phytosanitary status of fruit flies (*Ceratitis* spp. and *Bactrocera dorsalis* (Hendel)), indigenous mealybugs and the need for a quicker alternative to cold disinfestation for FCM, Vapormate may be able to play an important role in the future, either alone or followed by a short cold treatment. This report covers research conducted with Vapormate on citrus involving a range of internal and external citrus pests between 2011 and 2017.

Stated objectives

1. Develop specific Vapormate treatments that will guarantee freedom from external phytosanitary pests such as the grain chinch bug, mealybugs, scale insects and Fullers rose beetle.
2. Develop the most effective combination of fumigation with Vapormate or carbon dioxide and a short cold treatment for the control of the internal phytosanitary pests fruit fly and false codling moth.

Materials and methods

All fumigation took place in chambers made with a steel frame, walls of galvanised sheet metal and a removable Perspex lid that was held tightly on a neoprene gasket with home-made G-clamps. The volume of each container was 280 L and each contained a small fan to circulate the gas mixture continually during fumigation. Gas entered the chamber through a valve at the bottom and left through a valve at the top on the

opposite side. A gas analyser was connected to the top outlet and when the desired concentration was reached the valves were closed and the chamber moved to a room with the required temperature for the fumigation period. Fruit was never placed directly on the floor of the container but empty wire baskets 10 cm high, were inverted on the floor and the first fruit placed in baskets on top of these.

Objective 1: External phytosanitary pests

Grain chinch bug

In Trial 17, specimens of the grain chinch bug (GCB) *Macchiademus (Blissus) diplopterus* were obtained from Citrusdal where they cause export rejections on citrus, deciduous fruit and pomegranates. Less than 100 bugs were received so after being held in the laboratory with a water supply, 30 were fumigated on 10 July 2012 with Vapormate at 225 g/m³ for 6 h at 25°C and 30 were kept as a control at 25°C. In each treatment the 30 GCB were split into 6 replicates of 5 each, placed in ventilated plastic specimen jars. The bugs were kept for a further 24 h at 25°C after the fumigation period before mortality was determined.

Another package of GCB was received from the Western Cape in the same month which allowed for a further trial on this pest in Trial 18. Eight replicates of 10 bugs each were used per treatment with the bugs contained in ventilated specimen jars. A water-saturated disk of blotting paper made by use of a paper punch was attached to the inner side of each jar to provide some moisture. The treatments were all conducted at 25°C and included an untreated control, Vapormate at 112 g/m³ for 6 h, 225 g/m³ for 4 h and 225 g/m³ for 6 h. After fumigation the bugs were kept at 25°C for 24 h before mortality was determined.

A third trial (20) was conducted with GCB on 24 July 2012 using bugs that had arrived on 18 July where the effect of Vapormate was compared with carbon dioxide alone at 70%. Nine replicates were used per treatment with 19 bugs per ventilated specimen jar per replicate and moist blotting paper disks to provide moisture. The treatments included an untreated control, carbon dioxide at 70% for 6 h, CO₂ at 70% for 24 h, and Vapormate at 112 g/m³ for 4 h. The trial was conducted at 25°C and mortality was determined after a further 24 h at this temperature.

For Trial 25, large numbers of GCB were provided by Dr Shelley Johnson of Stellenbosch University on 24 April 2013 and a trial was conducted with small petri dishes containing 10 bugs each and eight replicates per treatment. The bugs were transported in corrugated cardboard and this was tapped over a sheet of white paper to dislodge the bugs which were then transferred to the dishes using a fine paint brush. The treatments of Vapormate were 112 g/m³ for 4 h, 112 g/m³ for 6 h, 170 g/m³ for 4 h and 170 g/m³ for 6 h. An additional treatment with 70% CO₂ alone for 4 h was included as well as an untreated control where the bugs were placed in a fumigation chamber with the fan running. The trial was conducted at 25°C and mortality was determined the next day, 15 h after fumigation ended. At the end of each fumigation, the containers with the bugs were placed in front of a fan for 30 min to accelerate the removal of the fumigant.

A further GCB trial (26) was conducted on 26 April 2013 using the small petri dishes with 10 bugs per dish and 12 dishes per treatment. The treatments were 112 g/m³ for 2 h, 112 g/m³ for 3 h, 170 g/m³ for 2 h and 170 g/m³ for 4 h and an untreated control. This time mortality was determined only 1 h after removal from the fumigation chamber.

A confirmatory GCB trial (27) was conducted on 29 April 2013 using the large ventilated containers in Figure 1. In each container, three squares (40 x 40 mm) were saturated with water before the bugs were transferred using a brush. One container with 100 bugs was used as a control and six other containers were used with from 219 to 1 266 bugs per container. Although such large numbers of bugs were used, care was taken to ensure that only live bugs were placed in the containers. The only treatment given to all replicates was Vapormate 170 g/m³ for 6 h and mortality was determined 24 h after treatment to ensure that none had recovered from the treatment.



Figure 1. Small petri dish used in some grain chinch bug trials and larger containers with bugs used in the confirmatory trial. Bugs were tapped from the cardboard onto the white sheet and transferred to the containers using the brush.

Trial 38

In early 2014, several shipping containers of pears were rejected in Europe for GCB so we decided to get some more trial data on the efficacy of Vapormate 170 g/m³ for 6 h at 25°C that was previously used on bugs in containers without fruit. Core Fruit provided us with Forelle pears and Shelley Johnson from Stellenbosch University provided us with GCB collected from behind *Eucalyptus* bark in the Western Cape. We placed 21 pears in each of 25 clear plastic containers and sprinkled from 70 to 110 bugs over the pears in each box, then closed the lid. The boxes were held at room temperature for 22 h in order to provide time for the bugs to hide in the styler openings of the pears. On 1 July 2014, the lids were tapped and opened and 5 boxes placed in each of 5 fumigation chambers of 280 L volume. One fumigation chamber was kept as a control at 23°C while the others were fumigated at the same temperature using Vapormate 170 g/m³ for 6 h (measured as 7.4% CO₂ in the air). After 6 h the CO₂ concentration in the fumigation chambers was checked before the lids were removed and a fan was used to blow fresh air into each open fumigation chamber for 5 minutes before removing the boxes of fruit. The lids of all the fruit boxes were then closed and mortality determined the next morning. The base of each pear was sliced off with a knife to progressively display more of the styler opening and any bug that was discovered was recorded as alive or dead (not moving after prodding). The numbers of bugs in or on fruit were also recorded as well as the numbers that were found on the box surface.

Trial 39 was a repeat of Trial 38 but used GCB on Boschhoek navel oranges and a treatment of Vapormate 197 g/m³ for 6 h (measured as 8.6% CO₂ in the air) at 23°C on 3 July 2014. Only four plastic boxes containing 15 navel oranges each were used in each fumigation chamber because fruit were larger and the numbers of bugs limited. Similar numbers of bugs were sprinkled over the fruit in each box and left for 18 h before fumigation. After fumigation the boxes were ventilated as before, then left overnight before the fruit were cut and mortality determined.

Further trials (43-50) were conducted during February and March 2015 with GCB while this pest was abundant in the Western Cape and focused on verifying a treatment that would be 100% effective at Probit 8.7 or higher. Previous research by the authors in 2014 had resulted in 99.8% corrected mortality when using Vapormate at 170 g/m³ for 6 h at 25°C for GCB on Forelle pears or at 198 g/m³ for 6 h at 25°C on Boshhoek navel oranges (Grout and Stoltz 2016). As deciduous fruit exporters would prefer a shorter treatment period, we decided to evaluate 250 and 315 g/m³ for periods of 4 and 6 h in order to facilitate registration of the same treatment for the same pest on multiple crops. With all trials we included 20 Valencia oranges and 5 Packham Triumph pears in the fumigation chamber to absorb fumigant and check for phytotoxicity, but we placed the bugs in ventilated plastic containers amongst the fruit so that they were easy to recover for mortality determination.

In Trial 43, fruit was brought from 4°C to room temperature the day before treatment. Grain chinch bug was collected from behind bark on *Eucalyptus* trees in the Western Cape and sent by courier to Nelspruit shortly before the trial was due to start. With moisture present the bugs survive for a long time. All treatments were applied on 18 February 2015 at 25°C to maximise respiration and included an untreated control, Vapormate at 250 g/m³ for 4 h and for 6 h, and Vapormate at 315 g/m³ for 4 h and for 6 h. Approximately 250 bugs were used per treatment, shared between 4 ventilated containers placed amongst the fruit in each fumigation chamber. After the fumigation was complete, the bug containers were removed from the chambers and placed on a rack at 25°C for 24 h before checking for mortality indicated by no limb movement on prodding. The fruit was also checked at this time for any signs of phytotoxicity.

Trial 44 only involved an untreated control and Vapormate 250 g/m³ for 4 h. The fumigation took place on 19 February 2015 at 25°C and the bugs were held for a further 24 h at this temperature before evaluating mortality.

Trial 45 involved a comparison between two treatment temperatures, 25°C which had been used previously and 15°C which is a more realistic temperature for packhouses in the Cape in winter. Two control treatments were therefore used with one at each temperature and two fumigation treatments, both using Vapormate at 250 g/m³ for 4 h. Fumigation took place on 1 March 2015 and after treatment the fruit were held at 25°C for 24 h before evaluating GCB mortality. Trials 46 and 47 were identical to Trial 45 with fumigation taking place on 6 March and 12 March 2015 and evaluations one day later.

Trials 48, 49 and 50 were conducted for verification purposes of the Vapormate 250 g/m³ for 4 h treatment at 15°C in order to show that no survivors were found at this temperature. Fumigation took place on 18 March, 20 March and 30 March, respectively. Trial 50 involved Abate Fetel pears that had been sent to us in apple boxes from the Western Cape with a request that we move the pears to 0°C immediately after fumigation. Two further trials with pears were conducted just to check for phytotoxic effects and off-tastes when using this treatment. Three different types of stone fruit were also fumigated and tasted afterwards. No insects were included in these trials.

Mealybug and false codling moth eggs

A provisional trial (Trial 1) was conducted with citrus mealybug *Planococcus citri*, and vine mealybug, *Planococcus ficus* using oranges and grapes, respectively. This trial evaluated a concentration of 370 g/m³ Vapormate or 1.91% of the active ingredient ethyl formate in air. Second or third instar mealybugs were transferred onto fruit held in separate ventilated containers. Ten oranges were used for citrus mealybug with at least 5 mealybugs transferred from the culture to each fruit using a fine paintbrush. In the case of vine mealybug, 3 or 4 grape berries were placed in each of 10 ventilated containers and 5 mealybugs were transferred onto them. Three fumigation chambers were used in this trial so that temperatures of 15°C and 27°C could be evaluated for fumigation and compared with a control at 20°C. Both fruit types and insects were placed in wire baskets and stacked in each chamber. The fumigation was conducted on 12 May 2011. After each chamber containing the right dosage of fumigant was sealed, it was moved to a room at the required temperature where it was held for 6 h before the lids were removed and the fruit taken out of the chamber and stored at 25°C. Mealybug mortality was evaluated 2 h after removal from the chambers.

As the control mortality of citrus mealybug on oranges in Trial 1 had been quite high we decided to use small sprouting potatoes as a substrate with one potato per ventilated container (11 cm diam.) and 10 per treatment in Trial 2. Five vine mealybug second or third instars were placed on each potato and 10 citrus mealybug were used per potato. We evaluated Vapormate at 225 g/m³ on both mealybug species for 4 h at both 15°C and 25°C. This dosage of fumigant contains 9.8% CO₂ so the dosage was determined by measuring the CO₂ and it corresponded with the use of 120 g fumigant from the cylinder. A third treatment containing 9.8% CO₂ alone was also included with the fruit held at 20°C, the same temperature at which the untreated control was held. The fumigation took place on 27 July 2011 and mortality in all treatments was determined 40 h after the fumigation period was complete on 29 July 2011.

In a third trial (3), sprouting potatoes were again used in separate containers but only citrus mealybug adults without egg sacs were transferred from the culture onto the sprouts. Ten adults were used per potato and 10 potatoes per treatment. All treatments were held at 25°C during fumigation but two different fumigation dosages were used: 112 g/m³ for 4 h and 225 g/m³ for 2 h. The former dosage contained 4.9% CO₂ and used 60 g fumigant mixture in the 280 L chamber. Fumigation was conducted on 1 August 2011 and mortality was evaluated after 44 h on 3 August 2011. At the end of the fumigation period, compressed air was used to dispel fumigant from the chamber in a short time.

In Trial 4, adult citrus mealybugs were again evaluated and the eggs of FCM, which is a serious quarantine pest of citrus, stone fruit and macadamia nuts. Rather than using potatoes, a water-saturated piece of blotting paper (10x4 cm) was placed in the bottom of each container and a small plastic lid (30 mm diameter) similar to a Petri dish was placed on the paper. Either 10 adult mealybug were placed in each lid or a piece of grease-proof paper having at least 50 FCM eggs on it (from an insectary egg-sheet supplied by River Bioscience, Addo) was placed in the lid. Treatments were held at 26°C and included an untreated control, fumigation at 112 g/m³ for 2 h and fumigation at 56 g/m³ for 4 h. The latter dosage contained 2.5% CO₂. Fumigation started at around 12h00 on 15 August 2011. Mealybug mortality was determined 24 h later and the numbers of hatched FCM larvae counted after 2 days and again after 9 days.

After a negligible effect on FCM eggs in Trial 4 we decided to evaluate FCM eggs and citrus mealybug egg sacs on an artificial substrate at increased dosages in Trial 5. The small plastic lid was once again used but in the case of FCM eggs it was placed in the middle of a piece of rigid plastic sheeting that was coated with a thin layer of polybutene. A piece of egg sheet with approximately 150 eggs was placed in each lid for fumigation. Ten replicates were used per treatment and after fumigation the number of FCM larvae that had hatched and died in the dish or the polybutene was recorded. For citrus mealybug eggs, two egg sacs were placed in each plastic lid for fumigation and only after fumigation was each lid placed on the sticky card to prevent crawlers from falling on the sticky card accidentally when the egg sacs were placed in the dishes. Ten replicate lids were again used per treatment. The treatments included an untreated control stored at 25°C, fumigation at 112 g/m³ for 6 h and fumigation at 112 g/m³ for 4 h, both stored at 25°C for the fumigation period. After fumigation the chamber was blown out with compressed air and after all lids with mealybug egg sacs were placed on the sticky surfaces, the containers were held for 11 days before the numbers of hatched FCM larvae and mealybug crawlers were determined per replicate. Fumigation took place on 1 September 2011 and evaluation on 12 September 2011.

The same technique was used for Trial 6 as for Trial 5 with FCM eggs and citrus mealybug eggs but the dosage of fumigant and exposure period was increased significantly to 225 g/m³ for 24 h and 112 g/m³ for 24 h. These were compared to an untreated control and all fruit were fumigated at 25°C. Fumigation started on 15 September 2011 and FCM larvae were counted on 19 September and mealybug crawlers in polybutene on 23 September 2011.

The same technique was again used in Trial 7 against FCM eggs and citrus mealybug eggs but the centre of the lids in which the two mealybug egg sacs were placed was roughened to make it easier to attach the egg sacs. Treatments included an untreated control, fumigation at 112 g/m³ for 18 h and 112 g/m³ for 24 h; all at 25°C. Fumigation started on 26 September 2011 and both mealybug and FCM were evaluated on 4 October 2011.

Further fumigation of FCM eggs with Vapormate took place in Trial 21. Eggs were received from River Bioscience and fumigated the next day on 25 July 2012. Pieces of egg sheet with similar densities of eggs (approximately 110) and dimensions 10 x 10 mm were cut out and placed in a small petri dish which was placed in the centre of a larger petri dish that had been coated with polybutene adhesive. Ten replicate dishes were used per treatment. Fumigation treatments were Vapormate at 225 g/m³ for 4 h and 6 h, Vapormate at 112 g/m³ for 4 h and 6 h and CO₂ at 70% for 24 h. All fumigation took place at 25°C and after fumigation eggs were stored at 25°C for 7 d to wait for all viable eggs to hatch. The numbers of larvae that had hatched out were then recorded and compared with the numbers hatching in the control, expressed as percentage hatch.

Trial 22 was conducted with citrus mealybug egg sacs to obtain more data on the susceptibility of the eggs to Vapormate and carbon dioxide. The egg sacs were obtained from DuRoi's insectary in Letsitele on 26 July 2012 and each sac was placed in a small petri dish which in turn was placed on polybutene adhesive in a larger petri dish. Ten replicates were used per treatment. The treatments included an untreated control held at 25°C for 8 days, fumigation with Vapormate at 112 g/m³ for 4 h, 225 g/m³ for 4 h and CO₂ 70% for 24 h. Fumigation took place at 25°C after which all treatments were held at 25°C until 3 August when the numbers of crawlers that had emerged were determined for each treatment.

A further trial (23) with mealybug egg sacs was conducted using the same technique as Trial 22 but with Vapormate at 170 g/m³ for 4 h and 6 h and in comparison with CO₂ 70% for 24 h. Fumigation took place on 28 August 2012 at 25°C and the eggs were stored at 25°C for 7 days to ensure that all viable eggs had hatched. The numbers of crawlers from each egg sac were then determined.

A further trial (24) with FCM eggs on a 1 cm square piece of egg-sheet in a small dish surrounded by polybutene was started with fumigation on 28 August 2012. The treatments were Vapormate at 170 g/m³ for 4 h and 6 h and in comparison with CO₂ 70% for 24 h. Dishes were held for 7 days at 25°C to ensure that all eggs had hatched before numbers of larvae were determined.

The above research had shown that motile life stages of both the citrus mealybug and the vine mealybug were very susceptible to Vapormate, but eggs were much more difficult to control. Trials 51 to 53 were conducted with citrus mealybug *Planococcus citri* during April 2015 to determine whether Vapormate 250 g/m³ for 4 h could prevent eggs of the citrus mealybug from hatching. Fumigation periods of 4, 6, 10 and 24 h were evaluated at 25°C and 24 h was also evaluated at 15°C. Nineteen to 38 replicates were used per trial with one egg sac in a small petri dish per replicate. After fumigation, all treatments were followed by at least 7 days at 25°C to allow the eggs to hatch and crawlers to be captured on Vaseline surrounding the small dish containing each egg sac. The numbers of crawlers produced relative to the number of egg sacs were compared between the treatments and untreated controls.

Trial 54 was conducted in the same way as the previous mealybug trials but used vine mealybug (*Planococcus ficus*) egg sacs to see whether the results were similar to those achieved with citrus mealybug.

Armoured scales

A trial (62) was conducted with Valencia oranges from Malalane that were heavily infested with red scale, *Aonidiella aurantii*. We used Vapormate 250 g/m³ in air for 4 h at 25°C for a fumigation treatment on 31 August. As it was not possible to determine whether scale insects were alive or dead shortly after fumigation we incubated the fruit at 28°C for 14 d before inspecting them for the presence of whitecaps on 15 September. One hundred infested fruit were used as a control and another 100 infested fruit were fumigated.

A similar trial (63) was conducted to evaluate Vapormate 250 g/m³ for 4 h at 25°C against mussel scale, *Lepidosaphes beckii*, on Valencia oranges from L.A. Visagie, outside Nelspruit. One hundred infested fruit were fumigated on 19 August 2015 and these plus 100 untreated fruit were stored at 28°C until 4 September when they were inspected for the presence or absence of whitecaps.

Oribatulid mites

A citrus grower in KwaZulu-Natal was having export oranges rejected for the presence of harmless oribatulid mites *Siculobata sicula* that were seeking refuge under the calyx at harvest. Two trials were conducted to evaluate GRAS fumigants against these mites. Trial 59 evaluated Vapormate 250 g/m³ for 4 h at 25°C using 14 ventilated containers holding approximately 190 mites in each and a control with 7 ventilated containers with approximately 100 mites per container. A dental roll containing 2 ml water was included in each container to provide moisture. Mortality was determined the next day. A similar trial (Trial 60) was conducted to evaluate CO₂ alone at 70% for 4 h at the same temperature, but using 21 replicate containers in the control and treatment with approximately 500 mites per container. Mortalities were again determined 24 h later.

Fuller's rose beetle

Insufficient numbers of this insect could be found to conduct any experiments with fumigants on eggs.

Objective 2: Internal phytosanitary pests

False codling moth

Trial 11

The susceptibility of third instar FCM in larval carrot medium was evaluated at 56 g/m³ for 24 h, 112 g/m³ for 24 h and 225 g/m³ for 24 h and compared with an untreated control. The FCM larvae were earlier reared together from eggs hatching on 2 March. Ten larvae (second and third instars) were then transferred to each of 8 Petri dishes with some medium for each treatment and fumigation started on 9 March 2012. Larval mortality was determined shortly after the 24 h fumigation period.

Trial 12

Navel oranges were picked from Crocodile Valley Citrus Co., Nelspruit and dipped in imazalil 0.67 g/L and Sporekill 1 ml/L on 2 April 2012. The next day, pieces of egg-sheet paper containing approximately 50 FCM eggs were placed on each of 108 fruit in individual containers and the neonate larvae left to penetrate each fruit naturally. The fruit were stored at 25°C for the larvae to develop and were fumigated on 11 April when the larvae would have been third instars. An untreated control was compared with 112 g/m³ for 24 h and 225 g/m³ for 24 h using 36 fruit per treatment. The fruit were stored at 25°C during the fumigation period and for another 24 h after fumigation to allow for any delayed effect of fumigant inside the fruit. The mortality of larvae was determined by cutting the fruit and inspecting the larvae on 13 April 2012.

Trial 13

This trial investigated the possible additive effect of a cold treatment to the fumigation of exposed FCM larvae in carrot medium. FCM eggs were placed on the larval medium on 5 April 2012 and 10 resultant larvae were transferred to individual Petri dishes with some medium on 16 April. Ten replicate dishes were used per treatment on 16 April, the treatments being an untreated control, stored for 5 days at 25°C, a control that was later stored for 5 days at 1°C, fumigation at 105 g/m³ for 24 h followed by 5 days at 25°C and fumigation at 210 g/m³ for 24 h followed by 5 days at 1°C. The fumigation took place at 25°C and larval mortality was determined in all treatments 24 h after the dishes were removed from the cold treatment into 25°C.

Trial 15

Pieces of FCM egg sheet were placed on navel oranges as before on 15 June 2012 and the fruit stored at 25°C until 25 June when they were treated. Twenty fruit were kept as a control at 25°C and 20 fruit were fumigated at 225 g/m³ for 24 h while being held at 25°C. After the fumigation period, 10 fumigated fruit and 10 control fruit were moved to a cold room at -1°C while the rest of the fruit were kept at 25°C. The fruit was held at these two temperatures for 5 days, then the fruit was removed from the cold room on 1 July and held at 25°C for 24 h before the fruit from all treatments was cut and larval mortality determined.

Earlier unpublished research showed that mortality of FCM after fumigation with Vapormate varied considerably. Several trials were therefore undertaken to determine whether there were consistent differences in permeability between cultivars. The first trial (55) compared the susceptibility of third instar FCM in three different cultivars of navel oranges (Bahianinha, M7 and Palmer) when treated for 24 h at 25°C with Vapormate at either 250 or 315 g/m³ or with CO₂ 70%. Fruit was harvested at the optimal time, dipped in a mixture of three different fungicides and stored at 4°C until the trial was due to start. Fruit was removed from cold storage and held at 25°C for 24 h before moving to a laminar flow bench on 15 May 2015. In the laminar flow bench, squares of wax paper 10 x 10 mm with approximately 30 FCM eggs laid on them that were about to hatch, were placed on each fruit. After one day in the flow bench adequate numbers of larvae had hatched and penetrated the fruit so the paper squares were removed and the fruit transferred to a room held at 25°C for the larvae to develop. Seven days after placing the paper squares on the fruit, the fruit were again dipped in the

fungicide mixture. Ten days after placing the egg papers on the fruit the fruit were divided into groups of 60 sound fruit per cultivar and each batch divided into 15 infested fruit per treatment. Fifteen fruit per cultivar type were kept as control fruit and the others were used for the 3 treatments per cultivar which were applied in 280 L fumigation chambers on 25 May. After 24 h fumigation at 25°C the fruit was placed on shelves in a room held at 25°C for 24 h, after which the fruit were dissected and larval mortality determined. A further trial (Trial 58), compared the late navel orange Boshhoek with Palmer navel that had been stored at 4°C until the harvest time for Boshhoek. The same treatments as above were used with 15 infested fruit per treatment and control. Fumigation took place on 15 July 2015 which was 12 days after egg papers were placed on fruit in a laminar-flow bench. Evaluation was done by fruit dissection 24 h after removal from fumigation.

Mediterranean fruit fly

A provisional trial (1) was conducted with Medfly, *Ceratitidis capitata*, using grapes, oranges, apples and plums. This initial trial was conducted at a concentration of 370 g/m³ Vapormate or 1.91% of the active ingredient ethyl formate. Forty eggs were inoculated into a hole drilled into a fruit which was then sealed with wax. Ten each of Granny Smith apples, plums and navel oranges were used. The fruit were inoculated with the eggs, 8 days before fumigation so that the larvae were in the third instar at fumigation. The fruit were stored at 25°C during incubation. Three fumigation chambers were used in this trial so that temperatures of 15°C and 27°C could be evaluated for fumigation and compared with a control at 20°C. The fumigation was conducted on 12 May 2011. After each chamber was sealed with the fumigant it was moved to a room at the required temperature where it was held for 6 h before the lids were removed and the fruit taken out of the chamber and stored at 25°C. Fruit fly mortality was evaluated 4 days after removal from the chambers to take advantage of any residual effect of fumigant inside the fruit.

Trial 8

Having determined that a high dosage and long exposure period was required to control FCM eggs and mealybug eggs, attention switched to fruit fly larvae (*C. capitata*) with a trial being conducted with larvae in medium which was very permeable to the fumigant. Ten replicates were used per treatment with each replicate containing 10 third instars in bran-based, larval-rearing medium in a Petri dish. An untreated control was compared with fumigation at 112 g/m³ for 24 h. Both treatments were held at 25°C during the fumigation period. Treatment started on 16 February 2012 and the numbers of live larvae (or pupae) were determined shortly after the fumigation period.

Trial 9

The treatment in Trial 8 was repeated with Medfly third instars in bran larval medium as before with the fumigation taking place at 25°C for 24 h. This treatment was compared with an untreated control and both 56 g/m³ for 24 h and 225 g/m³ for 24 h, all at 25°C. Ten larvae were again used per replicate and 10 replicates per treatment. Fumigation started on 23 February 2012 and treatments were evaluated shortly after treatment was complete on 24 February.

Trial 10

The low dosage of Vapormate at 56 g/m³ for 24 h was used in combination with cold to determine whether the combination caused more mortality than cold alone. Ten replicates each with 10 third instar Medfly in bran medium in Petri dishes were used. Fumigation took place at 25°C on 29 February 2012 and after the 24 h period, the dishes were moved to two different cold rooms; one at 15°C and one at 1°C. A control with 10 replicates was also moved into each cold room at the same time. The larvae were kept in the cold rooms for 5 days then moved into a 25°C room for 24 h before determining mortality. Any pupae that had formed were kept until 22 March so that only those that emerged as adults were called alive.

Trial 14

To fumigate Medfly larvae in fruit, navel oranges were pricked with a row of eight spikes in three different places and hung in a cage of adult Medfly for 24 h for the females to oviposit in the holes. Twenty pricked fruit were hung in the Medfly cages on 7 June 2012 for 24 h, then stored at 25°C until 13 June (second instar) when 10 fruit were kept as an untreated control and 10 fruit were fumigated with Vapormate at 225 g/m³ for 24 h.

During the 24 h fumigation both the treated fruit and the control fruit were held at 25°C. The fruit were cut on 14 June, shortly after removing from the fumigation chamber and larval mortality determined.

Trial 16

Fresh Shamouti oranges were picked from Crocodile Valley Citrus Co. on 25 June 2012 and were each pricked in three places as before and hung in cages with Medfly on 27 June. After the 24 h oviposition opportunity the fruit were stored at 25°C until fumigation on 3 July when the larvae would have been second instars. Twenty fruit were used in the untreated control, another 20 were fumigated with Vapormate at 225 g/m³ for 24 h and for the first time another 20 fruit were fumigated with CO₂ only at 70% for 24 h as a comparison. All the fruit were stored at 25°C during fumigation then half the fruit from each treatment were moved into a coldroom at -0.5°C for 5 days while the other half of the fruit were kept at 25°C for 2 d before evaluation on 6 July. When the cold period was finished the fruit was stored at 25°C for 24 h before evaluating mortality on 10 July. In this trial a blind organoleptic test was conducted on 6 July on uninfested fruit that were treated as for the infested fruit at 25°C.

Results and discussion

Objective 1: External phytosanitary pests

Grain chinch bug

Trial 17

Fumigation of grain chinch bug at 225 g/m³ for 6 hours at 25°C gave 100% mortality and involved 30 insects. However, the control mortality was high with 33.3% of the 30 insects dying. This trial was therefore repeated at the same and other concentrations.

Trial 18

The results (Table 1) confirmed those in Trial 17 where 100% mortality was obtained with 420 g/m³ for 6 h. However, the 4 h exposure of this dosage and the 6 h exposure of 112 g/m³ for 6 h also gave 100% mortality. These results are similar to those found with immature or adult citrus and vine mealybug.

Table 1. Fumigation of grain chinch bug adults in ventilated containers

Treatments	Mortality (%)
Control 6 h at 25°C, followed by 24 h at 25°C	0
Vapormate 112 g/m ³ for 6 h at 25°C, followed by 24 h at 25°C	100
Vapormate 225 g/m ³ for 4 h at 25°C, followed by 24 h at 25°C	100
Vapormate 225 g/m ³ for 6 h at 25°C, followed by 24 h at 25°C	100

Trial 20

In the third trial, the carbon dioxide treatment of either 6 h or 24 h was completely effective (Table 2) and Vapormate at 112 g/m³ for 4 h gave 97.8% mortality. Control mortality was high at 23% but by now the bugs in this trial had been in the laboratory for one week.

Table 2. A comparison of CO₂ and Vapormate against grain chinch bug adults in ventilated containers in July 2012

Treatments	Alive in 90 bugs	Mortality (%)
Control 6 h at 25°C, followed by 24 h at 25°C	69	23.3
Vapormate 112 g/m ³ for 4 h at 25°C, followed by 24 h at 25°C	2	97.8

CO ₂ 60% for 6 h at 25°C, followed by 24 h at 25°C	0	100
CO ₂ 60% for 24 h at 25°C, followed by 6 h at 25°C	0	100

Trial 25

In the first GCB trial of April 2013, one survivor was obtained in the 170 g/m³ for 4 h treatment but none in the lower dosage treatments (Table 3). The shorter 4 h carbon dioxide treatment was completely ineffective so perhaps in the previous trial where a 6 h carbon dioxide treatment had caused 100% mortality the bugs were weak and more susceptible to it.

Table 3. Further comparisons of Vapormate treatments with CO₂ against grain chinch bug adults in ventilated containers in April 2013

Treatments	Alive in 80 bugs	Mortality (%)
Control 6 h at 25°C, followed by 24 h at 25°C	79	1.3
Vapormate 112 g/m ³ for 4 h at 25°C, followed by 24 h at 25°C	0	100.0
Vapormate 112 g/m ³ for 6 h at 25°C, followed by 24 h at 25°C	0	100.0
Vapormate 170 g/m ³ for 4 h at 25°C, followed by 24 h at 25°C	1	98.8
Vapormate 170 g/m ³ for 6 h at 25°C, followed by 24 h at 25°C	0	100.0
CO ₂ 60% for 4 h at 25°C, followed by 24 h at 25°C	80	0.0

Trial 26

In the fifth GCB trial lower dosages of Vapormate over time resulted in no survivors and there was no mortality in the control (Table 4). However, due to the one survivor in the previous treatment of 170 g/m³ for 4 h we decided to do a final confirmatory treatment at this dosage but for 2 h longer to ensure that no survivors were obtained.

Table 4. Further evaluations of Vapormate treatments against grain chinch bug adults in ventilated containers in April 2013

Treatments	Alive in 120 bugs	Mortality (%)
Control 4 h at 25°C, followed by 1 h at 25°C	120	0.0
Vapormate 112 g/m ³ for 2 h at 25°C, followed by 1 h at 25°C	0	100.0
Vapormate 112 g/m ³ for 4 h at 25°C, followed by 1 h at 25°C	0	100.0
Vapormate 170 g/m ³ for 2 h at 25°C, followed by 1 h at 25°C	0	100.0
Vapormate 170 g/m ³ for 4 h at 25°C, followed by 1 h at 25°C	0	100.0

Trial 27

The results of this final trial with Vapormate 170 g/m³ for 6 h were no survivors out of 3 543 treated bugs and seven dead bugs out of the 100 control bugs. If this result is combined with the previous result of this treatment shown in Table 3 it shows that no survivors were obtained in a total of 3 623 bugs.

Trial 38

The trial to evaluate Vapormate at 170 g/m³ for 6 h against grain chinch bug on Forelle pears gave 99.80% mortality or 4 survivors in 1942 individuals (Table 5). The percentage of bugs found in the boxes after fumigation that were either on or in the fruit was 36.9, the same percentage for control fruit was 58.2 and the overall percentage of bugs found on or in fruit was 41.2.

Table 5. The susceptibility of adult grain chinch bug to one treatment of Vapormate when given the opportunity to hide in the styler openings of Forelle pears

Outcome	Mean (%)	Standard error	Number of bugs involved
Percentage of live or dead bugs on treated fruit	36.9	2.04	707
Percentage of live or dead bugs on control fruit	58.2	2.95	290
Percentage of live or dead bugs on all fruit	41.2	2.44	997
Mortality in the untreated control	16.94	2.28	497
Mortality after Vapormate 170 g/m ³ for 6 h	99.80	0.11	1942

Trial 39

The trial to evaluate Vapormate at 197 g/m³ for 6 h against grain chinch bug on Boschhoek navel oranges gave 99.76% mortality or 4 survivors in 1588 individuals (Table 6). The percentage of bugs found in the boxes after fumigation that were either on or in the fruit was 12.7, the same percentage for control fruit was 36.4 and the overall percentage of bugs found on or in fruit was 17.5. Some extra bugs were left in plastic ventilated containers with *Eucalyptus* bark and one container was fumigated at the same time with some of the oranges and one was kept as a control. The mortality obtained of these extra bugs was 100% while the control mortality was 18.4% (Table 6). Comparing the percentage of bugs in each replicate box that were found on or in the fruit in this trial and the previous one on pears, it is clear that the phytosanitary risk with the pears is higher than these navel oranges because the proportion of bugs per replicate that were found on or in pears was twice as high as the proportion found on navel oranges.

Table 6. The susceptibility of adult grain chinch bug to one treatment of Vapormate when given the opportunity to hide in the styler openings of Boschhoek navel oranges

Outcome	Mean (%)	Standard error	Number of bugs involved
Percentage of live or dead bugs on treated fruit	12.7	1.42	205
Percentage of live or dead bugs on control fruit	36.4	8.68	123
Percentage of live or dead bugs on all fruit	17.5	2.90	328
Mortality in the untreated control	17.77	1.42	324
Mortality after Vapormate 197 g/m ³ for 6 h	99.76	0.17	1588
Mortality of extra bugs on bark in container after Vapormate 197 g/m ³ for 6 h	100.0	-	255
Mortality of untreated extra bugs on bark in container	18.4	-	125

All fumigation treatments in Trial 43 resulted in 100% mortality (Table 7), so we decided to continue with the lowest dose/shortest time combination of 250 g/m³ for 4 h in Trial 44 at the same temperature and that still provided 100% mortality of 1 208 bugs. When using Vapormate 250 g/m³ for 4 h against GCB at 15°C and 25°C no survivors were found at either temperature in any of the trials (Table 8).

Table 7. Fumigation of adult grain chinch bug with Valencia oranges and pears using Vapormate at 25°C

Trial	Fumigation treatment	Number of adult GCB treated	Mortality (%)
43	Untreated at 25°C	275	2.2
	Vapormate 250 g/m ³ for 4 h at 25°C	234	100.0
	Vapormate 250 g/m ³ for 6 h at 25°C	279	100.0
	Vapormate 315 g/m ³ for 4 h at 25°C	225	100.0
	Vapormate 315 g/m ³ for 6 h at 25°C	271	100.0
44	Untreated at 25°C	150	2.0
	Vapormate 250 g/m ³ for 4 h at 25°C	1208	100.0

Table 8. Fumigation of adult grain chinch bug with Valencia oranges and pears using Vapormate at 15 and 25°C

Trial	Fumigation treatment	Number of adult GCB treated	Mortality (%)
45	Untreated at 15°C	229	7.9
	Untreated at 25°C	284	4.9
	Vapormate 250 g/m ³ for 4 h at 15°C	512	100.0
	Vapormate 250 g/m ³ for 4 h at 25°C	480	100.0
46	Untreated at 15°C	242	9.1
	Untreated at 25°C	295	14.2
	Vapormate 250 g/m ³ for 4 h at 15°C	232	100.0
	Vapormate 250 g/m ³ for 4 h at 25°C	257	100.0
47	Untreated at 15°C	275	8.7
	Untreated at 25°C	236	3.8
	Vapormate 250 g/m ³ for 4 h at 15°C	544	100.0
	Vapormate 250 g/m ³ for 4 h at 25°C	504	100.0

Further verification trials (48-50) confirmed the efficacy of Vapormate 250 g/m³ for 4 h at 15°C against adult GCB. No survivors were found after treating 37 573 GCB adults in the three trials (Table 9). After adding the numbers of bugs killed in previous trials using the same treatment and temperature the total number of bugs killed without a survivor using this treatment was 38 861 (Grout and Stoltz 2016). Some of the Abate Fetel pears that were bruised in transit showed accelerated waste in the form of dark brown patches within days after fumigation but sound pears showed no deleterious effects and had no off-taste. Stone fruit and Packham Triumph pears were also unaffected by this treatment and no off-tastes were detected. Valencia oranges were not adversely affected in any way by this relatively short treatment.

Table 9. Fumigation of adult grain chinch bug with Valencia oranges and pears using Vapormate at 15°C

Trial	Fumigation treatment	Number of adult GCB treated	Mortality (%)
48	Untreated at 15°C	290	3.8
	Vapormate 250 g/m ³ for 4 h at 15°C	5 075	100.0
49	Untreated at 15°C	502	1.4
	Vapormate 250 g/m ³ for 4 h at 15°C	31 494	100.0
50	Untreated at 15°C	178	4.5
	Vapormate 250 g/m ³ for 4 h at 15°C	1 004	100.0

Mealybug and false codling moth eggs

Trial 1

The chambers used for fumigation purposes worked well, provided the pressure from the compressed air carrier was kept low, otherwise the gas mixture was too dilute and the chamber lid began to bulge. Results were promising with citrus mealybug and vine mealybug. At both storage temperatures, there was 100% mortality of all mealybugs (Table 10). However, there were signs of phytotoxicity (browning) around the stem on grapes at this high dosage.

Table 10. Results of fumigation for 6 h with Vapormate at 370 g/m³ with fruit at two different temperatures

Pest	Fruit type	Treatment	Live	Dead	Total	% Mort
Citrus MB	Oranges	Control	61	11	72	15.3
Citrus MB	Oranges	Vapormate 15°C	0	70	70	100.0
Citrus MB	Oranges	Vapormate 27°C	0	60	60	100.0

Vine MB	Grapes	Control	45	4	49	8.2
Vine MB	Grapes	Vapormate 15°C	0	47	47	100.0
Vine MB	Grapes	Vapormate 27°C	0	52	52	100.0

Trial 2

The second and third instar mealybugs of both species were very susceptible to 225 g/m³ for 4 h at both temperatures evaluated with 100% mortality being obtained in both cases (Table 11). Carbon dioxide alone at 9.8% caused a significant amount of mortality but much less than the fumigant, so it is clear that it is the active ingredient in the fumigant that is causing most mortality and the contribution from the CO₂ carrier is not important. Slight browning was observed on the growing tips of some potato sprouts after fumigation which was not observed on sprouts in the control.

Table 11. Fumigation of two mealybug species with Vapormate at two different temperatures and CO₂ alone

Treatments	Citrus mealybug 2 nd & 3 rd instars			Vine mealybug 2 nd & 3 rd instars		
	Total alive	Total dead	Mortality (%)	Total alive	Total dead	Mortality (%)
Control	145	2	1.4 a	48	3	5.9 a
CO ₂ only at 9.8% for 4 h at 20°C	119	27	18.5 b	46	9	16.4 b
Vapormate 225 g/m ³ for 4 h at 13°C	0	106	100.0 c	0	49	100.0 c
Vapormate 225 g/m ³ for 4 h at 25°C	0	118	100.0 c	0	47	100.0 c

Mean mortalities in the same column followed by the same letter are not significantly different at $\alpha=0.05$ (SNK)

Trial 3

Adult citrus mealybugs were highly susceptible to both treatments with both causing 100% mortality (Table 12).

Table 12. Fumigation of citrus mealybug adults on potatoes at different dosages and for different periods

Treatments	Citrus mealybug adults		
	Total alive	Total dead	Mortality (%)
Untreated control at 25°C	154	4	2.5
Vapormate 112 g/m ³ for 4 h at 25°C	0	157	100.0
Vapormate 225 g/m ³ for 2 h at 25°C	0	166	100.0

Trial 4

The control mortality of adult citrus mealybug when using an artificial substrate was extremely low (Table 13) so this technique was justified. However, the adults were once again extremely susceptible to the fumigant and both treatments resulted in 100% mortality. On the other hand, both treatments appeared to have no effect on the viability of exposed FCM eggs (Table 13) so higher dosages will be required to control both pests.

Table 13. Fumigation of citrus mealybug adults and FCM eggs in a plastic dish at different dosages and for different periods

Treatments	Citrus mealybug adults			Total hatched FCM larvae
	Total alive	Total dead	Mortality (%)	
Untreated control at 26°C	109	2	1.8	593
Vapormate 112 g/m ³ for 2 h at 26°C	0	117	100.0	612

Vapormate 56 g/m ³ for 4 h at 26°C	0	115	100.0	611
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Trial 5

Repeating the 112 g/m³ dosage at 4 h and increasing it to 6 h still had no effect on FCM egg hatch and did not reduce the numbers of citrus mealybug crawlers hatching from egg sacs (Table 14).

Table 14. Fumigation of citrus mealybug eggs and FCM eggs with results on 1st instars 11 days later

Treatments	Mean no. crawlers emerged/ replicate	FCM larvae	
		Mean hatched/ replicate	Non-hatch (%)
Untreated control at 25°C	87.9 a	148.7	21.3 a
Vapormate 112 g/m ³ for 4 h at 25°C	66.0 a	156.8	18.9 a
Vapormate 112 g/m ³ for 6 h at 25°C	92.8 a	110.7	24.8 a

There were no significant differences between treatments at $\alpha=0.05$ (SNK)

Trial 6

A drastic increase in the fumigation dose and period resulted in complete control of FCM eggs and almost complete control of mealybug eggs (Table 15).

Table 15. Fumigation of citrus mealybug eggs and FCM eggs with results on first instars

Treatments	Mean no. MB crawlers emerged/ replicate	Approx. MB mortality relative to control (%)	FCM larvae	
			Mean hatched/ replicate	Non-hatch (%)
Untreated control at 25°C	103.6	-	82.4	22.8
Vapormate 225 g/m ³ for 24 h at 25°C	0.1	99.9	0	100
Vapormate 112 g/m ³ for 24 h at 25°C	0.3	99.7	0	100

Trial 7

Repeating the fumigation at 112 g/m³ for 24 h confirmed the previous results with 100% mortality of FCM eggs and 99.8% mortality of citrus mealybug eggs (Table 16). Reducing the fumigation period to 18 h with the same concentration reduced the mortality slightly for both insects.

Table 16. Fumigation of citrus mealybug eggs and FCM eggs with results on first instars

Treatments	Mean no. MB crawlers emerged/ replicate	Approx. MB mortality relative to control (%)	FCM larvae	
			Mean hatched/ replicate	Non-hatch (%)
Untreated control at 25°C	109.7	-	91.3	23.3
Vapormate 112 g/m ³ for 18 h at 25°C	1.9	98.3	0.1	99.9
Vapormate 112 g/m ³ for 24 h at 25°C	0.2	99.8	0	100

Trial 21

Previously in Trial 5, Vapormate at 112 g/m³ for 6 h did not appear to have any effect on the numbers of FCM neonate larvae but in this trial after the same treatment only 36.7% of the eggs hatched relative to the control (Table 17). The high rate of Vapormate at 225 g/m³ for 6 h resulted in only 5.5% hatch and 70% CO₂ for 24 h reduced the hatch to only 12.1%.

Table 17. The effect of Vapormate and CO₂ on the ability of FCM eggs on wax paper to hatch

Treatments	Total no. neonate larvae	Relative hatch (%)
Control 8 d at 25°C	1113	100.0
Vapormate 112 g/m ³ for 4 h at 25°C, followed by 7 d at 25°C	643	57.8
Vapormate 112 g/m ³ for 6 h at 25°C, followed by 7 d at 25°C	408	36.7
Vapormate 225 g/m ³ for 4 h at 25°C, followed by 7 d at 25°C	456	41.0
Vapormate 225 g/m ³ for 6 h at 25°C, followed by 7 d at 25°C	61	5.5
CO ₂ 70% for 24 h at 25°C, followed by 7 d at 25°C	134	12.1

Trial 22

The mealybug eggs appeared to be more susceptible to Vapormate 225 g/m³ for 4 h than the FCM eggs in Trial 21 because the relative hatch percentage was lower with mealybug (Table 18). However, the results of the CO₂ treatment appeared similar for the eggs of both these insects.

Table 18. The effect of Vapormate and CO₂ on the hatching of citrus mealybug eggs and numbers of crawlers

Treatments	Total no. crawlers	Relative hatch (%)
Control 8 d at 25°C	956	100.0
Vapormate 112 g/m ³ for 4 h at 25°C, followed by 8 d at 25°C	317	33.2
Vapormate 225 g/m ³ for 4 h at 25°C, followed by 8 d at 25°C	29	3.0
CO ₂ 70% for 24 h at 25°C, followed by 7 d at 25°C	190	19.9

Trial 23

The results of the second fumigation of mealybug egg sacs at a dosage rate between the two rates previously used were as expected (Table 19) but with very little difference between the fumigation periods. The relative hatch after the CO₂ treatment was similar to that found previously too.

Table 19. The effect of Vapormate and CO₂ on the hatching of citrus mealybug eggs and numbers of crawlers

Treatments	Total no. crawlers	Relative hatch (%)
Control 7 d at 25°C	1005	100.0
Vapormate 170 g/m ³ for 4 h at 25°C, followed by 7 d at 25°C	76	7.6
Vapormate 170 g/m ³ for 6 h at 25°C, followed by 7 d at 25°C	74	7.4
CO ₂ 70% for 24 h at 25°C, followed by 6 d at 25°C	114	11.3

Trial 24

These results confirmed earlier results in Trial 21 (Table 17) which showed CO₂ for 24 h to have an almost identical effect on FCM eggs (Table 20). The results with Vapormate at 170 g/m³ in this trial are similar to those with Vapormate at 112 g/m³ in the previous FCM egg trial (Table 17) with once again a large difference in efficacy with exposure time.

Table 20. The effect of Vapormate and CO₂ on the hatching of FCM eggs and numbers of larvae

Treatments	Total no. neonate larvae	Relative hatch (%)
Control 7 d at 25°C	1828	100.00

Vapormate 170 g/m ³ for 4 h at 25°C, followed by 7 d at 25°C	1342	73.4
Vapormate 170 g/m ³ for 6 h at 25°C, followed by 7 d at 25°C	742	40.6
CO ₂ 70% for 24 h at 25°C, followed by 6 d at 25°C	244	13.3

Trials 51 to 54

Trials against mealybug showed that it was not possible to completely eliminate eggs of either *Planococcus citri* or *P. ficus* using Vapormate at 250 g/m³ for up to 24 h. Egg hatch was reduced to 2.2% after a 4 h fumigation and reduced to 0.1% with a 24 h fumigation, when treated at 25°C. Treating at 15°C for 24 h resulted in 4.6% egg hatch, but the increased survival was not significant ($P>0.05$). The effect on egg hatch of vine mealybug was similar with 3.4% egg hatch after a 24 h treatment at 25°C. There is therefore not much to gain from fumigating for 24 h rather than 4 h. Both treatments would need to be combined with another treatment such as cold storage to guarantee complete freedom from mealybug.

Armoured scales

Trials 62 and 63

Vapormate at 250 g/m³ for 4 h at 25°C prevented the formation of whitecaps on 100% of the fruit infested by both scale insects, whereas all of the untreated fruit had whitecaps (Figs. 2 and 3).



Figure 2. Control on left showing red scale with whitecaps and treated red scale on the right with no white caps



Figure 3. Control on left showing mussel scale with whitecaps and treated mussel scale on the right with no white caps

Oribatulid mites

Trials 59 and 60

The oribatid beetle mite was very susceptible to Vapormate 250 g/m³ for 4 h at 25°C and 100% mortality was obtained of 2 659 mites. Carbon dioxide at 70% in air for 4 h was not quite as effective and caused 94% mortality of 10 420 mites. This mite is causing rejections at the port so at least we know that the Vapormate

treatment that is so effective against the grain chinch bug hitchhiker is also effective against this mite. A slightly longer treatment of 6 h with carbon dioxide would result in more than 95% mortality.

Objective 2: Internal phytosanitary pests

False codling moth

Trial 11

It was noted that the larval medium had become hard and dry in the 225 g/m³ treatment and the FCM larvae appeared to be partially decomposed. Only 6.25% mortality was obtained in the untreated control but all three fumigation dosages gave 100% mortality, including 56 g/m³ for 24 h. This shows that exposed FCM larvae are more susceptible to the fumigant than exposed Medfly larvae because the lowest dosage of 56 g/m³ for 24 h only caused 26.3% mortality in Medfly (Table 27).

Trial 12

The fumigation at 112 g/m³ did have a significant effect on FCM larvae in fruit (Table 21) and the higher dosage was significantly better, but not close to the efficacy required for a phytosanitary pest.

Table 21. Fumigation of FCM larvae in navel oranges with Vapormate

Treatments	Mortality of 3 rd instars (%)
Untreated control at 25°C	2.1 a
Vapormate 112 g/m ³ for 24 h at 25°C	29.8 b
Vapormate 225 g/m ³ for 24 h at 25°C	70.6 c

Mean mortalities followed by the same letter are not significantly different at $\alpha=0.05$ (SNK)

Trial 13

The cold treatment at 1°C for 5 days did not appear to contribute to the mortality of FCM third instars in carrot larval medium (Table 22) and the mortality obtained with fumigation at 56 g/m³ for 24 h was very similar to the 100% obtained previously with exposed FCM larvae in medium (Trial 11). Both of which were much higher than the mortality of around 60% obtained with the same treatment against exposed Medfly larvae.

Table 22. Fumigation of third instar FCM in carrot larval medium followed by cold treatment

Treatments	Mortality of 3 rd instars (%)
Control 24 h at 25°C, 5 d at 1°C, 24 h at 25°C	15.0 a
Vapormate 56 g/m ³ for 24 h at 25°C, 5 d at 1°C, 24 h at 25°C	98.6 b
Control 24 h at 25°C, 6 d at 25°C	9.5 a
Vapormate 56 g/m ³ for 24 h at 25°C, 6 d at 25°C	98.8 b

Mean mortalities followed by the same letter are not significantly different at $\alpha=0.05$ (SNK)

Trial 15

It appeared from the results (Table 23) that the fumigation did not work and the cold alone caused a very high level of mortality. Previously a temperature of 1°C for 5 d only caused 15% mortality with exposed larvae in medium (Table 22). It is possible that the vaporiser unit did not reach operating temperature after the usual amount of time due to cold weather at the time. We still do not have a gas meter for the fumigant so are relying on the concentration of CO₂ to determine dosage. We therefore could not detect that the level of fumigant in the final mixture was unusually low. This trial will be repeated.

Table 23. Fumigation of third instar FCM in navel oranges followed by cold treatment at -1°C

Treatments	Mortality of 3 rd instars (%)
Control 24 h at 25°C, 5 d at -1°C, 24 h at 25°C	96.0
Vapormate 225 g/m ³ for 24 h at 25°C, 5 d at -1°C, 24 h at 25°C	95.6
Control 24 h at 25°C, 6 d at 25°C	5.1
Vapormate 225 g/m ³ for 24 h at 25°C, 6 d at 25°C	6.9?

Trial 55

Mortalities of second and third instars of FCM from both Vapormate dosages and carbon dioxide were surprisingly high for all navel orange selections (Table 24). This is in contrast with some earlier results and many Valencia selections.

Table 24. Mortality of FCM larvae in three different early navel orange cultivars after fumigation

Fumigation treatment	Cultivar	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	Bahianinha navel	468	1.1
Vapormate 250 g/m ³ for 24 h at 25°C		369	97.6
Vapormate 315 g/m ³ for 24 h at 25°C		379	97.6
CO ₂ 70% for 24 h at 25°C		314	85.7
Untreated 24 h at 25°C	M7 navel	519	0
Vapormate 250 g/m ³ for 24 h at 25°C		396	92.2
Vapormate 315 g/m ³ for 24 h at 25°C		511	98.0
CO ₂ 70% for 24 h at 25°C		425	88.7
Untreated 24 h at 25°C	Palmer navel	301	0
Vapormate 250 g/m ³ for 24 h at 25°C		338	94.4
Vapormate 315 g/m ³ for 24 h at 25°C		373	99.5
CO ₂ 70% for 24 h at 25°C		363	89.8

Trial 58

Carbon dioxide fumigation of FCM larvae in Palmer navel oranges caused 41% mortality (Table 25) which was similar to the 36% obtained in another trial but much lower than in two other trials. Mortalities of larvae in Boshhoek navel were much higher than had been recorded in the previous season when it appeared that both gases were not penetrating the fruit. This confirms the extreme variability in efficacy of these fumigants against internal pests.

Table 25. Mortality of FCM larvae in early and late navel orange cultivars after fumigation

Fumigation treatment	Cultivar	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	Palmer navel	282	0
Vapormate 250 g/m ³ for 24 h at 25°C		274	71
Vapormate 315 g/m ³ for 24 h at 25°C		303	82
CO ₂ 70% for 24 h at 25°C		231	41
Untreated 24 h at 25°C	Boshhoek navel	162	0
Vapormate 250 g/m ³ for 24 h at 25°C		182	87
Vapormate 315 g/m ³ for 24 h at 25°C		173	93
CO ₂ 70% for 24 h at 25°C		204	54

Mediterranean fruit fly

Trial 1

The chambers used for fumigation purposes worked well, provided the pressure from the compressed air carrier was kept low, otherwise the gas mixture was too dilute and the chamber lid began to bulge. The Medfly eggs placed in Granny Smith apples did not hatch, perhaps due to high acidity. Medfly larval mortality in plums was also high due to all the liquid. However, there were no indications that the fumigant was increasing mortality of fruit fly larvae (Table 26). There were signs of phytotoxicity (browning) around the stem on grapes at this high dosage.

Table 26. Results of fumigation for 6 h with Vapormate at 700 g/m³ with fruit at two different temperatures

Pest	Fruit type	Treatment	Live	Dead	Total	% Mort
Medfly	Oranges	Control	42	0	42	0.0
Medfly	Oranges	Vapormate 15°C	90	14	104	13.5
Medfly	Oranges	Vapormate 27°C	87	3	90	3.3
Medfly	Plums	Control	44	55	99	55.6
Medfly	Plums	Vapormate 15°C	113	52	165	31.5
Medfly	Plums	Vapormate 27°C	112	37	149	24.8
Medfly	GS Apples	No larvae hatched in any treatments including the control				

Trial 8

There was only 2% survival of 100 third instar Medfly after fumigation at 112 g/m³ for 24 h compared with 100% survival in the control. It is therefore clear that when fruit fly larvae are exposed to the fumigant they are as susceptible as mealybug eggs.

Trial 9

These results (Table 27) confirmed those in Trial 8 with fumigation at 112 g/m³ for 24 h again causing exactly 98% mortality of third instar Medfly. Halving the dosage gave a significantly poorer result of only 26.3% mortality and doubling the dosage increased the mortality to 100%.

Table 27. Fumigation of third instar Medfly in bran larval medium

Treatments	Mortality of 3 rd instars (%)
Untreated control at 25°C	0.0 a
Vapormate 56 g/m ³ for 24 h at 25°C	26.3 b
Vapormate 112 g/m ³ for 24 h at 25°C	98.0 c
Vapormate 225 g/m ³ for 24 h at 25°C	100.0 c

Mean mortalities followed by the same letter are not significantly different at $\alpha=0.05$ (SNK)

Trial 10

A surprisingly high mortality of 65% occurred by storing the larvae at 15°C and there was no contribution to the mortality by fumigation at 105 g/m³ for 24 h (Table 28).

Table 28. Fumigation of third instar Medfly in bran larval medium followed by cold treatment

Treatments	Mortality of 3 rd instars (%)
Control 24 h at 25°C, 5 d at 1°C, 24 h at 25°C	72 a

Vapormate 105 g/m ³ for 24 h at 25°C, 5 d at 1°C, 24 h at 25°C	57 a
Control 24 h at 25°C, 5 d at 15°C, 24 h at 25°C	65 a
Vapormate 105 g/m ³ for 24 h at 25°C, 5 d at 15°C, 24 h at 25°C	67 a

There were no significant differences between treatments at $\alpha=0.05$ (SNK)

Trial 14

Only 5 of the 10 control fruit contained Medfly larvae but these had an average of 19.2 larvae per fruit. Six of the 10 fumigated fruit contained larvae with an average of 7.1 larvae per fruit. The percentage mortality in the control was 0% compared with mortality of 85.9% after fumigation with Vapormate at 225 g/m³ for 24 h at 25°C. This was a higher level of mortality than FCM larvae in fruit after the same treatment but a lower level of mortality than for exposed Medfly larvae in medium after the same treatment.

Trial 16

There was no mortality in the larvae in the control fruit kept at 25°C but 100% mortality in both Vapormate 225 g/m³ for 24 h and CO₂ 70% for 24 h (Table 29). Trial 14 had given 85.9% mortality with the same dosage of Vapormate but Trial 9 had given 100% mortality with exposed larvae. However, there was also 100% mortality in the control fruit that was kept at -1°C for 5 d so the cold treatment was a bit too severe to see additive effects of the fumigant. The CO₂ treatment with cold also gave 100% mortality but surprisingly there appeared to be some survivors in the Vapormate treatment with cold. The organoleptic test showed that the control and CO₂-treated fruit tasted similar but the fruit treated with Vapormate 225 g/m³ for 24 h had a watery taste. This may have been for this particular cultivar or batch as we did not encounter this on other citrus types.

Table 29. Fumigation of second instar Medfly in Shamouti oranges followed by cold treatment at -0.5°C

Treatments	Mortality of 2 nd instars (%)	Number of larvae per treatment
Control 24 h at 25°C, 5 d at -0.5°C, 24 h at 25°C	100	137
Vapormate 225 g/m ³ for 24 h at 25°C, 5 d at -0.5°C, 24 h at 25°C	84.2	95
CO ₂ 70% for 24 h at 25°C, 5 d at -0.5°C, 24 h at 25°C	100	149
Control 24 h at 25°C, 2 d at 25°C	0	127
Vapormate 225 g/m ³ for 24 h at 25°C, 2 d at 25°C	100	133
CO ₂ 70% for 24 h at 25°C, 2 d at 25°C	100	223

Conclusion

As a result of this research, Vapormate is now registered as a fumigant treatment for the control of grain chinch bug and some other external pests on citrus in South Africa. However, it requires the installation of expensive equipment that may deter growers who do not have a serious problem with grain chinch bug. Against the internal pests FCM and Medfly, results were very variable and are not worth pursuing. Further research will focus on sequential carbon dioxide fumigation and short cold treatments.

Future research

Although Vapormate can be useful for disinfesting fruit of external pests, no further research is recommended for the control of internal pests.

Technology transfer

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2.4.3 FINAL REPORT: Carbon dioxide as a shock treatment to shorten cold disinfestation treatments for internal pests of citrus fruit

Summary

Although elevated levels of carbon dioxide have been used in controlled atmosphere treatments with low oxygen levels for a number of deciduous crops, the use of much higher concentrations of carbon dioxide as a shock treatment in combination with a short cold treatment for citrus was only suggested as a viable option in 2005. Carbon dioxide alone, for a period of 24 hours at concentrations up to 70% in air caused variable levels of mortality of false codling moth and Medfly larvae in various citrus types, but never completely controlled these pests. By following fumigation with a short cold treatment, complete control of these pests was achieved and results with the combined treatment were more consistent. The efficacy of this combined treatment was reduced when it took longer than 12 hours to reduce the temperature of the fruit to 6°C after fumigation. Taste tests sometimes found that fumigated fruit was more mature so if this treatment were to be commercialised, harvest would have to be at the beginning of the picking window. Confirmatory tests with pulp temperatures of 3, 4 and 5°C for various periods after 70% CO₂ for 24 hours will now be conducted to arrive at commercial treatments that could be used for export of citrus to the EU.

Opsomming

Hoewel verhoogde vlakke van koolstofdioksied al in beheerde atmosfeer behandelings met lae suurstofvlakke vir 'n aantal bladwisselende gewasse gebruik is, is die gebruik van baie hoër konsentrasies koolstofdioksied as 'n skok behandeling, in kombinasie met 'n kort koue-behandeling vir sitrus, slegs as 'n lewensvatbare opsie in 2005 voorgestel. Koolstofdioksied alleen, vir 'n periode van 24 uur teen konsentrasies so hoog as 70% in lug, het variërende vlakke van mortaliteit van valskodlingmot en Mediterreense vrugtevlieglarwes in verskeie sitrustipes tot gevolg gehad, maar het hierdie plaë nooit heeltemal beheer nie. Deur beroking met 'n kort koue-behandeling op te volg, is volledige beheer van hierdie plaë verkry, en resultate met die gekombineerde behandeling was meer konsekwent. Die effektiwiteit van hierdie gekombineerde behandeling is verlaag wanneer dit langer as 12 ure geneem het om die temperatuur van die vrugte na 6°C te verlaag ná beroking. Smaak toetse het soms gevind dat berookte vrugte meer ryp was, so indien hierdie behandeling gekommersialiseer gaan word, sal oes tydens die begin van die plukvenster moet geskied. Bevestigende toetse met pulp temperature van 3, 4 en 5°C vir verskeie periodes ná 70% CO₂ vir 24 uur, sal nou uitgevoer word om by kommersiële behandelings uit te kom wat vir uitvoer van sitrus na die EU gebruik kan word.

Introduction

The only post-harvest disinfestation treatment currently in commercial use for false codling moth (FCM) *Thaumotobia 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 Clementines 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. 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 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. We therefore conducted a series of experiments to evaluate the impact of carbon dioxide fumigation on FCM and Medfly larvae in citrus fruit and its value in being able to shorten the postharvest cold treatment required to disinfest fruit of these pests.

Stated objectives

3. Develop specific Vapormate treatments that will guarantee freedom from external phytosanitary pests such as the grain chinch bug, mealybugs, scale insects and Fullers rose beetle.
4. Develop the most effective combination of fumigation with Vapormate or carbon dioxide and a short cold treatment for the control of the internal phytosanitary pests, fruit fly and false codling moth.

Materials and methods

Preliminary research involving FCM

From the field there was a perception that navel oranges were more likely to be infested with FCM than Valencia orange types. However, the season during which navel oranges are available is quite short so we tested whether Valencia oranges were suitable for FCM larval development. A small test was conducted where similar sized pieces of egg sheets (wax paper with FCM eggs) were placed on each of 10 navel oranges and 10 Valencia oranges on 16 Sep 2011 and the neonate larvae left to penetrate the fruit naturally. After 11 days, the fruit were dissected and the number and size of larvae recovered from the different fruit types determined.

A small test was conducted in July 2012 to determine what effect 70% CO₂ for 24 h would have on the hatchability of FCM eggs. Approximately 2000 eggs were cut out of wax paper egg sheets. Half of these were kept at the ambient temperature in the laboratory and the other half were fumigated at ambient temperature. The pieces of egg sheet were placed in a small dish in the centre of a large petri dish that was coated with polybutene. All neonate larvae leaving the inner dish would stick to the polybutene and could be counted. The eggs were left for a week before the numbers of hatched larvae were determined.

In practice, fumigation would probably take place in degreening rooms before fruit is waxed and packed. The question of whether the waxing of the fruit after fumigation would have any effect on the mortality of FCM larvae in the fruit was investigated in one trial (57) using Bahianinha navel oranges infested with third instars. Treatments included an untreated control, fumigation with CO₂ 70% for 24 h at 25°C and the same fumigation followed by a cold treatment at 2°C for either 6 d or 7 d. Twenty-four fruit were used for each treatment but half of the fruit were waxed with polyethylene fruit wax (Citrashine Poly Orange at 1 ml per kg fruit) immediately after fumigation and half were not. Fruit were cut and larval mortality determined (larvae that moved on prodding were considered alive) after storage for 24 h at 25°C after each treatment was complete.

Sequential CO₂ fumigation and cold treatment in navel and Valencia oranges

Early research with carbon dioxide fumigation of FCM in citrus started in August 2011 under project 965 using navel oranges. Previous researchers such as Hofmeyr had found that there were great losses of FCM-infested fruit beyond the third instar due to decay so most of our research initially involved third instars while we developed techniques to minimise decay and allow us to conduct trials with significant numbers of fifth instars in fruit.

The first trial with third instar FCM in navel oranges was conducted in the laboratories at Nelspruit using FCM eggs laid on wax paper at the River Bioscience insectary, Addo and sent by courier to Nelspruit. Fumigation periods of 6 h at concentrations of 10% and 35% CO₂ followed by 5 d at -0.5°C were compared with the cold treatment alone. Fumigation took place on 4 August 2011. After the cold treatment, the fruit was moved to 25°C for 24 h before being cut and larval mortality determined.

A second trial was conducted with navel oranges along the same lines as the previous one, but higher concentrations of carbon dioxide were used (35 and 70%) and the fumigation period was increased to 12 h.

Fumigation took place on 25 August 2011 and fruit were waxed by dipping within 20 min after fumigation, then immediately moved to the cold room for 5 d at -0.5°C as before.

In July 2012, another trial was conducted with FCM third instars in Valencia C100 oranges and CO₂ 70% in air for 24 h at 25°C. In one treatment the fumigation was followed by a cold treatment of 5 d at -1.0°C and there was also a cold treatment alone. Fumigation took place on 23 July 2012 and fruit were cut for larval mortality determination 24 h after each treatment had ended.

Trial 31

This trial was conducted with Delta Valencias from Crocodile Valley, Nelspruit. Seventy fruit were dipped in a mix of three fungicides and held at 25°C. The next day, 26 September 2013, FCM eggs on squares (10 x 10 mm) of paper were placed on each fruit in a laminar flow bench and left for 24 h for the eggs to hatch and the larvae to penetrate the fruit. The fruit were then moved to a controlled environment room at 25°C. After 8 days the fruit were dipped in the same fungicide mix again. When the larvae were 11 days old the fumigation treatments started on 7 October 2013 using 10 fruit per treatment. Twenty of the fruit were not fumigated but held at 25°C. Another 20 were fumigated with Vapormate 170 g/m³ (measured as 7.4% CO₂ in air in the chamber) for 24 h at 25°C and 20 fruit were fumigated with 70% CO₂ for 24 h at 25°C. After 24 h the lids were removed and a fan used to blow remaining fumigant out of the chambers. Each group of 20 fruit was then split in half with 10 fruit being kept at 25°C for 24 h before being evaluated and another 10 fruit of each treatment being moved into a cold room held at 1°C. The fruit in the cold room was protected from a direct blast from the chiller unit by surrounding it with plastic crates of fruit. After 7 days cold exposure, the fruit from the three cold treatments were moved to 25°C for 24 h before evaluating all fruit for FCM mortality. For evaluation the fruit were dissected and any moving larva was recorded as being alive.

Trial 33

Valencias from Mahela Boerdery were used in another trial with FCM similar to Trial 31. Eggs were placed on fruit in a laminar flow bench on 13 November, fruit were dipped in fungicide on 19 November and fumigation started on 22 November when the larvae were 9 days old. After 24 h fumigation at 25°C half the fruit in each treatment were moved into a cold room at 1°C for 8 days while the remaining three treatments were evaluated after being held at 25°C for a further 24 h. After the cold treatment the fruit was moved into 25°C for 24 h before being evaluated.

Trial 42

Two more Valencia cultivars were compared for the efficacy of CO₂ 70% against FCM larvae in the fruit. Du Roi Valencia and Old Clone Valencia were harvested in mid-September 2014 near Letsitele, dipped in a mixture of fungicides and stored at 4°C until required. On 30 October the fruit was moved into a room at 25°C and on 1 November FCM eggs on squares (10 x 10 mm) of wax paper were placed on each of 120 fruit (60 per cultivar) in a laminar flow bench and left for 24 h for the eggs to hatch and the larvae to penetrate the fruit. The fruit were then moved to a controlled environment room at 25°C. Seven days after egg placement on 8 November the fruit were dipped in the same fungicide mix again. On 11 November, the infested fruit were fumigated at 25°C for 24 h. Only one treatment of CO₂ 70% was used for fumigation purposes but this fumigation was followed by three different cold treatments of 7, 8 or 9 days in a room at 2°C. After the cold period was complete the fruit was moved into a room at 25°C for 24 h before they were dissected for evaluation of larval mortality.

Trial 55

Compared the efficacy of third instar FCM in three different types of navel oranges when treated for 24 h at 25°C with Vapormate at either 250 or 315 g/m³ or CO₂ 70%. [Trial 56](#) was similar and compared FCM larval mortality in two types of navel oranges when using CO₂ 70% but the fumigation was also followed by cold treatments at 2°C for 6, 7 or 8 days.

Trial 58

This trial was the same as Trial 55 but involved one late navel (Boshoek) in comparison with an earlier Palmer navel that was used in Trial 55 but had been kept in cold storage. This was to verify the results achieved with Trial 40.

Trial 61

This trial was conducted with Boshhoek navel oranges from Crocodile Valley, Nelspruit. Two hundred fruit were taken from picking bins on 17 June 2015, dipped in a mix of three fungicides and stored at 4°C until 20 August when 100 fruit in good condition were moved into a room at 25°C for 24 h. On 21 August 2015, FCM eggs on squares (10 x 10 mm) of wax paper were placed on each of the fruit in a laminar flow bench and left for 24 h for the eggs to hatch and the larvae to penetrate the fruit. The fruit were then moved to a controlled environment room at 25°C. Seven days after egg placement the fruit were dipped in the same fungicide mix again and the fruit randomly placed on trays. Twelve days after egg placement the fumigation treatments started on 2 September 2015 against the third instars. Third instars were used to minimise fruit decay as this increases with time. Fifteen infested fruit were used per treatment with five treatments and one control. The control fruit were not fumigated but held at 25°C. The treatments included:

- CO₂ 70% for 24 h at 25°C followed by a further 24 h at 25°C before evaluation.
- No fumigation but held at 25°C for 48 h then moved into 2°C for 6 d followed by 24 h at 25°C before evaluation.
- CO₂ 70% for 24 h at 25°C followed by immediate transfer to 2°C for 6 d followed by 24 h at 25°C before evaluation.
- No fumigation but held at 25°C for 48 h then moved into 2°C for 7 d followed by 24 h at 25°C before evaluation.
- CO₂ 70% for 24 h at 25°C followed by immediate transfer to 2°C for 7 d followed by 24 h at 25°C before evaluation.

After fumigation the lids were removed and a fan used to blow remaining fumigant out of the chambers. For evaluation, fruit were dissected under a stereomicroscope and any larva that moved when prodded was recorded as being alive. Due to decay of some fruit, the numbers of fruit evaluated were reduced to 14 per treatment.

The susceptibility of third and fifth instar FCM in citrus to carbon dioxide fumigation followed by a short cold treatment

Previously, most work with FCM larvae in fruit has involved third instars because many fruit are lost to decay when waiting for the larvae to develop to fifth instar. However, after gaining a lot of information on sequential treatments of carbon dioxide fumigation and short cold treatments we needed to compare third instar efficacy with that of fifth instars. The practice of keeping the hatching eggs on fruit on a laminar flow bench and dipping the fruit in a combination of three fungicides seven days later, reduced losses due to decay and made these trials possible. Trial 66 evaluated both instars for susceptibility to cold, fumigation and both treatments using Valencia C100 oranges. Fumigation of third instars took place on 27 Jan 2016 and fumigation of fifth instars on 31 Jan 2016. Fruit was held at 25°C for 24 h before being cut to determine larval mortality. This trial was repeated in Trial 67, again using Valencia C100 fruit that had been in cold storage. Fumigation of third instars took place on 24 Feb 2016 and fumigation of fifth instars on 28 Feb 2016.

A series of trials was conducted with different citrus fruit types using FCM fifth instars at around the same time that a similar series was conducted with Medfly larvae. The trials were 69, 70, 72-75, 81 and 83 and started in May 2016. Fruit were placed on trays in a laminar flow bench and pieces of paper with hatching FCM eggs placed on each fruit so that neonate larvae could move onto the fruit. After 24 h, the trays of fruit were moved to a 25°C incubation room. One hundred and fifty fruit were used per trial, per cultivar or citrus type. After incubation for 7 days, decaying fruit were removed and the remainder dipped in a mixture of postharvest fungicides, the fruit were randomised before being placed on trays in the incubation room. Fifteen days after moving the fruit into the incubation rooms it was fumigated with CO₂ 70% in air for 24 h. The fruit were then waxed by hand and moved to a cold room at 2°C for 9 to 13 days. Thereafter, fruit were held at 25°C for 24 h before cutting and evaluating mortality. Treated, but uninfested fruit were tasted at this time and again after storing for a month at 4°C to see whether there were differences in taste between those receiving fumigation plus cold and those only being stored at 4°C for the same time.

In February 2017, further comparisons were conducted between FCM third and fifth instars with regard to their susceptibility to cold and the sequential CO₂ – cold treatment. In order to reduce variation, the same batch of eggs from River Bioscience was used for each comparison. Pieces of egg sheet were placed on Valencia oranges in a laminar flow bench as described above and the same process of incubation at 25°C and dipping in fungicide used. For third instars, fruit was fumigated 11 days after moving to the incubation room and fifth instars were fumigated 4 days later. The cold treatment at 2°C was reduced to 6 days in order to ensure some survival and some infested fruit with both ages of instars were treated with cold only to again demonstrate the value of the combined treatment. Four such comparisons were successfully conducted in Trials 85 to 92, but further attempts were scrapped because the fruit was too old and control mortalities were too high due to fungal infection.

In June 2017, further comparisons between third and fifth FCM instars in Valencia C100 oranges were conducted using eggs from River Bioscience, although the same egg batches were not used for both ages of instars. These trials were conducted with three different concentrations of carbon dioxide and compared the susceptibility of both larval ages, but without sequential cold treatments. Fumigation with 35%, 55% and 70% CO₂ for 24 h at 25°C was followed by 48 h at 25°C before cutting the fruit and determining larval mortality. Trials 93 to 96 were first conducted with third instars and these were followed in July by trials 97 to 101 on fifth instars.

Trial 102 only involved fifth instar FCM and compared carbon dioxide fumigation treatments at 50% and 70% alone and followed by a short cold treatment of 5 d at 3°C pulp temperature. Fruit were held for 48 h at 25°C after fumigation when not followed by cold, or after the cold treatment, before determining larval mortality.

Trials with Medfly

Most research conducted with sequential carbon dioxide fumigation and cold treatment was with FCM larvae because the cold treatment requirement for FCM is much longer than that for fruit flies. A few trials were conducted with Medfly (*Ceratitis capitata*) larvae to ensure that treatments developed for FCM would also be effective against them. Full cold treatments developed for Medfly have sometimes been developed using second instars (De Lima et al. 2007, Grout et al. 2011) and sometimes with third instars (Gazit et al. 2014). We conducted two trials with third instars followed by a series of trials in different citrus species and cultivars using second instars.

Delta Valencia oranges were also used in Trial 32 with Medfly third instars. Forty fruit that had been pricked in three positions with a row of eight spikes were hung in Netlon bags in Medfly culture cages on 27 September 2014 and left for 48 h for oviposition. The fruit were then moved into a 25°C room and dipped in a mixture of three fungicides on 6 October. On 9 October, 12 days after oviposition, fumigation started. Twenty of the fruit were not fumigated but held at 25°C to be used in untreated and cold-only controls. Twenty fruit were fumigated with 70% CO₂ for 24 h at 25°C. After 24 h the fruit were removed from the chambers. Ten fruit were kept at 25°C for 24 h before being evaluated and the remaining 10 fruit were moved into a cold room held at 1°C. Ten control fruit were also moved into the cold room at the same time. The fruit in the cold room was protected from a direct blast from the chiller unit by surrounding it with plastic crates of fruit. After 7 days cold exposure, the fruit from the two cold treatments were moved to 25°C for 24 h before evaluating all fruit for Medfly mortality. For evaluation the fruit were dissected and any larva that moved when prodded was recorded as being alive.

A similar trial (34) was conducted with Valencia oranges infested with third instar Medfly. The pricked fruit were hung in the Medfly rearing cages on 15 November 2013 and removed 48 h later. The fruit were held at 25°C for 8 days before being dipped in a fungicide mixture. They were then kept for another two days before being divided into two batches for fumigation on 27 November. After half the fruit were fumigated with 70% CO₂ for 24 h, half the fumigated fruit were moved into a cold room at 1°C for 8 days with the cold-treated control fruit, while the remaining control and fumigated fruit were evaluated after being held at 25°C for a further 24 h. After the cold treatment was completed, the fruit were moved into 25°C for 24 h before being evaluated.

Trials 68, 71, 76-80, 82, 84 were conducted with second instar Medfly using different types and cultivars of citrus (Satsuma mandarin, Nova mandarin, Clementine, Nadorcott mandarin hybrid, navel oranges, Star Ruby grapefruit, Turkey Valencia, Valencia). Holes were pricked in three places around the perimeter of 30 fruit per treatment using a tool with eight spikes in a row. The fruit were then suspended for 24 h in cages with Medfly adults that were ovipositing. After oviposition the fruit were moved to an incubation room and held at 25°C for 5 days to be sure that most larvae had reached second instar before fumigation. On the fifth day the fruit were all dipped in a mixture of postharvest fungicides, randomised and placed on trays in the incubation room. The next day, the fruit were fumigated with CO₂ 70% in air for 24 h before being hand-waxed using a gloved hand and transferred to a cold room at 2°C for 9 to 13 days. The cold treatment started at 9 days but when larvae were found that showed some movement the treatment was lengthened with later cultivars. After the cold treatment, fruit were held at 25°C for 24 h before being cut and larval mortality determined. A panel of people tasted some fruit that had not been infested with fruit fly but were fumigated and cold treated and other fruit that had received these treatments were stored for a month at 4°C before being tasted. These were compared with fruit that had been held at 4°C for the same time.

Consequences of delaying cold treatment after CO₂ fumigation

After determining that fumigation with carbon dioxide alone was inadequate to consistently kill internal citrus pests and required a sequential short cold treatment, the question of whether the benefit of the fumigation could be lost with too long an interval before the cold treatment needed to be addressed. The first trial was conducted with third instar FCM in Valencia oranges on 28 Oct 2011. Two fumigation treatments were used (35% and 70%) but for only 12 h. This treatment was used alone but also followed by 5 d at -0.5°C. All fruit was waxed immediately after fumigation by brushing with polyethylene fruit wax (Citrashine Poly Orange at 1 ml per kg fruit), then in one case the fruit was moved immediately into the cold room whereas in another it was stored for 24 h at 25°C before then moving into a cold room for the same period. After the cold treatment the fruit treated without the gap was stored for 48 h at 25°C and the fruit with the gap treatment was stored for 24 h at 25°C before these treatments were evaluated by dissecting the fruit and checking for live larvae (showing movement on prodding).

A further trial (35) was conducted with Valencia oranges that had been stored at 4°C for four months. The best 110 fruit were dipped again in a mixture of three fungicides, then warmed to 25°C before FCM eggs were placed on them on a laminar flow bench on 19 January 2014. Only carbon dioxide at 70% for 24 h was used as a fumigation treatment in this trial and the cold treatment was 7 d at 1°C. Third instars (10 d old) were used in the trial and some fruit were moved into the cold room immediately after fumigation as before, while other fruit were held for a further 24 h at 25°C after fumigation before being moved into the cold room. Fumigation was started on 29 January with 70% CO₂ for 24 h at 25°C. All evaluations took place after holding fruit at 25°C for 24 h after fumigation or after the cold treatments ended. This trial was repeated twice (36 & 37) using the same fruit source and third instar FCM but the length of the cold treatment at 1°C was reduced to 6 d in order to get more survivors. Fumigation took place on 11 March 2014 with 70% CO₂ for 24 h at 25°C and the gap used was 24 h at 25°C. Efficacy was evaluated as before, 24 h after the end of the cold treatments.

The above trials investigated intervals of 24 h before cold treatment, but Trial 64 involved shorter intervals. This trial was conducted with M7 navel orange fruit and third instar FCM to determine the maximum length of the gap between CO₂ fumigation and the cold treatment that will still provide an additive mortality effect. Carbon dioxide fumigation was at 70% for 24 h at 25°C and a short cold treatment of 5 d at 2°C was chosen. The intervals between fumigation and moving the fruit into the cold were immediate, 6 h, 12 h, 18 h and 24 h. Treatments were evaluated after 24 h at 25°C subsequent to the removal from the cold room. Fumigation took place on 4 November 2015.

Due to differences in cooling rates with different cooling systems, we also decided to investigate a gap in terms of cooling rate rather than time. In this trial we infested Valencia C100 oranges with third instar FCM but instead of varying the time before going into 2°C, two step-down temperatures of either 6°C or 12°C were used for the first 24 h after fumigation, then moved to 2°C for a further 4 d. These treatments were compared with fruit going from fumigation immediately into 2°C for 5 d and fruit that was held at 25°C for 24 h after fumigation

before going to 2°C for 5 d. Controls that had the same cold treatment but no fumigation were also included, as well as a control at 25°C. All treatments were held for 24 h at 25°C after removal from the cold treatment before being evaluated for larval mortality. Fumigation took place on 2 December 2015.

Results and discussion

Preliminary research involving FCM

The simple comparison of host suitability between navel oranges and Valencia oranges resulted in almost twice as many larvae being found in Valencia fruit compared to navels and although the average larval length was slightly shorter in Valencia, the size range was similar (Table 1). Research therefore focused on the use of both these types of oranges, which once infested, generally lasted longer than thinner skinned mandarins.

Table 1. Results of dissecting 10 Navel oranges and 10 Valencia oranges to compare FCM larval development.

Fruit type	Total Larvae	Size range	Avg length
Navel orange	88	3-13 mm	7.8 mm
Valencia	152	3-9 mm	6.4 mm

When FCM eggs were fumigated with CO₂ at 70% in air for 24 h there was an 88% reduction in hatch (Table 2) so this would be an extra benefit of fumigation, although eggs are unlikely to survive the packing procedure.

Table 2. Susceptibility of FCM eggs to CO₂ 25/7/2012

Treatments	Total no. hatched eggs	Percentage hatch relative to control
Untreated control	1112	-
70% CO ₂ 24 h only	134	12.1

Trial 57 demonstrated that waxing fruit immediately after fumigating with carbon dioxide had no effect on FCM larval mortality in Bahianinha navel oranges either with or without a cold treatment (Table 3).

Table 3. The efficacy of CO₂ 70% followed by a short cold treatments of 6 or 7 d against third instar FCM larvae in Bahianinha navel oranges with or without waxing in June 2015

Treatment	Storage conditions	Waxed	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	No	103	0.0
		Yes	113	0.0
CO ₂ 70% for 24 h at 25°C	1 d at 25°C	No	90	67.8
		Yes	95	66.3
	6 d at 2°C followed by 1 d at 25°C	No	128	100.0
		Yes	120	100.0
	7 d at 2°C followed by 1 d at 25°C	No	148	100.0
		Yes	130	100.0

Sequential CO₂ fumigation and cold treatment in navel and Valencia oranges

The first investigation of sequential carbon dioxide fumigation and cold treatment showed that 10% CO₂ for 6 h had no noticeable effect on increasing mortality above what was caused by the cold treatment alone (Table 4). The 35% CO₂ treatment for 6 h actually reduced the mortality caused by the cold by approximately 50% so in some way this treatment may have increased the insect's cold hardiness. In the next trial when the 35% CO₂ fumigation period was extended to 12 h it did not have a significant ($P>0.05$) influence on the mortality caused by a sequential cold treatment and the 35% CO₂ treatment alone did not cause significantly more mortality than occurred in the untreated control (Table 5). Fumigation with 70% CO₂ in air for 12 h did cause significantly more mortality than occurred in the untreated control and when followed by a short cold treatment the combination did increase mortality significantly ($P<0.05$) above that observed for the cold treatment alone (Table 5).

Table 4. Fumigation of FCM-infested navel oranges 04/08/2011

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	8	197	4.1	
Cold ONLY at -0.5°C for 5 d	143	227	63.0	61.4
10% CO ₂ (6h) + cold (-0.5) for 5 d	99	161	61.5	59.9
35% CO ₂ (6h) + cold (-0.5) for 5 d	56	189	29.6	26.7

Table 5. Fumigation of FCM-infested navel oranges 25/08/2011

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	9	199	4.5 d	
Cold ONLY at -0.5°C for 5 d	155	215	72.1 b	70.8
35% CO ₂ (12h) only	21	173	12.1 d	8.0
35% CO ₂ (12h) + cold (-0.5) 5 d	125	211	59.2 b	57.3
70% CO ₂ (12h) only	69	179	38.5 c	35.6
70% CO ₂ (12h) + cold (-0.5) 5 d	259	276	93.8 a	93.5

The trial conducted in July 2012 with a cold treatment of -1°C for 5 d and FCM third instars in Valencia oranges resulted in this cold treatment alone killing all larvae without the addition of fumigation (Table 6). However, fumigation alone with 70% CO₂ for 24 h caused similar mortality to that concentration for half the time in the previous trial.

Table 6. Susceptibility of FCM third instars in Valencia C100 oranges fumigated with CO₂ on 23/7/2012

Treatments	Dead larvae	Total larvae	Mortality (%)
Untreated control at 25°C	0	115	0
Cold only at -1°C for 5 d	148	148	100
70% CO ₂ 24 h at 25°C	41	115	35.7
70% CO ₂ 24 h then -1°C for 5 d	127	134	94.8

Trial 31

The CO₂ 70% for 24 h treatment alone caused 68% mortality whereas the addition of 7 days of 1°C increased the mortality to 100% in Delta Valencia (Table 7).

Table 7. Mortality of FCM third instars in Delta Valencia after fumigation only or fumigation followed by cold treatment

Fumigation treatment	Storage conditions	Number of larvae treated	Mortality (%)
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Untreated 24 h at 25°C	1 d at 25°C	92	0.0
CO ₂ 70% for 24 h at 25°C		117	68.4
Untreated 24 h at 25°C	7 d at 1°C followed by 1 d at 25°C	130	74.6
CO ₂ 70% for 24 h at 25°C		155	100.0

The mortality from the CO₂ treatment in Trial 33 was slight, but it did increase the mortality in the cold treatment to 100% (Table 8).

Table 8. Mortality of FCM third instars in Valencia after fumigation only or fumigation followed by cold treatment

Fumigation treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	56	1.8
CO ₂ 70% for 24 h at 25°C		74	35.1
Untreated 24 h at 25°C	8 d at 1°C followed by 1 d at 25°C	90	94.4
CO ₂ 70% for 24 h at 25°C		146	100.0

Trial 42

This trial showed no difference between Du Roi Valencia and the Old Clone Valencia as far as mortality of FCM larvae from CO₂ was concerned (Table 9). Fumigation alone without a cold treatment caused around 60% mortality in both cultivars compared with zero mortality in the control. Following the fumigation with 7, 8 or 9 days at 2°C caused 100% mortality in all cases.

Table 9. The efficacy of CO₂ 70% followed by a short cold treatment against FCM larvae in two different Valencia cultivars.

Treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	137	0.0 a
CO ₂ 70% for 24 h at 25°C		130	61.1 b
CO ₂ 70% for 24 h at 25°C	7 d at 2°C followed by 1 d at 25°C	186	100.0 c
	8 d at 2°C followed by 1 d at 25°C	150	100.0 c
	9 d at 2°C followed by 1 d at 25°C	163	100.0 c
Du Roi Valencia: CO ₂ 70% for 24 h at 25°C	1 d at 25°C	83	62.7 z
Old Clone Valencia: CO ₂ 70% for 24 h at 25°C		47	59.6 z

Means followed by the same letter were not significantly different at P=0.05 (SNK)

Trial 55

Mortalities of second and third instars of FCM from carbon dioxide without cold treatment were surprisingly high for all three navel orange selections (Table 10). This was in contrast with many other results for oranges.

Table 10. Mortality of FCM larvae in three different early navel orange cultivars after fumigation.

Fumigation treatment	Cultivar	Number of larvae treated	Mortality (%)
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Untreated 24 h at 25°C	Bahianinha navel	468	1.1
CO ₂ 70% for 24 h at 25°C		314	85.7
Untreated 24 h at 25°C	M7 navel	519	0
CO ₂ 70% for 24 h at 25°C		425	88.7
Untreated 24 h at 25°C	Palmer navel	301	0
CO ₂ 70% for 24 h at 25°C		363	89.8

Trial 56

Whereas the mortality of FCM larvae in M7 and Palmer navels was high in Trial 55 for carbon dioxide fumigation, the mortality dropped by around 55% in Trial 56 with the same batch of fruit that had been stored at 4°C for 3 weeks longer than in Trial 55 (Table 11). Once again there was no difference between the two navel selections. Even though the fumigation alone caused only 33% mortality, 100% mortality was obtained when this was followed immediately by 6 days at 2°C.

Table 11. The efficacy of CO₂ 70% followed by a short cold treatment against FCM larvae in two different navel cultivars.

Treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	402	0.5
CO ₂ 70% for 24 h at 25°C		334	33.2
CO ₂ 70% for 24 h at 25°C	6 d at 2°C followed by 1 d at 25°C	600	100.0
	7 d at 2°C followed by 1 d at 25°C	607	100.0
	8 d at 2°C followed by 1 d at 25°C	659	100.0
M7 navel: CO ₂ 70% for 24 h at 25°C	1 d at 25°C	186	31.2
Palmer navel: CO ₂ 70% for 24 h at 25°C		148	35.8

Trial 58

Carbon dioxide fumigation of FCM larvae in Palmer navel oranges caused 41% mortality (Table 12) which was similar to the 36% obtained in the previous trial (Table 11) but much lower than in two previous trials. Mortalities of larvae in Boshhoek navel were much higher than had been recorded in the previous season when it appeared that both gases were not penetrating the fruit. This confirms the extreme variability in efficacy of fumigants alone when used against internal pests.

Table 12. Mortality of FCM larvae in early and late navel orange cultivars after fumigation.

Fumigation treatment	Cultivar	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	Palmer navel	282	0
CO ₂ 70% for 24 h at 25°C		231	41
Untreated 24 h at 25°C	Boshhoek navel	162	0
CO ₂ 70% for 24 h at 25°C		204	54

Trial 61

The results in this trial clearly showed the benefit of following a CO₂ fumigation with a short cold treatment, although the combination with 6 d cold or 7 d cold both gave 100% mortality (Table 13). CO₂ alone caused 41% mortality and 6 d cold caused 47% mortality. Cold alone for 7 d caused 79% mortality.

Table 13. The efficacy of CO₂ 70% followed by a short cold treatments of 6 or 7 d against third instar FCM larvae in Boshhoek navel oranges in September 2015

Treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	112	0.0 a
CO ₂ 70% for 24 h at 25°C		121	41.3 b
Untreated 24 h at 25°C	6 d at 2°C followed by 1 d at 25°C	128	46.9 b
CO ₂ 70% for 24 h at 25°C	6 d at 2°C followed by 1 d at 25°C	171	100.0 d
	7 d at 2°C followed by 1 d at 25°C	151	100.0 d
Untreated 24 h at 25°C	7 d at 2°C followed by 1 d at 25°C	153	79.1 c

Means followed by the same letter were not significantly different at P=0.05 (Tukey HSD)

The susceptibility of third and fifth instar FCM to carbon dioxide fumigation followed by a short cold treatment

Trial 66

After cold treatment alone and after the sequential treatment of fumigation followed by cold treatment, the mortality for third instars was higher than for fifth instars, so cold treatments will need to be longer to control the fifth instars (Table 14).

Table 14. Mortality of third and fifth instar FCM in C100 Valencia after fumigation and/or a short cold treatment in January 2016

Fumigation treatment	Instar	Storage conditions	Number of larvae treated	Mortality (%)
Untreated for 24 h at 25°C	3	None	236	0.0 a
Untreated for 24 h at 25°C	5		215	2.8 a
Untreated for 24 h at 25°C	3	6 d at 2°C followed by 1 d at 25°C	207	47.8 c
Untreated for 24 h at 25°C	5		219	36.1 b
CO ₂ 70% for 24 h at 25°C	3	1 d at 25°C	253	49.8 c
CO ₂ 70% for 24 h at 25°C	5		231	57.1 c
CO ₂ 70% for 24 h at 25°C	3	6 d at 2°C followed by 1 d at 25°C	383	99.2 d
CO ₂ 70% for 24 h at 25°C	5		259	87.3 d

Means followed by the same letter were not significantly different at P=0.05 (SNK)

Trial 67

The repeat trial with third and fifth instar FCM gave similar results to those in Trial 66, although the mortality caused by cold alone was lower for both larval ages than in the previous trial, despite being in the same batch of fruit (Table 15). After fumigation and cold, the mortality of third instars was 16% higher than fifth instars.

Table 15. Mortality of third and fifth instar FCM in C100 Valencia after fumigation and/or a short cold treatment in February 2016

Fumigation treatment	Instar	Storage conditions	Number of larvae treated	Mortality (%)
Untreated for 24 h at 25°C	3	None	165	1.2 a

Untreated for 24 h at 25°C	5		153	0.7 a
Untreated for 24 h at 25°C	3	6 d at 2°C followed by 1 d at 25°C	163	11.0 a
Untreated for 24 h at 25°C	5		125	4.8 a
CO ₂ 70% for 24 h at 25°C	3	1 d at 25°C	169	43.2 c
CO ₂ 70% for 24 h at 25°C	5		209	34.0 b
CO ₂ 70% for 24 h at 25°C	3	6 d at 2°C followed by 1 d at 25°C	296	91.6 e
CO ₂ 70% for 24 h at 25°C	5		230	76.1 d

Means followed by the same letter were not significantly different at P=0.05 (SNK)

In the sequential treatments of fumigation and cold for the control of fifth instar FCM in different types of citrus, a moving larva was discovered in two fruit types when the cold treatment was only 9 days (Table 16). After extending it to 11 days, one moving larva was found in navel oranges. The further treatments with 13 day cold treatments had no moving larvae in Valencia types. None of the moving larvae found survived longer than 24 h after placing them on citrus pulp and none had sufficient energy to burrow into the pulp but just lay on the surface. However, due to inspectors basing rejections on moving larvae, this criterion was used. Once again, there were no detrimental effects of the sequential treatments on taste, although some people thought the fruit tasted more mature. After storage for another month, these differences seemed even less detectable.

Table 16. Mortality of fifth instar false codling moth in different citrus types after a sequential combination of 24 h fumigation with CO₂ at 70% and a short cold period at 2°C.

Fruit type	Untreated control		Days of cold at 2°C	Fumigation and cold		Larvae killed
	Mortality %	SEM		Mortality %	SEM	
Satsuma mandarin	15.25	6.663	9	99.76	0.238	259
Nova mandarin	10.98	2.801	9	99.72	0.278	319
Clementine	6.66	2.709	11	100	0	326
Nadorcott	3.13	2.545	11	100	0	418
Star Ruby grapefruit	7.94	5.069	11	100	0	328
Navel orange	0.59	0.428	11	99.64	0.357	243
Turkey Valencia	10.89	2.138	13	100	0	262
Valencia	3.36	1.847	13	100	0	176

Despite using only a 6-day cold treatment after fumigation in the comparisons between third and fifth instar FCM, 100% mortality of third instars was obtained in three out of four trials whereas there were some survivors in each of the trials with the fifth instars (Table 17). Control mortality was generally low, although fruit that had collapsed due to fungal infection were discarded. The mortality caused by cold alone was variable, but the mean mortality for the third instars was higher than for the fifth instars and both were always a small fraction of that caused by the sequential fumigation and cold treatment.

Table 17. Mortality in FCM caused by 6 days at 2°C alone, or preceded by fumigation with CO₂ at 70%

Batch comparisons	Third instar mortality (%) and (N)			Fifth instar mortality (%) and (N)		
	Untreated	Cold only	CO ₂ and cold	Untreated	Cold only	CO ₂ and cold
1	0.78 (257)	26.23 (530)	99.77 (864)	0.00 (364)	30.97 (649)	99.87 (760)
2	3.76 (186)	2.90 (449)	100.00 (659)	0.00 (232)	1.89 (159)	96.66 (898)
3	3.42 (146)	5.43 (442)	100.00 (915)	0.30 (332)	1.83 (327)	98.33 (779)
4	2.11 (142)	40.71 (280)	100.00 (473)	0.92 (326)	2.54 (118)	99.20 (251)
Mean (ΣN)	2.52 (731)	18.82 (1701)	99.94 (2911)	0.31 (1254)	9.31 (1253)	98.52 (2688)

Third instar mortalities in batches 2 and 4 were very similar but batches 1 and 3 were significantly different to each other and to 2 and 4 ($P < 0.05$) (Table 18). Although third instar mortalities were similar for each batch when using 55% CO₂, they varied considerably with the 70% CO₂ treatment between batches, resulting in a mean mortality for 70% CO₂ that was significantly lower than for 55% CO₂. Mortalities of fifth instars were more similar between batches for each treatment with only batches 2 and 5 showing overall significant differences ($P < 0.05$). The impact of 35% CO₂ was once again significantly different to the untreated, although the mean mortality remained below 5%. The mean mortalities for 55% and 70% CO₂ were significantly different, with the mortality from 55% CO₂ being almost half that obtained with 70% CO₂. If fumigation alone were to be used to control the larvae, these results would suggest that we should work with third instars because the mean mortality obtained with 70% CO₂ was lower than that obtained with the same concentration for fifth instars. However, we are interested in the combined treatment of fumigation with cold and we know that fifth instars are more cold tolerant than third instars which was confirmed in the previous series of trials. In these, cold alone at 2°C for 6 days caused 19% mortality of third instars but only 9% mortality of fifth instars and when these treatments were preceded by 24 h fumigation with 70% CO₂ the mean mortality of third instars was 99.9% while the mean mortality for fifth instars was 98.5%. From further results in Table 19 it is clear that 70% CO₂ leads to significantly higher mortality of fifth instars after a short cold period than when the cold follows fumigation at 50% CO₂. As the objective of this research is to find a combined fumigation-cold treatment that will be as short as possible, we will proceed with the use of 70% CO₂ in confirmatory trials.

Table 18. Mortality in FCM caused by three different concentrations of CO₂ for 24 h without any cold treatment

Batches	Third instar mortality (%) and (N)				Fifth instar mortality (%) and (N)			
	Untreated	35% CO ₂	55% CO ₂	70% CO ₂	Untreated	35% CO ₂	55% CO ₂	70% CO ₂
1	3.3 (578)	11.2 (830)	77.8 (877)	31.9 (790)	2.9 (560)	2.0 (664)	50.7 (568)	98.4 (432)
2	3.2 (626)	8.7 (703)	84.2 (741)	85.7 (756)	0.8 (505)	4.1 (662)	36.4 (349)	98.2 (669)
3	0.8 (122)	6.5 (835)	81.1 (704)	67.5 (160)	0.1 (769)	2.8 (675)	-	99.3 (705)
4	0.5 (597)	5.8 (812)	85.2 (965)	96.9 (477)	0.0 (602)	5.4 (369)	48.2 (342)	100.0 (557)
5	-	-	-	-	0.0 (450)	7.4 (340)	52.0 (373)	99.8 (501)
Mean (ΣN)	1.95 a (1923)	8.05 b (3180)	82.08 d (3287)	70.50 c (2183)	0.76 w (2886)	4.34 x (2710)	46.83 y (1632)	99.14 z (2864)

Means followed by the same letter were not significantly different at P=0.05 (Tukey HSD); 3rd and 5th instars did not come from the same batches.

Table 19. Mortality in FCM 5th instars caused by two rates of CO₂ fumigation for 24 h compared to fumigation followed by 5 days at 3°C (Trial 102)

Mortality (%) and (N) after fumigation			Mortality (%) and (N) after fumigation and cold		
Untreated	50% CO ₂	70% CO ₂	Cold only	50% CO ₂	70% CO ₂
5.7 a (388)	47.7 b (572)	73.7 c (649)	6.8 a (324)	56.5 b (637)	94.1 d (725)

Means followed by the same letter were not significantly different at P=0.05 (Tukey HSD)

Trials with Medfly

The results from Trial 32 showed that CO₂ without cold caused very little mortality in this cultivar (Table 20). However, the combined effect of CO₂ followed by 7 d cold did cause more mortality than cold alone. This suggests that even though the fumigation alone did not cause much mortality, it made the larvae more susceptible to the cold. In the repeated trial (34) a different cultivar was used which may have been the reason for higher mortality levels in both the control and fumigation alone (Table 21). The longer cold treatment also resulted in higher mortality but the sequential combination with CO₂ did not quite get the mortality to 100%.

Table 20. Mortality of Medfly third instars in Delta Valencia after fumigation only or fumigation plus cold treatment

Fumigation treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	113	0.9
CO ₂ 70% for 24 h at 25°C		282	2.1
Untreated 24 h at 25°C	7 d at 1°C followed by 1 d at 25°C	193	66.3
CO ₂ 70% for 24 h at 25°C		315	98.1

Table 21. Mortality of Medfly third instars in Valencia after fumigation only or fumigation plus cold treatment

Fumigation treatment	Storage conditions	Number of larvae treated	Mortality (%)
Untreated 24 h at 25°C	1 d at 25°C	141	11.3
CO ₂ 70% for 24 h at 25°C		199	25.1
Untreated 24 h at 25°C	8 d at 1°C followed by 1 d at 25°C	203	88.7
CO ₂ 70% for 24 h at 25°C		189	99.5

In the Nova mandarin trial (Table 22) where the cold treatment was 2°C for 9 days, one moving larva was found but with the longer treatments for other cultivars, no survivors were found. No negative off-tastes were detected but sometimes treated fruit tasted more mature and where the fruit was mature to start with, fumigation could make the fruit taste old. For commercial use, fruit would have to be picked at the start of the picking window, or a little earlier than normal.

Table 22. Mortality of second instar Medfly in different citrus types after a sequential combination of 24 h fumigation with CO₂ at 70% and a short cold period at 2°C

Fruit type	Untreated control		Days of cold at 2°C	Fumigation and cold		Larvae killed
	Mortality %	SEM		Mortality %	SEM	
Satsuma mandarin	1.36	0.885	11	100	0	343
Nova mandarin	0	0	9	99.31	0.694	202
Clementine	0	0	13	100	0	1935
Nadorcott	0	0	11	100	0	809
Star Ruby grapefruit	0	0	13	100	0	393
Navel orange	2.38	1.885	13	100	0	300
Turkey Valencia	0	0	13	100	0	928
Valencia	0	0	13	100	0	239

Consequences of delaying cold treatment after CO₂ fumigation

When a 1 day gap was introduced between the fumigation with 35% CO₂ for 12 h and the cold treatment, the corrected mortality dropped to 25.3% which was less than the effect of cold only (Table 23). It therefore appears that the 1 day interval allows the larvae to recover and prepare for the cold after the 12 h fumigation with 35% CO₂. The use of 70% CO₂ for 12 h caused significantly more mortality than 35% CO₂ for 12 h and when the former treatment was followed by a cold treatment it also caused significantly more mortality than the latter treatment followed by cold (Table 23). Having a 1-day interval between the 70% CO₂ treatment and the cold treatment caused a significant drop in mortality to the level obtained with 35% CO₂ followed immediately by cold, but this still caused significantly more mortality than cold alone (Table 23). This led to further research with 70% CO₂ because the likelihood of getting a shorter cold treatment with the sequential treatment was greater.

Table 23. Treatment of Valencia oranges infested with FCM third instars.

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	7	189	3.7 e	
Cold ONLY at -0.5°C for 5 d	78	183	42.6 c	40.4
35% CO ₂ (12 h, no gap) + Cold (-0.5)	120	189	63.5 b	62.1
35% CO ₂ (12 h, 1-day gap) + Cold	46	164	28.0 d	25.3
35% CO ₂ only 12 h	9	200	4.5 e	0.8
70% CO ₂ (12 h no gap) + Cold	168	195	86.2 a	85.6
70% CO ₂ (12 h 1-day gap) + Cold	108	164	65.9 b	64.5
70% CO ₂ 12 h only	37	180	20.6 d	17.5

One way ANOVA after Asin (SNK 0.05)

Trial 35

The CO₂ fumigation at 70% followed immediately by 7 d at 1°C killed 100% of the FCM third instar larvae but when this cold treatment was delayed by 24 h the additional mortality caused by the CO₂ was lost (Table 24), although the difference was not significant (P>0.05). Due to the high mortality caused by cold alone, the trial was repeated with a 6-day cold period.

Table 24. Mortality of FCM third instars in Valencia after fumigation only or fumigation plus immediate or delayed cold treatment.

Treatments	Number of larvae treated	Mortality (%)
Untreated 1 d at 25°C followed by 1 d at 25°C	58	1.7 a
Untreated 1 d at 25°C followed immediately by 7 d at 1°C then a further 1 d at 25°C	115	94.8 b
Untreated 2 d at 25°C then 7 d at 1°C followed by a further 1 d at 25°C	136	94.9 b
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C	103	90.3 b
CO ₂ 70% for 1 d at 25°C followed immediately by 7 d at 1°C then a further 1 d at 25°C	115	100.0 b
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C then 7 d at 1°C followed by a further 1 d at 25°C	79	94.9 b

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

With the cold treatment shortened to 6 days (trial 36) there was not much FCM larval mortality caused by the cold alone (Table 25), although there were differences between the two cold treatments where one was held for an extra day at 25°C before going into the cold. The mortality caused by the CO₂ alone was 85% and CO₂ fumigation followed immediately by cold treatment once again caused 100% mortality. Delaying the cold treatment for 1 d after fumigation resulted in a significant loss of efficacy in the order of 20%.

Table 25. Mortality of FCM third instars in Valencia oranges after fumigation only or fumigation plus immediate or delayed cold treatment.

Treatments	Number of larvae treated	Mortality (%)
Untreated 1 d at 25°C followed by 1 d at 25°C	72	0.0 a
Untreated 1 d at 25°C followed immediately by 6 d at 1°C then a further 1 d at 25°C	47	29.8 b
Untreated 2 d at 25°C then 6 d at 1°C followed by a further 1 d at 25°C	68	11.8 a
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C	66	84.8 cd
CO ₂ 70% for 1 d at 25°C followed immediately by 6 d at 1°C then a further 1 d at 25°C	78	100.0 d
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C then 6 d at 1°C followed by a further 1 d at 25°C	29	79.3 c

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

Trial 37

In Trial 37, lower numbers of FCM larvae penetrated the fruit due possibly to the extreme age of this fruit. The lower numbers led to more variability within treatments but the results showed the same trend as in Trials 35 and 36 with 100% mortality being achieved with CO₂ 70% for 1 d when followed immediately by 6 days at 1°C (Table 26). Delaying the cold treatment by 24 h after the fumigation again resulted in 20% less mortality, but in this case the difference was not significant ($P > 0.05$). We have now shown reduced mortality from cold treatment following fumigation with CO₂ when a gap of 1 d is included between the two treatments in three trials where the fumigation was for 24 h and another earlier trial where fumigation was for 12 h.

Table 26. Mortality of FCM third instars in Valencia oranges after fumigation only or fumigation plus immediate or delayed cold treatment.

Treatments	Number of larvae treated	Mortality (%)
Untreated 1 d at 25°C followed by 1 d at 25°C	32	0.0 a
Untreated 1 d at 25°C followed immediately by 6 d at 1°C then a further 1 d at 25°C	26	61.5 b
Untreated 2 d at 25°C then 6 d at 1°C followed by a further 1 d at 25°C	29	41.4 b
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C	77	83.1 c
CO ₂ 70% for 1 d at 25°C followed immediately by 6 d at 1°C then a further 1 d at 25°C	37	100.0 c
CO ₂ 70% for 1 d at 25°C followed by 1 d at 25°C then 6 d at 1°C followed by a further 1 d at 25°C	15	80.0 c

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

Trial 64

The short cold treatment of 5 d at 2°C caused very little mortality and only made a noticeable difference after fumigation when there was no gap or an interval of 6 h. Intervals between fumigation and cold treatment of 12, 18 or 24 h appeared to allow the insects to recover and the combined benefit in terms of mortality was lost with all mortalities being approximately 20% less (Table 27). Fumigation alone with 70% CO₂ for 24 h caused 76% mortality.

Table 27. Mortality of third instar FCM in M7 navel oranges after fumigation and a short cold treatment when the interval between these two treatment components was varied.

Fumigation treatment	Interval at 25°C between fumigation and cold	Storage conditions	Number of larvae treated	Mortality (%)
Untreated control 24 h at 25°C	None	1 d at 25°C	72	0.0
Untreated for 24 h at 25°C	None	5 d at 2°C followed by 1 d at 25°C	122	12.3
Untreated for 24 h at 25°C	6 h		155	2.6
Untreated for 24 h at 25°C	12 h		137	0.7
Untreated for 24 h at 25°C	18 h		187	2.1
Untreated for 24 h at 25°C	24 h		112	2.7
CO ₂ 70% for 24 h at 25°C	None	1 d at 25°C	121	76.0 a
CO ₂ 70% for 24 h at 25°C	None	5 d at 2°C followed by 1 d at 25°C	160	97.5 b
CO ₂ 70% for 24 h at 25°C	6 h		187	92.0 b
CO ₂ 70% for 24 h at 25°C	12 h		137	79.6 a
CO ₂ 70% for 24 h at 25°C	18 h		178	73.0 a
CO ₂ 70% for 24 h at 25°C	24 h		196	79.1 a

Trial 65

Moving the fruit after fumigation into 6°C for 24 h before going into a 2°C room gave the same result as moving the fumigated fruit directly into 2°C for 5 d (Table 28). However, moving the fruit to 12°C for 24 h before going to 2°C resulted in an intermediate loss of mortality between that found for gaps of 6 h and 24 h. It may therefore be feasible to apply this combined treatment where it may take a day to reach the set temperature of 2°C, provided the fruit reaches around 6°C within 12 h.

Table 28. Mortality of third instar FCM in C100 Valencia oranges after fumigation and a short cold treatment with a step-down temperature interval between these two treatment components

Fumigation treatment	Step-down temperature between fumigation and cold	Storage conditions	Number of larvae treated	Mortality (%)
Untreated control 24 h at 25°C	None	None	139	0.0
Untreated for 24 h at 25°C	None	5 d at 2°C followed by 1 d at 25°C	154	26.0
Untreated for 24 h at 25°C	6°C for 24 h	4 d at 2°C followed by 1 d at 25°C	118	11.9
Untreated for 24 h at 25°C	12°C for 24 h		124	4.0
Untreated for 24 h at 25°C	25°C for 24 h	5 d at 2°C followed by 1 d at 25°C	136	9.6
CO ₂ 70% for 24 h at 25°C	None	1 d at 25°C	144	67.4 a
CO ₂ 70% for 24 h at 25°C	None	5 d at 2°C followed by 1 d at 25°C	160	97.5 b
CO ₂ 70% for 24 h at 25°C	6°C for 24 h	4 d at 2°C followed by 1 d at 25°C	199	95.0 b
CO ₂ 70% for 24 h at 25°C	12°C for 24 h		143	86.7 b
CO ₂ 70% for 24 h at 25°C	25°C for 24 h	5 d at 2°C followed by 1 d at 25°C	215	80.5 a

Conclusion

A high dose of carbon dioxide (70%) for 24 h was found to reduce FCM egg hatch by 88% while the same treatment caused from 31 to 90% mortality of third instar FCM in oranges. A low dose of 10% CO₂ for 6 h had no effect on third instar FCM mortality caused by a subsequent short cold treatment while a dose of 35% CO₂ for 6 h appeared to reduce mortality in a subsequent cold treatment. When the fumigation period was increased to 12 h the mortality caused by the fumigation alone was not significantly worse than for untreated third instars, but a subsequent cold treatment did not have less mortality than cold alone. Thirty-five percent CO₂ for 24 h did cause significant mortality in third and fifth instar FCM in oranges and 55% CO₂ for 24 h caused more mortality in third instars than fifth instars, but 5 d of 3°C after 50% CO₂ for 24 h did not have significantly higher mortality than 50% CO₂ for 24 h alone in fifth instars. In all cases where 70% CO₂ for 24 h was followed by a short cold treatment the mortality was higher than for cold alone or fumigation alone for both third and fifth instars. As fifth instar FCM are known to be more tolerant to cold treatment than third instars, fumigation at 70% CO₂ will be required to shorten the required cold treatment for all life stages. The benefit of preceding the cold treatment with carbon dioxide fumigation was reduced if the temperature was not lowered to around 6°C within 12 h of fumigation. Fumigation with 70% CO₂ for 24 h followed by 13 d at 2°C was successful in controlling all fifth instar FCM and second instar Medfly in a range of different citrus cultivars. Fumigation with 70% CO₂ for 24 h sometimes made the fruit taste more mature, although many people could not detect any differences. If fumigation is to be used commercially, fruit should probably be picked at the beginning of the picking window or even a week earlier.

Future research

Further research will be conducted under Project 1197 to fine-tune sequential treatments of CO₂ at 70% in air followed by cold treatments at 2 or 3°C for 13 or 15 days, respectively.

Technology transfer

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2.4.4 FINAL REPORT: The natural enemies and biological control of *Delottococcus aberiae* Project 1150 (April 2016 – March 2018) by S D Moore, W Kirkman (CRI) and M Goddard (RU)

Summary

Delottococcus aberiae, previously incorrectly identified as *D. elizabethae*, is one of the seven mealybug species recorded to infest citrus in South Africa, and has been considered extremely rare and very difficult to find, with no more than nominal pest status. However, since around 2012 fairly dramatic outbreaks of this species have been recorded on citrus in the Letsitele region, with an apparent gradual spread of the pest in the area. Due to the limited knowledge of the pest and its biological control, we proposed to regularly collect samples from infested orchards in Letsitele with the objective of monitoring the species of parasitoids and the levels of parasitism. Four collections of mealybug infested grapefruit were made from December 2015 until February 2016. These were used both to try and establish a *D. aberiae* laboratory culture on citrus seedlings and for inspection for parasitism by placing samples of individuals in eclosion chambers. *Delotococcus aberiae* infestation was lower than it had been for the previous three years and thus it was not possible to establish a laboratory culture. Also, no parasitoids were found. As was the case during the previous season, infestation levels of *D. aberiae* on citrus in the Letsitele region were again very low during the 2016/7 season. Two collections were conducted but no parasitoids emerged from either of these collections. Contact has been made with a Spanish postgraduate student, who is also working on parasitism of *D. aberiae*, including in the Letsitele region, and results from this study will become available to us in time. Additionally, a first record of a new mealybug species in South Africa, *Phenacoccus solenopsis*, has been made on citrus. Surveys will also be conducted for this species and its natural enemies.

Opsomming

Delottococcus aberiae, voorheen bekend as *D. elizabethae*, een van die sewe witluis spesies wat op sitrus in Suid-Afrika aangeteken is, is as baie skaars en moeilik om te vind beskou, en het daarom net nominale plaagstatus. Van omtrent 2012 is redelike dramatiese uitbrake van die spesie op sitrus in die Letsitele streek egeter plaasgevind, met 'n oënskynlike geleidelike verspreiding van die plaag in die omgewing. As gevolg van die beperkte kennis van die plaag en sy biologiese beheer, ons het voorgestel om gereeld monsters van besmette boorde in Letsitele te versamel met die doel om parasiet spesies en vlakke van parasitisme te monitor. Vier versamelings van witluis besmette pomelos is van Desember 2015 tot Februarie 2016 gemaak. Hierdie is gebruik albei om 'n *D. aberiae* laboratorium kultuur op sitrus saailinge te probeer stig en vir ondersoek vir parasitisme deur om individuele monsters in uitbroeiings kaste te sit. *Delotococcus aberiae* besmetting was laer as wat dit vir die vorige drie jaar was en dus was dit nie moontlik om 'n laboratorium kultuur te vestig. Daar is ook geen parasiete gekry nie. Soos wat die geval gedurende die vorige seisoen was, was besmettings vlakke van *D. aberiae* op sitrus in die Letsitele streek weer gedurende die 2016/7 seisoen baie laag. Monsters is op twee verskillende geleenthede versamel maar geen parasiete is gekry nie. Kontak is met 'n Spaanse nagraadse student gemaak, wat ook op parasitisme van *D. aberiae* werk, insluitend in die Letsitele streek, en resultate van hierdie studie sal in die toekoms vir ons beskikbaar wees. Bonop, is die voorkoms van 'n nuwe witluis spesie in Suid-Afrika, *Phenacoccus solenopsis*, vir die eerste keer op sitrus aangeteken. Opnames sal ook vir hierdie spesie en sy natuurlike vyande gemaak word.

Introduction

It has been known for many years that there are seven mealybug species that occur on citrus in southern Africa (Hattingh et al., 1998; Grout & Moore, 2015). One of these species, *Delottococcus aberiae*, previously incorrectly identified as *D. elizabethae* (Miller and Giliomee, 2011), has been considered extremely rare and very difficult to find, with no more than nominal pest status. However, in the last three years, fairly dramatic outbreaks of this species have been recorded on citrus in the Letsitele region, with an apparent gradual spread of the pest in the area.

As this pest has been so rare in the past, nothing was known about its natural enemies and potential for biological control, until a snap survey was conducted by Beltra et al. (2015). They made several collections of *D. aberiae* from ornamental plants in the Western Cape between January and March 2012, identifying the following parasitoids attacking *D. aberiae*: *Anagyrus aurantifrons*, *Lamennasia* sp., *Pachyneuron* sp., *Aenasius comperei* and one member of each of the superfamilies, Cynipoidea and Proctotrupeoidea. However, very limited surveying was done on citrus and on only one occasion (29 January 2014) was *D. aberiae* infestation recorded – this was at four locations in the Letsitele area. Only one incidence of parasitism was recorded, being an unidentified *Anagyrus* species.

We believe that Beltra et al.'s (2015) survey is therefore insufficient to ascertain the biological control potential for *D. aberiae* and thus propose to regularly collect samples from infested orchards in Letsitele with the objective of monitoring the species of parasitoids and the levels of parasitism. Additionally, a laboratory culture can be established in order to test the relative susceptibility to parasitism by the two most common parasitoids of citrus mealybug, *Planococcus citri*, namely *Coccidoxenoides perminutus* and *Anagyrus* nr sp *pseudococci*, both of which are reared commercially by insectaries in South Africa.

Objectives

- To determine the species of parasitoid species attacking *D. aberiae*
- To determine the level of parasitism of *D. aberiae*
- To determine the susceptibility of *D. aberiae* to commercially available mealybug parasitoids

Materials and methods

An initial visit was made to the Letsitele region to make contact with growers and consultants in the region who are currently familiar with the recent *D. aberiae* outbreaks recorded and to identify orchards most suitable for sample collection. As soon as mealybug was first noted, samples were collected from orchards and sent to CRI for identification. Once *D. aberiae* was recorded, the collection and despatching of additional samples of infested fruit was arranged. This was done on an approximately monthly basis, making up a total of four collections. Two further collections were made in 2017.

On receipt, samples were screened to determine whether the species present were indeed *D. aberiae*. Mealybug samples were also examined under a dissecting microscope for mummies, in order to isolate mummified mealybugs into glass vials as per Beltra et al. (2015) in order to observe for any parasitoid emergence.

The plan was to collect any parasitoids in 70% ethanol and store them at -20°C. Samples would be sent to the Biosystematics Unit of the PPRI-ARC for identification. If necessary, further molecular analyses of samples can be conducted by sequencing of the Cytochrome Oxidase 1 (CO1) gene and comparison with genes of relevant parasitoid species recorded on Genbank (Beltra et al., 2015; Marsberg et al., 2015).

Unparasitised mealybug were used to initiate a laboratory culture on citrus seedlings. Once a small culture is established, trials can be conducted to test the level of parasitism achieved with *C. perminutus* and *A. sp nr pseudococci* by exposure of a definite number mealybug of uniform life stage to a specific number of each parasitoid for a predetermined period of time. This can be done with each life stage of mealybug. This will also be conducted in comparison with citrus mealybug, as a standard.

As was the case during the previous season, infestation levels of *D. aberiae* on citrus in the Letsitele region were again very low during the 2016/7 season. Two collections were conducted but no parasitoids emerged from either of these collections. Contact has been made with a Spanish postgraduate student, who is also working on parasitism of *D. aberiae*, including in the Letsitele region, and results from this study will become available to us

in time. Additionally, a first record of a new mealybug species in South Africa, *Phenacoccus solenopsis*, has been made on citrus. Surveys will also be conducted for this species and its natural enemies.

Results and discussion

Task table

Objective / Milestone	Achievement
A: <i>Delotococcus aberiae</i> surveys	
A1: To determine the species of parasitoid species attacking <i>D. aberiae</i>	Surveys were conducted but no parasitoids were found.
A2: To determine the level of parasitism of <i>D. aberiae</i>	As above.
A3: To determine the susceptibility of <i>D. aberiae</i> to commercially available mealybug parasitoids	Due to very low levels of <i>D. aberiae</i> in orchards, it was not possible to establish a laboratory culture.
B: <i>Phenacoccus solenopsis</i> surveys	Due to the unexpected appearance of this new species of mealybug in South Africa, these objectives have now been added to the project and will be addressed during the 2016/17 season.
B1: To determine the species of parasitoid species attacking <i>P. solenopsis</i>	
B2: To determine the level of parasitism of <i>P. solenopsis</i>	
B3: To determine the susceptibility of <i>P. solenopsis</i> to commercially available mealybug parasitoids	

Delotococcus aberiae infestation in the Letsitele citrus growing region was lower than recorded during the previous three seasons. It was therefore only possible to collect very low numbers of *D. aberiae*. No parasitism was recorded and hence no parasitoids could be collected. It was therefore also not possible to establish a laboratory culture, despite attempts to do so. Further collections in 2017 also revealed no parasitoids.

Conclusions to date

As we initiated the study earlier than proposed (i.e. during the 2015/16 season, before the project funding came into effect), and as there is now a Spanish student, Javier Puig Ochoa, who has conducted a similar study starting in January 2017, and as levels of *D. aberiae* in the field have declined, this study was terminated after the 2016/17 season. *Phenacoccus solenopsis*, was also included in his survey.

Technology transfer

None yet.

Future research

No further work is planned, but results are awaited from the thorough study conducted by the Spanish students, including the *Phenacoccus solenopsis* study, once the work has been published.

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

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

Summary

The humidity requirement of seven selected fungal isolates and two commercial mycopesticides (Broadband® a.i. *B. bassiana* PPRI 5339 and Real IPM a.i. *M. anisopliae* ICIPE 69) was assessed by determining the virulence against FCM 5th instars at 3 concentrations (10^5 , 10^6 , and 10^7 conidia/mL) and four humidity levels (12, 43, 75 and 98% RH), under laboratory conditions. High mortalities were recorded for the *M. anisopliae* isolates and the two commercial products, regardless of humidity. At the highest tested concentration, three *M. anisopliae* isolates, G 11 3 L6 (Ma1), FCM Ar 23 B3 (Ma2) and G OL R8, from which two (Ma1 and Ma2) have been recommended for further studies, 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 the two commercial mycopesticides, Real IPM (75.8%, 83.3%, 82.5% and 85.8% at 12, 43, 75 and 98% RH respectively) and Broadband® (78.3%, 80.0%, 76.7% and 82.5% at 12, 43, 75 and 98% RH respectively) at all humidities tested. However, the LT₅₀ (lethal time to cause 50% pupal mortality) for Ma1 and Ma2 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). *Beauveriana bassiana* isolates, G Ar 17 B3 (Candidate Bb1), GB Ar 23 B3, G14 2 B5 and FCM 10 13 L1, showed reduced virulence against FCM, with pupal mortalities of the highest tested concentration being 40.0%, 57.5%, 60.0% and 50.8% at 12% RH, 47.5%, 58.3%, 57.5% and 55.8% at 43% RH, 46.7%, 63.3%, 59.2% and 55.0% at 75% RH, and 45.8%, 60.0%, 60.8% and 58.3% at 98% RH, respectively. Endophytic potential and UV sensitivity of these isolates is currently being tested.

Opsomming

Die vogtigheidsvereiste van sewe geselekteerde swamisolate en twee kommersiële swamplagdoders (Broadband® ai *B. bassiana* PPRI 5339 en Real IPM ai *M. anisopliae* ICIPE 69), is evalueer deur bepaling van virulensie teen VKM 5de instars teen 3 konsentrasies (10^5 , 10^6 , en 10^7 konidia/mL) en vier humiditeitsvlakke (12, 43, 75 en 98% RH), onder laboratoriumtoestande. Hoë mortaliteit is aangeteken vir die *M. anisopliae*-isolate en die twee kommersiële produkte, ongeag humiditeit. By die hoogste getoetste konsentrasie is drie *M. anisopliae*-isolate, G 11 3 L6 (Ma1), FCM Ar 23 B3 (Ma2) en G OL R8, waarvan twee (Ma1 en Ma2) aanbeveel is vir verdere studies, het papie mortaliteit veroorsaak van 77,5%, 83,3% en 80% teen 12% RH, 85,8%, 90,0% en 82,5% teen 43% RH, 91,7%, 91,7% en 80,8% teen 75% RH en 85,8%, 90,8% en 82,5% teen 98% RH, onderskeidelik. Hierdie mortaliteit het nie beduidend verskil van die twee kommersiële swamplagdoders, Real IPM (75,8%, 83,3%, 82,5% en 85,8% onderskeidelik 12, 43, 75 en 98% RH) en Broadband® (78,3%, 80,0%, 76,7% en 82,5% onderskeidelik 12, 43, 75 en 98% RH) teen alle humiditeits vlakke wat getoets is. Die LT_{50} (tydsduur om 50% papie mortaliteit te veroorsaak) vir Ma1 en Ma2 was egter aansienlik laer by hoë humiditeit (4.6-4.8 dae teen 43%, 4.1-4.2 dae teen 75% en 4.1-4.4 dae teen 98% RH) as by 12% RH (5.6-6.7 dae). *Beauveria bassiana* isolaat, G Ar 17 B3 (Kandidaat Bb1), GB Ar 23 B3, G14 2 B5 en FCM 10 13 L1, het verminderde virulensie teen VKM getoon, met papie mortaliteit van die hoogste getoetste konsentrasie van 40,0%, 57,5%, 60,0% en 50,8% teen 12% RH, 47,5%, 58,3%, 57,5% en 55,8% teen 43% RH, 46,7%, 63,3%, 59,2% en 55,0% teen 75% RH en 45,8%, 60,0%, 60,8% en 58,3% teen 98% RH, onderskeidelik. Endofitiese potensiaal en UV-sensitiwiteit van hierdie isolate word tans getoets.

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

Project 1174 (Apir 2017 – April 2019) by Sean Moore and Wayne Kirkman (CRI) and Mellissa Peyper (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. One trial was conducted in the Eastern Cape and another in the Western Cape. In both trials, four monthly applications were made with each of the three products, starting in October. In the Eastern Cape, these were applied in adjacent identical Navel orange orchards, one under nets and the other open. In the Western Cape, the treatments were only applied to a Navel orange orchard in the open. At the Eastern Cape site, three evaluations were conducted for red scale and mealybug infestation and thrips damage. No statistically significant differences were recorded between treatments. However, red scale infestation was significantly higher under the net and mealybug infestation and thrips damage were significantly higher outside the net. At the Western Cape trial site, although differences between treatments were not significant, red scale and mealybug infestation were lowest for the only *Metarhizium anisopliae* product treatment. A third corrective trial was applied with the same treatments at a site heavily infested with red scale and mealybug. Results are not yet available.

Opsomming

Tans word daar minstens drie produkte gebaseer op entomopatogeniese swamme (EPS) aktief bemark en verkoop vir beheer van sitrusplae in Suid-Afrika. Dit is bekend dat verskeie ander EPS produkte deur kleiner maatskappye vervaardig word en in nis-markte verkoop word. Al is hierdie produkte nie altyd teen die teikenplae geregistreer nie, is hulle gebruik in die bedryf besig om toe te neem. Bewerings van goeie beheer van 'n reeks sitrusplae word al hoe meer algemeen, maar hierdie bewerings bots gereeld met die ondervindinge van wetenskaplikes in die bedryf. Die doel van hierdie projek is om die waarheid oor die doeltreffendheid en

bruikbaarheid van hierdie produkte op te klaar. Een proef is in die Oos-Kaap uitgevoer en nog een in die Wes-Kaap. In albei proewe is die drie produkte elk maandeliks gespuit met die eerste bespuiting in Januarie. In die Oos-Kaap proef is die behandelings in twee boorde langs mekaar toegedien, een onder nette en die ander in die ope. In die Wes-Kaap proef is die drie produkte net in een Nawellemoenboord in die ope toegedien. By die Oos-Kaap perseel is drie evaluasies uitgevoer vir rooidopluis en witluis besmetting en blaaspootjie skade. Geen statisties betekenisvolle verskille is tussen behandelings aangeteken nie. Rooidopluis besmetting is egter betekenisvol hoër onder die net en witluis besmetting en blaaspootjie skade is betekenisvol hoër buite die net. By die Wes-Kaap perseel, al was die verskille nie betekenisvol nie, is dopluis en witluis besmetting die laagste vir die enigste *Metarhizium anisopliae* produk behandeling. 'n Derde korrektiewe proef is met dieselfde behandelings toegedien by 'n perseel waar dopluis en witluis besmetting hoog was. Resultate is nog nie beskikbaar nie.

2.5 PROGRAMME: NON-PHYTOSANITARY KEY PESTS

Programme Coordinator: Tim G Grout (CRI)

2.5.1 Programme summary

Although there are key cosmetic pests such as citrus thrips and bollworm that cause damage early in the season, there are many management options for these. The current challenges being addressed in this programme are the limited options for the control of late season infestations of citrus thrips, leafhoppers, woolly whitefly and our citrus psylla the African citrus triozid (ACT). In addition to ACT, we are also preparing for the arrival of the Asian citrus psyllid *Diaphorina citri* (ACP). CRI research has led to the emergency registration of an organic product for the control of the green citrus leafhopper (2.5.2), and a second organic product has also given promising results. These may be of value for other pests such as ACT and citrus thrips on the March growth flush, but this could not be evaluated. Further screening of prospective systemic insecticides against aphids has shown that some new products may be effective as soil drenches or stem treatments for the control of ACP and ACT (2.5.3). Preparations are being made in Kenya for these products to be evaluated on potted citrus plants against ACP. Attempts to control both ACT and ACP in Mauritius using products available in South Africa are continuing and a comparison of different sticky traps has given an indication of the most effective one to use (2.5.4). The next challenge to be faced in Mauritius is to see whether plant protection products used in South Africa are effective in keeping newly planted trees free from infection by Huanglongbing. All these projects will continue in 2018/9.

Program-opsomming

Hoewel daar sleutel kosmetiese plae soos sitrus blaaspootjies en bolwurm is wat skade vroeg in die seisoen veroorsaak, is daar baie bestuurs-opsies vir hierdie plae. Die huidige uitdagings wat in hierdie program aangespreek word, is beperkte opsies vir die beheer van laat-seisoen infestaties van sitrus blaaspootjies, bladspringers, wollerige witvlieg en ons sitrus bladvlooi, die Afrika sitrus triozid (ACT). Bykomend tot ACT, berei ons ook voor vir die arrivering van die Asiatiese sitrus bladvlooi, *Diaphorina citri* (ACP). CRI navorsing het tot die nood registrasie van 'n organiese produk vir die beheer van die groen sitrus bladspringer gelei (2.5.2), en 'n tweede organiese produk het ook belowende resultate gelewer. Hierdie kan van waarde vir ander plae soos ACT en sitrus blaaspootjies op die Maart groeistuwing wees, maar dit kon nie geëvalueer word nie. Verdere evaluering van voornemende sistemiese plaagdoders teen plantluis het getoon dat sommige nuwe produkte effektief as grondrengings of stambehandelings vir die beheer van ACP en ACT kan wees (2.5.3). Voorbereidings word in Kenia gemaak ten einde hierdie produkte op gepotte sitrusplante teen ACP te evalueer. Pogings om beide ACT en ACP in Mauritius te beheer, deur gebruik te maak van produkte wat in Suid-Afrika beskikbaar is, gaan voort, en 'n vergelyking van verskillende kleeflokvalle het 'n aanduiding gegee van die mees effektiewe een om te gebruik (2.5.4). Die volgende uitdaging wat in Mauritius wag, is om te sien of plantbeskermingsprodukte wat in Suid-Afrika gebruik word, effektief is om nuut-aangeplante bome vry van infeksie deur Huanglongbing te hou. Al hierdie projekte sal in 2018/9 voortgaan.

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

Project 1061 (2013/4 – 2019/20) by Tim G Grout and Peter R Stephen (CRI)

Summary

Although field trials with citrus thrips, citrus psylla and woolly whitefly were not possible, two trials were conducted with leafhoppers in the Marble Hall area. Two unregistered organic products with short preharvest intervals, Kangroshield100 and Xterminator, were both compared at 500 ml/hl water to a Phosdrin standard at 30 ml/hl. The two products were as effective as Phosdrin against the green citrus leafhopper for the first week after treatment but not effective against the citrus leafhopper. This trial led to the emergency registration of Xterminator for green citrus leafhopper on citrus. Further field trials will be conducted on autumn pests with similar products if opportunities arise. Unfortunately, Requiem, that had shown some promise in laboratory trials against woolly whitefly, is no longer available in South Africa.

Opsomming

Hoewel veldproewe met sitrus blaaspootjies, sitrus bladvlooi en wollerige witvlieg nie moontlik was nie, is twee proewe met bladspringers in die Marble Hall area uitgevoer. Twee nie-geregistreeerde organiese produkte met kort voor-oes intervalle, Kangroshield100 en Xterminator, is beide teen 500 ml/hl water met 'n Phosdrin standaard teen 30 ml/hl vergelyk. Die twee produkte was net so effektief as Phosdrin teen die groen sitrus bladspringer vir die eerste week ná behandeling, maar nie effektief teen die sitrus bladspringer nie. Hierdie proef het tot die nood registrasie van Xterminator vir groen sitrus bladspringer op sitrus gelei. Verdere veldproewe sal op herfs plaes met soortgelyke produkte uitgevoer word, indien geleenthede opduik. Requiem, wat belofte in laboratoriumproewe teen wollerige witvlieg getoon het, is ongelukkig nie meer in Suid-Afrika beskikbaar nie.

2.5.3 **PROGRESS REPORT: New systemic insecticides for citrus**

Project 1148 (2016/7 – 2020/1) by Tim Grout and Peter Stephen (CRI)

Summary

With the likely arrival of *Diaphorina citri* within a few years from Tanzania it is important for us to find more systemic treatments that can be used to control this vector in nurseries and for non-bearing trees. Further trials with aphids (*Toxoptera citricidus*) on potted citrus plants gave promising results but a field trial with aphids gave variable results due possibly to two different species being present and the influence of natural enemies. Preparations are now being made with ICIPE for pot trials against *D. citri* on citrus in Kenya with the construction of an insect-proof shade-house and the sourcing of potted citrus plants. Screening trials are expected to start later in 2018.

Opsomming

Met die waarskynlike arrivering van *Diaphorina citri* binne die volgende paar jaar vanaf Tanzanië, is dit belangrik vir ons om meer sistemiese behandelings te vind wat gebruik kan word om hierdie vektor in kwekerye te beheer, en vir nie-draende bome gebruik kan word. Verdere proewe met plantluise (*Toxoptera citricidus*) op gepotte sitrusplante het belowende resultate gelewer, maar 'n veldproef met plantluise het variërende resultate gegee, moontlik weens twee verskillende spesies wat teenwoordig was, en die invloed van natuurlike vyande. Voorbereidings word nou met ICIPE getref vir potproewe teen *D. citri* op sitrus in Kenia, met die konstruksie van 'n insek-bestande skaduhuis en die verkryging van gepotte sitrusplante. Daar word verwag om met evaluasieproewe later in 2018 te begin.

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

Project 1158 (2016/7 – 2018/9) by Aruna Manrakhan, Glynnis Cook, Rochelle Clase, Tim Grout, Herbert Wiehe (Domaine de Labourdonnais, Mauritius), Preaduth Sookar (Entomology Division, Ministry of Agro-Industry and Food Security, Mauritius), Malini Alleck (Entomology Division, Ministry of Agro-Industry and Food Security, Mauritius)

Summary

The citrus industry of South Africa is under threat of introduction of the Asian Citrus Psyllid (ACP), *Diaphorina citri*, currently present in Tanzania and Kenya. Between 2016 and 2017, a treatment programme recommended by CRI for control of the African Citrus Triozid (ACT), *Trioza erytreae*, was tested in a Valencia orchard at Labourdonnais farm in the north-west of Mauritius. This consisted of a trunk treatment with acetamiprid 20% SL in October followed a month later by soil drenching with imidacloprid 305 SC and followed five months later by a trunk treatment with acephate 350 AL. The mean numbers of ACP per trap per week in the treated and untreated blocks were 0.05 ± 0.01 and 0.14 ± 0.02 respectively. The efficacy of three trap types: Chempac yellow trap (8.5 cm x 20 cm), Alpha Scents yellow trap (18 cm X 14 cm) and Alpha Scents lime green ACP trap (18 x 14 cm) for monitoring of ACP was determined in three pummelo orchards at Labourdonnais farm. The trial was carried out over 11 weeks between October 2017 and January 2018. There were 12 traps of each type checked on a weekly basis. A total of only 10 ACP individuals were trapped over the study period. The Alpha Scents yellow trap was the most effective trap for ACP. The mean numbers of ACP per trap per week were 0.00 ± 0.00 , 0.06 ± 0.02 and 0.01 ± 0.01 for the Chempac Yellow trap, Alpha Scents yellow trap and Alpha Scents lime green ACP trap respectively. The dominant wavelengths measured for the Chempac Yellow, Alpha Scents yellow trap and Alpha Scents lime green ACP trap were 580, 578 and 574 nm respectively. Disease free Valencia plantings are currently being prepared in a quarantine facility at the Ministry of AgroIndustry and Food Security in Mauritius for trials to determine whether the South African ACT treatment programme will be able to prevent transmission of Huanglongbing by ACP.

Opsomming

Die Suid-Afrikaanse sitrusbedryf word deur die moontlike indringing van die Asiatiese sitrus-bladvlooi (ACP), *Diaphorina citri*, bedreig wat tans in Tanzanië en Kenia teenwoordig is. Tussen 2016 en 2017 is 'n behandelingsprogram, wat deur CRI vir die beheer van die Afrika sitrus-bladvlooi, *Trioza erytreae* (ACT), aanbeveel is, in 'n Valencia boord op die plaas Labourdonnais, wat in die noord-westelike dele van Mauritius geleë is, getoets. Die behandelingsprogram het bestaan uit 'n stambehandeling met acetamiprid 20% SL in Oktober, wat 'n maand later gevolg is met 'n grondtoediening van imidacloprid 305 SC en vyf maande later gevolg met 'n stambehandeling met acephate 350 AL. Die gemiddelde getal ACP per lokval per week in die behandelde blokke en onbehandelde blokke was onderskeidelik 0.05 ± 0.01 en 0.14 ± 0.02 . Die drie lokval tipes: "Chempac yellow" (8.5cm x 20cm), "Alpha Scents yellow" (18cm x 14cm) en "Alpha Scents lime green ACP" (18cm x 14cm) se effektiwiteit is in drie pompelmoes boorde op die Labourdonnais plaas bepaal. Die proef het vir 11 weke tussen Oktober 2017 en Januarie 2018 geduur. Daar was 12 lokvalle van elke soort wat weekliks gemonitor is. 'n Totaal van slegs 10 ACP is gedurende die 11-weke proef gevind. Die mees doeltreffende lokval was die "Alpha Scents yellow". Die gemiddelde getal ACP per lokval per week was 0.00 ± 0.00 vir die "Chempac yellow" lokval, 0.06 ± 0.02 vir die "Alpha Scents yellow" lokval en 0.01 ± 0.01 vir die "Alpha Scents lime green" lokval. Die dominantste golflengtes gemeet was 580 nm vir die "Chempac yellow" lokval, 578 nm vir die "Alpha Scents yellow" lokval en 574 nm vir die "Alpha scents lime green" lokval. Virusvrye Valencia saailinge word tans in 'n kwarantyn fasiliteit by the Ministerie van Landboubedryf en Voedselveiligheid in Mauritius voorberei vir proewe om te bepaal of die Suid-Afrikaanse behandelingsprogram vir ACT die verspreiding van Haunglongbing deur ACP kan verhoed.

2.6 PROGRAMME: MINOR PESTS AND MITES

Programme Coordinator: Tim G Grout (CRI)

2.6.1 Programme summary

For some growers in particular microclimates or those wanting to grow citrus organically, minor pests can become extremely serious. Woolly whitefly can become a serious pest where plant protection products are not being used for the control of scale insects and mealybug. The parasitoid that CRI imported to control this pest has become established in parts of the North West Province and Mpumalanga, and recently appeared in the Western Cape (2.6.2). Further attempts to establish it in the Eastern Cape will continue under a new project. Beetle mites, which are often used as indicators of healthy ecosystems, are causing export rejections in southern KZN. Research has shown that these mites are not moving from the soil into the tree but maintain populations in the tree canopies throughout the year that reach peak abundance in autumn, resulting in high levels of fruit infestation at harvest (2.6.3). Most citrus acaricides have little effect on these mites so further research will investigate management approaches.

Program-opsomming

Vir sommige produsente in spesifieke mikro-klimatiese omgewings of dié wat sitrus organies wil verbou, kan minder belangrike plaë uiters ernstig word. Wollerige witvlieg kan 'n ernstige plaag word waar plantbeskermingsprodukte nie vir die beheer van dopluis en witluis gebruik word nie. Die parasitoïed wat CRI ingevoer het om hierdie plaag te beheer, het in dele van die Noordwesprovinsie en Mpumalanga gevestig, en het onlangs in die Wes-Kaap verskyn (2.6.2). Verdere pogings om dit in die Oos-Kaap te vestig, sal onder 'n nuwe projek voortgaan. Kewer myte, wat dikwels as indikatore van gesonde ekosisteme gebruik word, veroorsaak uitvoer verwerpinge in suidelike KZN. Navorsing het getoon dat hierdie myte nie vanaf die grond in die boom in beweging is, maar handhaaf deur die jaar populasies in die boomlower, wat piek volopheid in herfs bereik, en tot hoë vlakke van vrug-infestasies tydens oes lei (2.6.3). Meeste sitrus mytdoders het min effek op hierdie myte, so verdere navorsing sal bestuursbenaderings ondersoek.

2.6.2 FINAL REPORT: Importing and releasing *Cales noacki* for the control of woolly whitefly Project 1082 (2014/5 – 2017/8) by T G Grout and P R Stephen (CRI)

Summary

Cales noacki, the parasitoid of woolly whitefly (WWF) that we imported from Spain, has become established in Mooinooi in the North West Province and in Nelspruit, but not where releases were made in the Eastern Cape. Populations of WWF were low in the north due to the drought and numbers of parasitoids were therefore too low to make further releases in the Eastern Cape. Surprisingly, *C. noacki* has become established in the Western Cape, even though we made no releases there. These populations will now be used to make further releases in the Western and Eastern Cape under project 1194.

Opsomming

Cales noacki, die parasitoïed van wollerige witvlieg (WWF) wat vanaf Spanje ingevoer is, het in Mooinooi in die Noordwesprovinsie en in Nelspruit gevestig, maar nie waar vrystellings in die Oos-Kaap gemaak is nie. Populasies van WWF was laag in die noorde weens die droogte, en parasitoïed getalle was dus te laag om verdere vrystellings in die Oos-Kaap te maak. *C. noacki* het verbasend in die Wes-Kaap gevestig hoewel ons geen vrystellings daar gemaak het nie. Hierdie populasies gaan nou gebruik word om verdere vrystellings in die Wes- en Oos-Kaap onder projek 1194 te maak.

Introduction

The woolly whitefly, *Aleurothrixus floccosus* (Maskell) arrived in the south of the country around 2006 and has slowly spread northwards and eastwards so that it now occurs in the Western and Eastern Cape, KwaZulu-Natal, the North West Province, the Mpumalanga lowveld and as far north as Polokwane. It is most common on lemon trees in home gardens but is becoming problematic in commercial citrus orchards where plant protection products

are not being applied for the control of red scale or mealybug. It therefore severely affects organic producers and those who attempt to maximise the biocontrol of sucking pests.

Throughout the world, the aphelinid parasitoid *Cales noacki* Howard has been introduced to control woolly whitefly and without exception has been extremely effective. This parasitoid was introduced to California from Chile in 1970 (DeBach and Rose 1976) and was introduced to Italy (Laudonia and Viggiani 1986) and Spain at about the same time. By the 1990s, woolly whitefly had spread to most citrus producing countries in the Mediterranean and *C. noacki* was introduced into Greece (Katsoyannos et al. 1997), Sicily, Tunisia, Turkey (Ulusoy et al. 2003), Israel, the Maltese islands and elsewhere. It was also introduced into Uganda, Kenya and Malawi where the pest had established in the 1990s.

A request was made to DAFF on 6 May 2009 to import and release *C. noacki* for the control of woolly whitefly in South Africa. We were told by Isabel Bezuidenhout, Acting Assistant Director: Pest Risk Analysis on 14 Sep 2009 that we were not permitted to release the parasitoid without conducting host specificity tests on indigenous species of whiteflies. As these species would first have to be found and reared, then exposed to *C. noacki* in quarantine in small cages we refused to proceed with this process because it would be extremely expensive and unlikely to succeed due to the unnatural conditions. Due to weed biocontrol researchers facing similar difficulties a committee of expert reviewers was formed to investigate the case and in July 2014 we were permitted to introduce and release *C. noacki*.

Research in California (Mottern et al. 2011) had shown that *C. noacki* is a species complex which explains why there are some records of it attacking some species other than woolly whitefly. Jason Mottern at UCR promised to do some molecular tests on the *C. noacki* that we hoped to import from Spain to confirm that it is *C. noacki sensu stricto* before we made final arrangements to import *C. noacki* from that region.

Stated objective

Import and release *Cales noacki* to biologically control woolly whitefly.

Materials and methods

We arranged with Prof Ferran Garcia-Mari of the Mediterranean Agroforestry Institute at the Polytechnic University of Valencia in Spain to provide *C. noacki* parasitoids in their pupal stage from citrus and send them to us. We placed these in emergence boxes and emerging adults were checked for identity before transferring them to potted citrus plants infested with local woolly whitefly. The parasitized local woolly whitefly was then used to start a colony of *C. noacki*. Once several thousand *C. noacki* had been reared, infested plants were exposed to adult parasitoids, then shortly before the next generation of adults were due to emerge the plants were placed in two citrus orchards with high infestations of woolly whitefly where pesticides are not being used in different climatic regions, Mooinooi, North West Province and Nelspruit, Mpumalanga. The plan was that when infestations in these release orchards were showing high levels of parasitism, infested branches from these orchards could be cut and placed in other infested orchards in the surrounding area. This has only happened at Mooinooi so far because numbers of *C. noacki* recovered from release sites around Nelspruit have remained low and populations of woolly whitefly have also remained low due to the drought and other natural enemy activity.

The small culture of Spanish *C. noacki* started in December 2014 increased successfully on potted citrus plants at CRI-Nelspruit. The first releases of *C. noacki* were made in an Empress mandarin orchard near Mooinooi, North West Province on 22 Jan 2015 by placing an infested potted citrus plant in the orchard from which parasitoids were emerging in addition to releasing approximately 100 adults. Multiple releases of adult *C. noacki* were also made in a citrus orchard at the ARC-TSC in Nelspruit. Home garden releases and releases at a shopping centre in Nelspruit also took place with from 50 to 200 parasitoids being released per time. However, the WWF culture on potted citrus plants in Nelspruit then started declining and attempts to culture WWF on floating citrus leaves

failed. Releases of low numbers of adult *C. noacki* from the mother culture were made in Port Elizabeth in March 2015 and in June, approximately 300 adults were released at Coerney Farm near Addo in the Sundays River Valley in an organic, Lane Late navel orange orchard. A further small release was made in Port Elizabeth at this time.

Attempts to recover *C. noacki* from release sites in the north were made between July and September 2016. Further releases in the Eastern Cape were not possible due to an inability to re-establish the WWF mother culture in Nelspruit and numbers in the field being inadequate for releases. This remained the case until the termination of the project in March 2018.

Results and discussion

Recovery of parasitoids from the release sites was lower than expected, perhaps due to competition from *Encarsia* spp. and the predatory beetle *Cybocephalus* sp. Sampling of WWF in November 2015 on trees in Nelspruit where releases had been made earlier in the year and at the Valencia release orchard only resulted in the recovery of two *C. noacki* from the Valencia orchard but quite high numbers of an *Encarsia* sp. In July 2016, 18 months after the initial release, approximately 20 infested leaves were picked from the release orchard at Mooinooi and placed in an emergence box. One hundred and twenty-four *C. noacki* emerged so this proved that the parasitoid was established at that site and the grower was encouraged to cut infested branches from there and move them to other orchards with WWF infestations. However, samples of WWF from an orchard 350 m from the release orchard yielded no *C. noacki*, so its dispersal appeared to be slow.

During September 2016, three samples of WWF taken from citrus trees at the Orchards Centre in Nelspruit yielded 26 *Cales noacki*. However, a sample taken from the Valencia ARC-TSC release orchard yielded no *Cales*. This sampling, conducted more than 18 months after multiple releases in these sites, indicates some establishment of *C. noacki* in Nelspruit but disappointingly, no establishment in an orchard close to the town.

After the initial establishment of a WWF culture, continuous challenges were experienced because although thousands of adult WWF were placed in cages with plants having tender new leaves, very few eggs were laid. We tried both high and no fertilisation regimes but this had no influence. We also moved the plants to different places with different light intensity and away from possible pesticide drift from the glasshouses, but this had no effect. Eventually the WWF culture collapsed completely and we were no longer able to rear the parasitoid, nor send more parasitoids to the Eastern Cape where establishment had not been confirmed.

In January 2017, a sample of WWF from citrus in the Robertson area of the Western Cape was received from Martin Gilbert and it yielded 39 *C. noacki*. Subsequently, reports of *C. noacki* from WWF on citrus in Stellenbosch were also received and Prof Jan Giliomee who had had WWF on the citrus trees in his garden since 2009 (Giliomee and Millar 2009) said that his trees were now clean. It is therefore apparent that *C. noacki* is established in the Western Cape, although we did not release it there.

Martin Gilbert now has a project (1194) in 2018/9 on *C. noacki* in the Western Cape in which he plans to assist in the establishment of *C. noacki* in the Eastern Cape.

Conclusion

Cales noacki was successfully imported from Spain and established in the North West and Mpumalanga provinces. It has also appeared in the Western Cape where it is noticeably reducing backyard infestations of WWF. Further distribution of *C. noacki* around the Western Cape and into the Eastern Cape will take place in project 1194 led by Martin Gilbert.

Future research

Further research will be conducted in project 1194 where Martin Gilbert will assist in distributing *C. noacki* in the Western Cape and attempt to get it established in the Eastern Cape.

Technology transfer

Included in presentations at CRI Integrated pest and disease management workshops.

Plan to include in an article on whiteflies on citrus for African Entomology.

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2.6.3 **PROGRESS REPORT: Preharvest management of oribatulid mites on citrus in KwaZulu-Natal** Project 1172 (2017/8 – 2018/9) by Tim G Grout and Peter R Stephen (CRI)

Summary

A citrus grower in southern KZN has 30-60% of his fruit infested with a harmless arboreal mite *Siculobata sicula* at harvest and these are resulting in export rejections. We have sampled in the trees and under the trees of three orange orchards every two months for a year and found that there is no obvious movement of these mites from the soil into the canopy that could be stopped with a trunk barrier. The mites remain in the canopy all year and become more abundant during late summer, resulting in high levels of fruit infestation at harvest. There is no obvious correlation with honeydew-producing pests and these mites are not known to be phytophagous, so there must be sufficient algae or fungi present to support them. Many of the acaricides registered for use on citrus have had little effect on mite populations other than chlorfenapyr applied as a medium cover spray soon after petal fall. Further research will be conducted on the management of these mites using various acaricides and high concentrations of horticultural mineral oil.

Opsomming

In suidelike KZN is 30-60% van 'n sitrusprodusent se vrugte met 'n skadelose boomlewende myt, *Siculobata sicula*, tydens oes geïnfesteer, en dit lei tot uitvoer verwerpinge. Ons het in die bome en onder die bome van drie lemoenboorde, elke twee maande vir 'n jaar, versamel, en gevind dat daar geen duidelike beweging van hierdie myte vanaf die grond in die boomlower in was, wat met 'n stamversperring gestop kon word nie. Hierdie myte bly in die lower deur die hele jaar en word meer volop gedurende láát somer, en dit lei tot hoë vlakke van vrug-infestasië tydens oes. Daar is geen duidelike korrelasie met heuningdou-produuserende plaë nie en hierdie myte

is nie bekend daarvoor dat hulle fitofagies is nie, so daar moet voldoende alge of swamme teenwoordig wees om hulle te onderhou. Baie van die mytdoders wat vir gebruik op sitrus geregistreer is, het min effek op mytopulasies gehad, behalwe vir chlorfenapyr was as 'n medium bedekkingsspuit spoedig ná blomval toegedien is. Verdere navorsing sal op die bestuur van hierdie myte uitgevoer word, deur gebruik te maak van verskeie mytdoders en hoë konsentrasies tuinboukundige minerale olie.

3 PORTFOLIO: DISEASE MANAGEMENT

3.1 PORTFOLIO SUMMARY

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

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

Within the Graft Transmissible Diseases (GTD) group “Huanglongbing” (HLB) or “Asian Greening” has been identified as an eminent threat to the South African industry. This was mainly due to the confirmed presence of ‘*Candidatus*’ *Liberibacter asiaticus* (Las) and its vector, *Diaphorina citri*, on the African continent. However, due to many years’ research done within CRI, the correct identification of the *Liberibacter* species present in East Africa was possible. Analysis of survey samples from this region identified less aggressive ‘*Candidatus*’ *Liberibacter africanus* (Laf) species as being present in Uganda, Kenya and Tanzania, confirming that Las is only present in Ethiopia (Project 1157). Project 1200 follows on 1157 and aims to validate the detection protocol for the various *Liberibacter* species that was developed in project 1157. Apart from molecular detection of the different *Liberibacter* species, project 1184 also aims to train a sniffer dog to detect ‘*Candidatus*’ *Liberibacter africanus* (Laf) in infected trees. Here also good progress was made with the dog being able to detect infected trees in a controlled environment. It is hoped that when HLB does arrive in South Africa, dogs can also be trained to detect and differentiate between Las and Laf infected trees. For the potential management of HLB, project 1160 is focused on the development of CTV infectious clones that can be used to deliver a payload to infected trees that could potentially eliminate or inhibit the development of Las within the infected tree.

Despite the focus on *Liberibacter* species, strong focus remains within GTD on *Citrus tristeza virus* (CTV) due to the use of cross-protection to mitigate the effects of this virus within the Citrus Improvement Scheme (CIS). Due to the progress made in CTV strain identification, the specific strains involved in disease expression and those required to mitigate the disease, can now be characterized accurately. Single strain sources can be studied in glasshouse and field trials to determine their impact on the host and their potential for use in cross-protection. More specific CTV-host interaction studies and advanced virus diagnostics using bioinformatics are enabling researchers to more deeply study the biology of this virus (Project 1100). Within project 1155 citrus viroids are also getting attention and several rootstocks are being evaluated for their sensitivity to the different viroids. As this project was only established recently, no clear results are evident at this stage.

Within grapefruit trials, results have shown that single strain CTV sources are more promising than mixed strain sources to be used in pre-immunization. Effect of climate on symptom expression is furthermore evident from these trials (Project 742). Project 1173 continues along the same vein by evaluating single vs. mixed strain sources for the pre-immunization of grapefruit, Valencia and navel cultivars. Investigation into CTV and its role in soft citrus is also being done (Project 968), while Project 1074’s results have already shown in a glasshouse trial that CIS material, that are free from viruses and viroids, are performing better than contaminated field-cut material.

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

Long-term studies of pre-and postplant management of nematodes and *Phytophthora* are showing that preplant fumigation results in trees in these treatments being taller with thicker trunks than trees in other treatments (Project 762). Evaluation of alternative, non-chemical treatments, to replace chemical nematicides, have shown that the products have variable results and that the best results are often achieved in regimes where the alternative is combined with one chemical application (Project 1030). Phosphonate applications on mandarin fruit were also shown to cause severe phytotoxic burn if these applications were done after colour break.

A complex of pathogens was found to be involved with a trunk rot and decline disease observed by growers in the Eastern Cape production region and that high soil and water pH in this region are possibly predisposing the Carrizo citrange and Swingle citrumelo rootstocks to this pathogen complex. This postulation is currently under further investigation (Project 1068) along with a study to determine where the sources of the pathogens involved are. Citrus decline is also seen as having a complex etiology of biotic and or abiotic factors and in Project 1092 these are investigated further to look for any factors that can be used as an early warning of decline problems. Citrus replant problems are more and more experienced due to old orchards being replaced and Project 1152 has been concluded. In this project it was found that apart from *Phytophthora* spp. and the citrus nematode, several *Fusarium* and *Neocosmospora* spp. are also involved in the citrus replant complex.

The rapid expansion in the industry as well as replant programmes implemented, is putting a strain on nursery production and in Project 1101, the preventative and curative soilborne disease management practices followed in citrus nurseries are being investigated through pathogen characterization and fungicide sensitivity work. Results from this project have already led to recommendations to nurseries. Further results have indicated that ten different *Pythium* spp. are present in South African citrus nurseries and that they vary greatly in their sensitivity to mefenoxam. Pathogenicity trials and chlorine sensitivity work with these species are currently underway.

Essential oils (EO) are known to have an effect on postharvest pathogens and the EO are therefore being tested as encapsulations of slow-release nano-or micro particles. Specialized equipment and methods were developed for this work and extensive data were gathered that are under analysis (PHI 66).

Alternative and new products were tested and the most successful found to be an azoxystrobin formulation against *Penicillium digitatum*. Combination products of hydrogen peroxide and acetic acid were seen to have no effect on the sensitivity of pyrimethanil and stability of propiconazole. A powder formulation of PAA was developed and tested and seen not to have the phytotoxic problems of liquid formulations. Water sanitation is also under focus with several products being tested (Project 123). Propiconazole has been registered in South Africa for sour rot control and this necessitated the determination of the baseline sensitivity of South African isolates of *G. citri-aurantii* causing sour rot and *Penicillium digitatum* causing green mould (Project 1141).

The role of phytochemicals, produced by citrus fruit, in inhibiting the infection of fruit by *Phyllosticta citricarpa* (citrus black spot, CBS), is being investigated. Cultivars with varying susceptibility to CBS are included and investigated with respect to their apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycosides) fractions in their rind phytochemistry. Included are Bitter Seville, which is accepted to have low susceptibility to CBS infection, Valencia orange with medium susceptibility, highly susceptible lemon, and kumquats as a resistant type (Project 1135).

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

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

Citrus black spot, caused by *Phyllosticta citricarpa*, occurs in many citrus growing areas but in the EU it is subject to phytosanitary legislation. The occurrence of *Phyllosticta* spp. in Europe was determined via surveys of citrus orchards, nurseries and in gardens in EU citrus regions (RCE-9). *Phyllosticta* spp. were found to occur with limited occurrence in the EU but never in association with infections. CBS symptoms were never observed, indicating that the fungi persists but does not cause disease.

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

Project RCE-6 is addressing knowledge gaps with regard to the epidemiology of the CBS pathogen. It was seen that high spore germination is achieved with spores older than 1 day and from the second and following spore generations formed by pycnidia. Tolerance of lime trees to CBS was furthermore seen not to be due to the germination process and that even after 3 h of dryness pycnidiospores can still germinate as early as 4 h after inoculation. An optimized qPCR protocol was developed to quantify *P. citricarpa* pycnidiospores obtained from spore traps. This protocol could however, not reliably quantify spore numbers below 1000 spores. Project RCE-7 incorporated different CBS related epidemiological models along with weather data into a web-based platform (PhytRisk) that can assist growers with CBS decision making. A mobile phone version was launched in October 2017 while the CBS model validation is also in progress. Validation and improvement of the CBS models are done in RCE-8 where new spore trap and weather data are used for this purpose. This follows on the termination of some of the objectives in RCE-8 due to the failure of planned and amended methodologies to yield any results for these objectives. Within project 1149 collaboration with researchers in the USA and Australia led to the evaluation of several models for the prediction of CBS. From the concluding report it is clear that all models performed equally well and a scientific paper is in the process of completion. These models, as well as ABS models, are consequently also employed within project 1187 to determine the effect of shade netting on the development of CBS and ABS within covered orchards. Findings from this study would lead to revised recommendations with regards to the management of these two diseases under shade netting.

Hand in hand with disease epidemiology goes fruit susceptibility and within project 1186, a collaboration with Brazilian researchers, the ontogenic resistance of sweet orange fruit is studied. This would also lead to improved control measures for CBS along with project 970 where developmental work is in progress to refine and renew existing CBS fungicide spray programmes.

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

PORTEFEULJE OPSOMMING

Die effektiewe bestuur van grondgedraagde siektes, blaar-en vrugsiektes, na-oes-siektes en ent-oordraagbare siektes maak 'n integrale deel van die produksie van uitvoersitrus van 'n hoë kwaliteit uit. Binne die Siektebestuurportefeulje is daar dus navorsingsprogramme wat elk deur 'n ekspert navorsers gelei word en wat op hierdie verskillende areas fokus. Die doel van die navorsingsprogramme is om huidige industriebehoefte aan te spreek, terwyl daar ook proaktiewe navorsing gedoen word op siektes wat verwag word om binnekort uitdagings aan die industrie te bied.

Binne die Ent-oordraagbare Siektes-groep is "Huanglongbing" (HLB) of Asiatiese Vergroening as 'n dreigende gevaar vir die Suid-Afrikaanse industrie geïdentifiseer. Dit was hoofsaaklik as gevolg van die bevestigde teenwoordigheid van '*Candidatus*' Liberibacter asiaticus (Las) en die vektor, *Diaphorina citri*, op die Afrika kontinent. Baie jare se navorsing binne CRI het egter daartoe gelei dat die Liberibacter spesie teenwoordig in Oos-Afrika, korrek geïdentifiseer kon word. Analise van opname-monsters uit hierdie areas het getoon dat 'n minder aggressiewe '*Candidatus*' Liberibacter africanus (Laf) spesie in Uganda, Kenia en Tanzanië teenwoordig is en dat Las dus huidiglik net in Ethiopië teenwoordig is (Projek 1157). Projek 1200 volg op 1157 en het ten doel om die waarnemingsprotokol van die verskeie Liberibacter spesies wat in projek 1157 ontwikkel is, te bekragtig. Behalwe vir molekulêre waarneming van die verskillende Liberibacter spesies, het projek 1184 ook 'n doelwit gestel om 'n snuffelhond op te lei om '*Candidatus*' Liberibacter africanus (Laf) in geïnfekteerde bome waar te neem. Hier is ook goeie vordering gemaak deurdat die hond in staat is om geïnfekteerde bome in 'n beheerde omgewing waar te neem. Daar word gehoop dat wanneer HLB in Suid-Afrika arriveer, honde ook opgelei kan word om Las- en Laf-geïnfekteerde bome waar te neem en tussen die twee te onderskei. Vir die potensiële bestuur van HLB, fokus projek 1160 op die ontwikkeling van CTV-besmette klone wat gebruik kan word om 'n "payload" aan geïnfekteerde bome te lewer, wat potensieel die ontwikkeling van Las binne die geïnfekteerde boom kan uitwis of inhibeer.

Ten spyte van die fokus op Liberibacter spesies, is daar steeds 'n sterk fokus op *Citrus tristeza virus* (CTV) as gevolg van die gebruik van kruisbeskerming binne die Sitrusverbeteringskema (SVS) om die effek van hierdie virus te verminder. As gevolg van die vordering wat daar gemaak is in die identifikasie van CTV-rasse, kan die spesifieke rasse betrokke by simptoom-uitdrukking, en dié wat nodig is om hul effek te verminder, nou akkuraat gekarakteriseer word. Enkel-ras bronne kan dus nou in glashuis- en veldproewe bestudeer word ten einde hul impak op die gasheer te bepaal, asook hul potensiaal vir kruisbeskerming. Meer spesifiekte CTV-gasheer-interaksies en gevorderde virusdiagnostiek deur middel van bio-informatika, maak dit nou vir navorsers moontlik om die biologie van die virus meer in diepte te bestudeer (Projek 1100). Sitrusviroïedes kry ook binne projek 1155 aandag, en verskeie onderstamme word geëvalueer vir hul sensitiwiteit teenoor die verskillende viroïedes. Aangesien hierdie projek onlangs gevestig is, is geen duidelike resultate op hierdie stadium waarneembaar nie.

In pomelo-proewe is gevind dat enkel-ras CTV-bronne meer potensiaal het as gemengde-ras bronne om in pre-immunisering gebruik te word. Die effek van klimaat op simptoom-uitdrukking was ook duidelik uit hierdie proewe (Projek 742). Projek 1173 gaan op dieselfde wyse voort, deurdat enkel- vs. gemengde-rasse geëvalueer word vir die pre-immunisering van pomelo's, Valencia en nawel variëteite. CTV en die rol binne sagte sitrus word ook ondersoek (Projek 968), terwyl dit in glashuisproewe reeds bewys is dat SVS-materiaal, wat vry is van virusse en viroïedes, beter vertoon as vuil materiaal wat in boorde gesny is (Projek 1074).

Grondgedraagde siektenavorsing fokus op grondgedraagde siektes en plae van sitrus. Die soektog na alternatiewe, meer volhoubare middels is voortgaande. Sommige studies ondersoek ook die komplekse oorsake van sitrus agteruitgang en herplantprobleme en kyk na faktore wat vroeë waarskuwings kan wees. Onbekende siektes en hul oorsake word ook ondersoek asook hoe om hulle te bestuur.

Langtermynstudies van vóór- en ná-plant bestuur van nematodes en *Phytophthora* dui aan dat vóór-plant beroking 'n effek het, met bome in hierdie behandelings wat hoër is met dikker stamme (Projek 762). Evaluasie van alternatiewe, nie-chemiese behandelings om chemiese aalwurmdoders te vervang, het getoon dat die produkte variërende resultate lewer en dat die beste resultate dikwels verkry word waar hierdie middels met een chemiese toediening gekombineer word (Projek 1030). Daar is ook gevind dat fosfonaat toedienings op mandaryne fitotoksiese skade op vrugte veroorsaak as dit ná kleurbreek gedoen word.

Daar is gevind dat 'n patogeen-kompleks betrokke is in die stamvrot en agteruitgang wat deur produsente in die Oos-Kaap waargeneem is en dat hoë grond en water pH moontlik Carrizo citrange en Swingle citrumelo onderstamme predisponeer vir infeksie deur bogenoemde patogeen-kompleks. Hierdie postulasie word tans verder ondersoek (Projek 1068), tesame met 'n studie om te bepaal waar die bronne van die patogene betrokke is. Sitrus agteruitgang het ook 'n komplekse oorsaak van biotiese en abiotiese faktore en in Projek 1092 word hierdie faktore ondersoek om was te stel of enige van hulle gebruik kan word as vroeë waarskuwing van probleme. Sitrus herplantprobleme word meer ondervind omdat ou boorde meer vervang word en Projek 1152 is voltooi. Daar is gevind dat, behalwe vir *Phytophthora* spp. en die sitrus aalwurm, verskeie *Fusarium* en *Neocosmospora* spp. ook in die sitrus herplantkompleks betrokke is. Die snelle groei van die industrie asook herplantprogramme wat geïmplimenter word, plaas druk op kwekeryproduksie en Projek 1101 ondersoek die voorkomende en uitwissende grondgedraagde siektebestuurspraktyke deur patogeenkarakterisering en swamdoder sensitiviteitswerk. Resultate van die projek het reeds tot aanbevelings aan kwekerye gelei. Verdere resultate het aangedui dat tien verskillende *Pythium* spp. in Suid-Afrikaanse sitruskwekerye teenwoordig is, en dat hulle grootliks in hul sensitiviteit teenoor mfenoxam varieer. Patogenisiteitsproewe en chloor sensitiviteitswerk met hierdie spesies is tans onderweg.

Essensiële olies (EO) is bekend daarvoor dat hulle 'n effek op na-oes patogene het en dus word EO getoets ten einde hulle in stadig-vrystellende nano- of mikro-partikels te enkapsuleer. Gespesialiseerde toerusting en metodes is vir hierdie werk ontwikkel en uitgebreide data is versamel wat tans geanaliseer word (PHI 66).

Alternatiewe en nuwe produkte is getoets en die beste blyk 'n azoksiestrobien-verbinding teen *Penicillium digitatum* te wees. Daar is gevind dat produkte wat waterstofperoksied en asynsuur (PPA) kombineer, geen effek op die sensitiviteit van pyrimethanil en die stabiliteit van propikonasool het nie. 'n Poelierformulasie van PAA is ontwikkel en getoets en gevind om nie die fitotoksiese probleme van vloeibare formulasies te hê nie. Daar word steeds op die sanitasie van water gefokus, met verskeie produkte wat getoets word (Projek 123). Propikonasool is ook in Suid-Afrika geregistreer vir suurvrotbeheer wat dit genoodsaak het dat die basissensitiviteit van Suid-Afrikaanse suurvrot- en *Penicillium digitatum*-isolate vasgestel moes word (Projek 1141).

Die rol van fito-chemikalieë, wat deur sitrusvrugte gevorm word, in die inhibisie van vrug-infeksies deur *Phyllosticta citricarpa*, word ondersoek. Kultivars wat verskil ten opsigte van hul sensitiviteit vir *Phyllosticta citricarpa* infeksies is ingesluit en word bestudeer ten opsigte van hul apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloidede, glikosiede) in die vrugskil. Huidig word Bitter Seville as 'n lae-vatbare tipe, medium-vatbare nawel en hoogsvatbare suurlemoen tipes gebruik, terwyl kumkwat as 'n nie-vatbare tipe bygevoeg is (Projek 1135).

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

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

Sitrus swartvlek, veroorsaak deur *Phyllosticta citricarpa*, kom in baie sitrus produksie-areas voor, maar in die EU is dit onderworpe aan fitosanitêre wetgewing. Die voorkoms van *Phyllosticta* spp. in die EU is bepaal deur opnames in sitrusboorde, kwekerye en huistuine in die EU sitrus-areas (RCE-9). Daar is gevind dat *Phyllosticta* spp. beperk in die EU voorkom, maar nooit in assosiasie met infeksies nie. SSV-simptome is nooit waargeneem nie wat aandui dat die patogeen vestig, maar nie siekte veroorsaak nie.

In projek 977 is 383 *P. citricarpa* isolate genotipeer en tesame met paringstipe analyses is onthul dat beide paringstipes in die populasies van Suid-Afrika, China, Australië en Brasilië in 'n 1:1 verspreiding teenwoordig is, maar dat die VSA populasie slegs een paringstipe bevat. Die populasies van hierdie patoëen in Suid-Afrika, Australië en Brasilië blyk met mekaar verbind te wees, wat toegeskryf word aan die uitruil van plantmateriaal tydens die vestiging van die sitrus-industrieë in hierdie lande.

Kennisgapings aangaande die epidemiologie van die SSV-patoëen word in RCE-6 aangespreek. Hoë spoorontkieming is behaal met spore ouer as 1 dag afkomstig van die 2de en 3de generasies spore afkomstig uit piknidia. Daar is gevind dat lemmetjebome se toleransie teen SSV onafhanklik is van die ontkiemingsproses en dat selfs ná 3 h van droogte, piknidiospore kan ontkiem, ook so vroeg as 4 h ná inokulasie. 'n qPKR protokol is ontwikkel en geoptimeer om *P. citricarpa* piknidiospore te kwantifiseer wat afkomstig is van spoorlokvalle. Hierdie protokol kon egter nie spoorhoeveelhede minder as 1000 akkuraat kwantifiseer nie. Binne projek RCE-7 is verskillende epidemiologiese modelle van SSV tesame met weerdata in 'n web-gebaseerde platform saamgevat (PhytRisk) wat produsente kan help met SSV-besluitneming. 'n Selfoon weergawe is in Oktober 2017 in gebruik geneem, terwyl bekragtiging van bestaande SSV-modelle ook voortgaan. Die toetsing en verbetering van SSV-modelle word in projek RCE-8 gedoen met behulp van nuwe spoorlokval- en weerdata wat versamel is. Dit volg op die terminering van sommige doelwitte in die projek omdat die beplande en aangepaste metodes nie enige betekenisvolle resultate vir hierdie doelwitte kon oplewer nie. Binne projek 1149 het samewerking met navorsers in die V.S.A. en Australië tot die evaluasie van verskeie modelle vir die voorspelling van SSV gelei. Uit die samevattende verslag is dit duidelik dat al die modelle ewe goed gevaar het en 'n wetenskaplike artikel word tans voltooi. Hierdie modelle, asook ABV-modelle, word gevolglik ook in projek 1187 gebruik om die effek van skadunette op die ontwikkeling van SSV en ABV binne die bedekte boorde te bepaal. Bevindinge vanuit hierdie studie sal tot aangepaste aanbevelings lei betreffende die bestuur van hierdie twee siektes onder skadunet.

Hand aan hand met siekte-epidemiologie, gaan vrugvatbaarheid, en binne projek 1186, word die ontogeniese weerstand van soetlemoenvrugte in samewerking met Brasiliaanse navorsers ondersoek. Dit sal ook tot verbeterde beheermaatreëls van SSV lei, tesame met projek 970, waar ontwikkelingswerk onderweg is om bestaande SSV-fungisied spuitprogramme te vernuwe en verfyn.

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

3.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: G. Cook (CRI)

3.2.1 Programme summary

Fortunately for the southern African citrus industry 'Haunglongbing' (HLB) or 'Asian Greening' does not yet occur in the region. 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. Identification of various Liberibacter species on the continent requires specific diagnostics for correct identification. Research, conducted over a number of years, investigating the alternate hosts of the African Greening pathogen, '*Ca*' L. africanus (Laf), has enabled the correct identification of Liberibacter species found in citrus in East Africa (3.2.3). This work highlighted limitations to diagnostic assays used widely and the need for improvement which will be addressed in a new project. The opportunity also exists to learn from approaches applied by countries already contending with the disease. Following the success of early detection

using sniffer dogs in the USA, a canine HLB detection project was initiated. A dog has been trained to detect Laf and training to transition the dog to work in orchards is underway (3.2.10). The development of an infectious *Citrus tristeza virus* (CTV) clone to combat HLB is a novel approach to use CTV as a vehicle to systemically deliver antimicrobial peptides or RNA 'signals' to arrest the insect vector or the Liberibacter pathogen of HLB (3.2.8) and follows from research done in the USA that has progressed to field trials. Characterising CTV isolates for use as potential cross-protection sources has provided background data to direct the selection of a CTV isolate for clone construction (3.2.5). Field trials evaluating performance of single-strain CTV sources in various citrus types and monitoring the CTV translocation to new growth of the trees are underway. This is done with the aim of testing the suitability of these isolates for use in CTV clone construction in addition to evaluating them as potential cross-protection sources (3.2.9). Field trials to assess the performance of various CTV sources in grapefruit are concluded and single-strain CTV sources show promise over sources consisting of strain mixtures. (Project 742). Two trials planted to investigate CTV in soft-citrus cultivars are showing tree decline not associated with the CTV sources and will be terminated (Project 968). A comparative trial to test the horticultural performance of field-cut propagation material compared to material supplied by the Citrus Improvement Scheme (CIS) is underway and results from both a glass-house and a field trial already demonstrated the benefit of CIS propagation material over field-cut sources (Project 1074). Commercial or potentially important rootstock selections will be tested for viroid sensitivity in a field trial. Plant preparation is underway (Project 1155).

Programopsomming

Gunstig vir die suidelike Afrikaanse sitrusbedryf is 'Haunglongbing' (HLB) of 'Asiese Vergroening' nog nie in die streek opgespoor nie. 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. Identifikasie van verskeie Liberibacter spesies op die vasteland vereis korrekte identifikasie en dus spesifieke toetse. Die ondersoek na alternatiewe gashere vir die Afrika-vergroeningspatogeen, '*Ca*' L. africanus (Laf), oor 'n geruime tydperk, het die korrekte analise van die teenwoordigheid van Liberibacter-spesies in sitrus in Oos-Afrika moontlik gemaak (Projek 1157). Hierdie werk beklemtoon beperkings van diagnostiese toetse wat algemeen gebruik word en die behoefte aan verbeterde toetse wat in 'n nuwe projek aangespreek sal word. Die geleentheid bestaan om te leer uit benaderings wat toegepas word deur lande wat reeds met die siekte worstel. Na aanleiding van die sukses van vroeë opsporing met snifferhonde in die VSA, is 'n HLB-opsporingprojek met honde geïnisieer. 'n Hond is opgelei om Laf op te spoor en opleiding is onderweg om die hond te leer om in boorde te werk (Projek 1184). 'n Benadering om 'n *Citrus tristeza virus* (CTV) kloon te gebruik om antimikrobiële peptiede of RNA 'seine' in die plant te lewer, om die insekvektor of die Liberibacter-patogeen van HLB te beheer, is geïnisieer. (Projek 1160) en volg uit navorsing wat in die VSA gedoen is, wat al tot veldproewe gevorder het. Jare van navorsing om CTV-isolate te karakteriseer vir gebruik as potensiële kruisbeskermingsbronne het agtergrond data verskaf om die keuse van 'n CTV-isolaat vir kloon konstruksie in te lig (Projek 1100). Veldproewe is onderweg om veldprestasie en CTV-translokasie in verskeie sitrus tipes te bepaal. Die doel hiermee is om die verskeie isolate te evalueer as kandidate vir gebruik in CTV-kloonkonstruksie, asook om hulle te evalueer as moontlike kruisbeskermingsbronne (Projek 1173). Veldproewe om die prestasie van verskeie CTV-bronne in pomelo's te evalueer, word afgesluit. CTV enkelras bronne het beter presteer as bronne wat bestaande uit rasmengsels. (Projek 742). Twee proewe wat geplant is om CTV in sagte-sitruskultivars te ondersoek, toon agteruitgang van bome wat nie met die CTV-bronne geassosieer is nie en sal dus beëindig word (Projek 968). 'n Vergelykende proef om die tuinbouprestasie van veldgesnyde voortplantingsmateriaal te vergelyk met materiaal wat deur die Sitrusverbeteringsskema (SVS) verskaf word, word uitvoer en resultate van beide 'n glashuis en 'n veldproef, het reeds die voordeel van SVS voortplantingsmateriaal bo veldgesnyde materiaal getoon (Projek 1074). Kommersiële of potensieel belangrike onderstamseleksies sal getoets word vir viroid sensitiviteit in 'n veldproef. Besig met die uitvoering van plantvoorbereiding. (Projek 1155).

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

Summary

CTV sources were collected from superior grapefruit trees from different grapefruit production areas of southern Africa to obtain mild *Citrus tristeza virus* (CTV) sources for cross-protection studies. These sources were evaluated in glasshouse trials and the four most promising field sources, Tambankulu 1, New Venture 41-2, ORE 8 and Tshipise 19-5, were further evaluated in field trials as potential pre-immunising sources for Marsh and Star Ruby grapefruit. They were compared to CTV sub-isolates, GFMS12-7 and GFMS12-9, as well as the four Beltsville sub-isolates; B389-1 and B389-4 of GFMS14, and B390-3 and B390-5 of a Mouton source. Trial controls included GFMS12, the previous standard CTV cross-protection source for white grapefruit at the time, GFMS35, the current standard cross-protection source for all grapefruit and un-inoculated trees. The Star Ruby trees were planted in the Letsitele area in Limpopo Province and the Marsh trees in the Malelane area in Mpumalanga province in 2007. A range of stem-pitting phenotypes was observed from large grooves, visible on tree trunks and limbs to porous wood pitting, referring to a high density of fine pits. The porous wood pitting was associated with decreasing horticultural performance in most cases. Star Ruby and Marsh trees containing the sub-isolated, single-strain sources, performed better than trees inoculated with the field sources and the current grapefruit cross-protection source, GFMS35. Star Ruby and Marsh trees inoculated with the GFMS12 source developed severe stem pitting. GFMS12 reduced tree size and decreased yield and fruit size significantly in Star Ruby. Although Marsh plants with this source were smaller, this influence was not as significant as seen on the more sensitive Star Ruby. CTV source, B390-5, was associated with good tree size, higher yields and good fruit size in both Marsh and Star Ruby and shows the most potential for further assessment.

Opsomming

Enthout is vanaf uitstaande pomelo bome uit verskillende pomelo gebiede van suider Afrika versamel om matige *Citrus tristeza virus* (CTV) bronne te verkry vir kruisbeskermingstudies. Die bronne is in 'n glashuisproef geëvalueer en die vier mees belowende veldbronne, Tambankulu 1, New Venture 41-2, ORE 8 en Tshipise 19-5, is verder in 'n veldproef geëvalueer vir moontlike kruisbeskermingsbronne vir Marsh en Star Ruby pomelos. Die bronne was vergelyk met CTV sub-isolate, GFMS12-7 en GFMS12-9, asook die vier Beltsville sub-isolate; B389-1 en B389-4 van GFMS14, en B390-3 en B390-5 van 'n Mouton bron. Proefkontroles sluit in GFMS12 (vorige CTV bron vir wit pomelos), GFMS35 (huidige CTV bron vir alle pomelos) en bome wat nie geïnkuleer is nie. In 2007 is die Star Ruby boompies in die Letsitele omgewing in Limpopo provinsie en die Marsh boompies in die Malelane omgewing in Mpumalanga provinsie geplant. 'n Reeks stamgleuf fenotipes is waargeneem vanaf groot groewe wat op die stam sigbaar is tot poreuse stamgleuf wat verwys na baie fyn stamgleuwe op die hout. Die poreuse stamgleuf is in meeste gevalle met 'n verminderde hortologiese prestasie geassosieer. Star Ruby en Marsh bome wat met sub-isolaat-enkelras-bronne geïnkuleer is, het beter presteer as bome wat met die veldbronne geïnkuleer is, insluitend die huidige pomelo kruisbeskermingsbron, GFMS35. Beide die Marsh en Star Ruby bome wat met GFMS12 geïnkuleer is, het ernstige stamgleuf getoon wat op Star Ruby beduidende boom groei onderdrukking en kleiner vruggrootte opgelewer het. Alhoewel die Marsh bome met hierdie bron kleiner was, was dieselfde invloed nie so merkwaardig op die meer sensitiewe Star Ruby gewees nie. CTV bron B390-5 was met goeie boomgroottes, hoër opbrengste en goeie vruggrootte in beide Marsh en Star Ruby geassosieer en toon ook die meeste potensiaal vir verdere ondersoek as 'n alternatiewe kruisbeskermingsbron.

Introduction

Due to the endemic presence of the aphid vector of *Citrus tristeza virus* (CTV) in South Africa, new orchards get infected with CTV within a few years after planting. Several strains of CTV exist and commonly occur as mixed strain populations in the citrus host (Harper, 2013). Factors such as the CTV strains, host cultivar and environment determine dominance of strains and symptom expression. CTV was a limiting factor in the production of grapefruit in South Africa due to severe stem-pitting (Oberholzer *et al.*, 1949). Decline in production and fruit size results in

early tree replacement and associated financial losses. Prior to any intervention strategies, the average lifespan of white grapefruit cultivars was reduced to 15 years and that of red cultivars to 10 years due to CTV stem-pitting (Marais, 1994). The only means to manage the deleterious effects of CTV where it occurs endemically is by applying cross-protection. This management strategy entails the deliberate infection of virus-free material with a known mild CTV source to mitigate the effects of severe strains. Mild sources for cross-protection are normally sourced from older healthy trees that produce good quality fruit which are found in severely affected orchards (Müller & Costa, 1987). Cross-protection, applied to grapefruit in South Africa, significantly increased the lifespan of grapefruit to 20-25 years (Marais, 1994) and research continues in order to identify optimal sources and understand the biological mechanisms that function in cross-protection.

This project is a continuation of a glasshouse trial (Project 49), where CTV sources were collected from different grapefruit production areas to obtain mild CTV sources for cross-protection studies. Budwood was collected from 108 superior grapefruit trees from different grapefruit production areas of southern Africa. These sources were established and evaluated in the glasshouse trials. Nineteen sources were found to be suitable for further evaluation as potential CTV cross-protection sources after initial screening in glasshouse evaluations on Mexican lime plants. The four most promising field sources, Tambankulu 1, New Venture 41-2, ORE 8 and Tshipise 19-5, indexed free for citrus viroids and these sources were evaluated in field trials as potential pre-immunising sources for Marsh and Star Ruby grapefruit. The sources were inoculated to Star Ruby and Marsh grapefruit trees for comparative evaluation to the Beltsville and ARC-ITSC sub-isolates of Nartia and also GFMS12 (standard CTV cross-protection source for white grapefruit at the time) and GFMS35 (current standard cross-protection source for all grapefruit) in field trials.

Objectives

To compare the performance of various CTV sub-isolates and field sources to existing CTV sources used for grapefruit cross-protection, under field conditions in Marsh and Star Ruby grapefruit.

Materials and methods

Virus-free 'Troyer' citrange rootstocks were propagated and budded with virus-free Star Ruby and Marsh grapefruit according to normal nursery practices. Once the scions had developed to approximately pencil thickness they were inoculated by patch-grafting with the various CTV sources. The field sources included Tabankulu 1, derived from a Star Ruby tree in Swaziland; New Venture 41-2, derived from a Star Ruby tree in the Nkwaleni Valley; ORE 8, derived from a Marsh tree in the Hoedspruit area and Tshipise 19-5, derived from a Marsh tree in Tshipise. Sub-isolates produced in Beltsville from CTV source GFMS14 include B389-1 and B389-4 and from a Mouton source; B390-3 and B390-5. Two ARC-ITSC sub-isolates, GFMS12-7 and GFMS12-9, were derived from GFMS12, the previous cross-protection source for grapefruit, also included in the trial. GFMS35 is the current cross-protection source for all grapefruit and was included as a comparative control and un-inoculated trees were included as negative controls. ELISA was used to confirm CTV transmission 3 months post inoculation. The trees were planted in two grapefruit production areas according to a randomised block design including five replicates for each treatment. The Star Ruby trees were planted in February 2007 at 'Bosveld Citrus' in the Letsitele area in Limpopo province and the Marsh trees in March 2007 at 'Riverside' in the Malelane area in the Mpumalanga province. These regions differ in climate. Letsitele is a hot and dry region, whereas the Malelane region is hot and humid.

The trees were evaluated annually for the development of stem-pitting as observed externally on the trunk using a severity scale of 0 to 3, where (0) represents a smooth trunk with no visible pits, (1) represents one to three grooves on the stem, (2) indicates multiple grooves and (3) is severe stem-pitting where the tree trunk has a knotted appearance. As a final assessment of stem-pitting, bark flaps of approximately 70cm² were cut from the stem of each Star Ruby tree after ten years in the field.

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

Fruit yield (kilograms per tree) was measured annually and a 4-year cumulative yield per tree for the sixth to ninth year obtained. Fruit size distribution was determined at harvest according to export size categories, and fruit of 86 mm in diameter and smaller was regarded as small fruit.

Strain components of the sources used were determined by testing currently available propagations of the various sources that were maintained in glasshouses using strain specific tests (Cook *et al.*, 2016).

Cambium scrapings of each bark flap removed from the Star Ruby plants at final evaluation was also tested to determine the CTV strain components present after 10 years in the field.

Results and discussion

CTV strain components of sources

Strain specific tests were not available at the time the trial was prepared. The CTV sources used for the trial were maintained on Mexican lime plants and were later tested for their CTV strain components and the results are presented in Table 1. All the sub-isolated CTV sources were shown to be single-strain isolates whereas the four field sources were strain mixtures. The original source of GFMS35 is known to contain the T68 strain and two RB variants, but the source used to prepare the trial only contained two RB variants. There is uncertainty regarding the original components GFMS12, but the source used to inoculate the trial contained only strain T68.

Field transmissions had occurred. CTV strain analysis of the field trees showed CTV strain components not present in the respective sources used for inoculations were detected in the field trees. Also all CTV strains were detected in all the trees planted virus free.

Table 1. The CTV strain components detected in plants used to propagate the various sources originally used to inoculate the trial plants.

CTV source	CTV strain							
	T68	RB1	RB2	HA16-5	VT	T30	T3	T36
GFMS35	-	√ ^a	√	- ^b	-	-	-	-
GFMS12	√	-	-	-	-	-	-	-
ITSC sub-isolates								
GFMS12-7	√	-	-	-	-	-	-	-
GFMS12-9	√	-	-	-	-	-	-	-
Beltsville sub-isolates								
B389-1	-	-	√	-	-	-	-	-
B389-4	-	√	-	-	-	-	-	-
B390-3	-	-	√	-	-	-	-	-
B390-5	-	-	√	-	-	-	-	-
Field sources								
New Venture 41-2	√	√	√	√	√	-	-	-
ORE 8	√	(√)	√	-	√	-	-	-
Tambankulu 1	√	-	√	-	√	-	-	-
Tshipise 19/5	√	-	√	-	√	-	-	-

^a (√) indicates strain detected

^b (-) indicates strain not detected

^c (√) indicates not present in all propagation sources

Tree canopy size

Star Ruby: The tree canopy volumes of the Star Ruby trees were determined for the last time in 2015 and the averages for trees inoculated with each CTV source are presented in Table 2 alongside the average external stem pitting evaluation results. Trees inoculated with GFMS12 were smaller on average compared to other treatments and canopy sizes differed significantly to trees inoculated with the sub-isolated CTV sources. Trees containing the CTV sub-isolates were also larger than trees containing the four field sources and cross-protection source, GFMS35, which were all shown to be strain mixtures. Trees containing sub-isolate B390-5 and the un-inoculated control trees were the largest on average.

Marsh: The average canopy volumes of the Marsh trees are presented in Table 4 together with the average external stem pitting results for each CTV treatment. Trees inoculated with the Tshipise 19-5, Tambankulu 1 and GFMS12 sources were on average the smallest. Similar to Star Ruby, Marsh trees containing sub-isolate B390-5 and the un-inoculated control trees were the largest.

Tree health

Star Ruby: The average external stem pitting rates for the various treatments for Star Ruby are presented in Table 2 along with the average canopy volumes. Trees inoculated with GFMS12 and sub-isolate GFMS12-7 displayed severe stem pitting, significantly more severe than those of other treatments. Stem pitting was associated with these sources shortly after planting and the stem pitting severity progressed over the years as shown in Table 3. The other CTV sub-isolates and sources induced stem pitting, but the external stem pitting evaluations indicated mild to moderate stem pitting.

The severe stem pitting observed on trees inoculated with GFMS12 correlated to diminished tree size. GFMS12-7 showed moderate to severe stem pitting, but this was not correlated to smaller tree size. Removal of bark flaps to view the stem pitting in the wood indicated a range of pitting phenotypes from long grooves to porous wood pitting (Figures 1-3). All trees containing GFMS12 displayed severe porous wood pitting and those with sub-isolate GFMS12-7 displayed similar, but less pronounced symptoms. The other GFMS12 sub-isolate, GFMS12-9, showed symptom expression milder than either GFMS12 or GFMS12-7. These observations correlated with the external stem pitting evaluations.

Field isolates Tambankulu 1 and Tshipise 19/5 induced various degrees of porous wood pitting, with isolate Tambankulu 1 showing finer pitting compared to Tshipise 19/5. Both these sources were associated with smaller trees, but this severe stem pitting was not observed with the external evaluation. Sources New Venture 41/2 and ORE8 were associated with a different pitting phenotype and long grooves were observed rather than porous pitting. Trees containing isolate New Venture 41/2 displayed more severe grooves that seen for trees containing isolate ORE8. Externally these symptoms were rated as mild stem pitting.

The Beltsville sub-isolates, B398-1, B389-4, B390-3 and B390-5, were associated with mild stem pitting showing occasional long groove pits. Very mild pitting was visible on control trees as well as trees containing GFMS35, apart from one tree displaying deeper grooves.

Marsh: The average external stem pitting rates obtained for treatments on Marsh grapefruit are presented in Table 4 along with average tree canopy sizes. Trees pre-immunised with GFMS12 showed severe stem pitting, significantly more than those of other treatments where predominantly mild stem pitting was observed. Trees containing CTV sources B389-1, B389-4, ORE 8, GFMS35 and those planted virus-free displayed no stem pitting that was externally visible on the tree trunk. Stem pitting in Marsh trees was less severe than in Star Ruby, but similarly, the progression of stem pitting associated with certain sources was seen (Table 5).

Production

Star Ruby: Trees were harvested and graded into the various export sizes yearly. Cumulative yields per tree (kg/tree) for each treatment over four years are presented in Table 6. Trees inoculated with GFMS35 yielded the least fruit of all treatments. GFMS12 inoculated trees were similarly associated with low yields, but these trees also yielded the highest percentage smaller fruit over a three year period (Table 7). The low yields and smaller fruit correlated with severe stem pitting. This same association is can be made with Tambankulu 1 inoculated trees. Yields were lower relative to most treatments and trees consistently yielded smaller fruit over the three

years. Of the field isolates, Tambankulu 1 was associated with the most severe porous wood pitting (Figure 3). The single-strain CTV sub-isolate treatments were mostly higher yielding than the treatments containing strain mixtures apart from trees containing the New Venture 41/2 source, which gave good yields. The long groove stem-pitting associated with New Venture 41/2 (Figure 3) did not reduce yield or influence fruit size. Un-inoculated, control trees were not the highest yielding, but produced the smallest percentage small fruit of all treatments.

Marsh: Differences in the 4 year cumulative yields between CTV treatments were not significant (Table 8), neither was the percentage smaller fruit obtained per treatment for the last two harvest seasons (Table 9), but similar to Star Ruby, the single-strain CTV sources did have higher yields on average compared to sources containing CTV strain mixtures.

Table 2. Average tree canopy volumes and external stem pitting rates for Star Ruby grapefruit trees inoculated with various CTV sources.

Treatment ^w	2015		2016	
	Canopy volume (m ³)		Stem pitting ^y	
B390-5	24	a ^x	1.0	bc
Control	24	a	0	a
B389-1	23	a	0.7	abc
B389-4	22	ab	0.1	a
B390-3	22	ab	0.4	ab
GFMS12-7	20	abc	2.7	d
GFMS12-9	18	abcd	0.5	ab
New Venture 41/2	17	bcde	1.4	c
ORE8	16	bcde	1.0	bc
Tambankulu 1	15	cde	1.4	c
Tshipise 19/5	15	cde	0.7	abc
GFMS35	14	de	1.1	bc
GFMS12	11	e	3.0	d
<i>Prob F treat</i> ^z	<0.0001		<0.0001	

^w CTV sources applied and un-inoculated control

^x Data presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^y Rating scale: 0 = Smooth trunk; 1 = one to three grooves on stem (mild); 2 = multiple grooves that do not coalesce (moderate); 3 = numerous grooves coalesce to form a knotted appearance (severe)

^z Probability value from analysis of variance for differences between treatments

Table 3. Average yearly external stem pitting rates for Star Ruby grapefruit trees inoculated with various CTV sources showing symptom progression over time.

Treatment ^x	Stem pitting							
	2009	2010	2011	2012	2013	2014	2015	2016
B389-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
B389-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
B390-3	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.4
B390-5	0.0	0.0	0.0	0.1	0.5	0.5	0.8	1.0
control	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0
GFMS12	0.8	0.8	2.3	2.6	2.8	2.8	3.0	3.0
GFMS12-7	0.5	0.5	1.7	2.0	2.3	2.3	2.3	2.7
GFMS12-9	0.0	0.0	0.3	0.2	0.6	0.6	0.5	0.5
GFMS35	0.0	0.0	0.0	0.1	0.5	0.4	0.5	1.1
New Venture 41/2	0.0	0.0	0.0	0.0	0.6	0.6	1.1	1.4
ORE8	0.0	0.0	0.3	0.0	0.2	0.2	0.2	1.0
Tambankulu 1	0.0	0.0	0.7	0.8	0.9	0.9	1.2	1.4
Tshipise 19/5	0.0	0.0	0.0	0.0	0.4	0.4	0.4	0.7

^x CTV sources applied and un-inoculated control

Table 4. Average tree canopy volumes and stem pitting rates for Marsh grapefruit trees inoculated with various CTV sources.

Treatment ^w	2015		2016	
	Canopy volume (m ³)		Stem pitting ^y	
B390-5	31	a ^x	0.7	ab
Control	30	ab	0	a
GFMS12-7	29	ab	0.7	ab
B389-4	26	abc	0	a
B390-3	26	abcd	0.9	b
GFMS12-9	26	abcd	0.4	ab
B389-1	26	abcd	0	a
New Venture 41/2	24	abcd	0.9	b
ORE8	23	abcd	0	a
GFMS35	23	bcd	0	a
GFMS12	21	cd	2.8	c
Tambankulu 1	21	cd	1.0	b
Tshipise 19/5	18	d	1.1	b
<i>Prob F treat^z</i>	<i>0.04</i>		<i>0.0001</i>	

^w CTV sources applied and un-inoculated control

^x Data presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^y Rating scale: 0 = Smooth trunk; 1 = one to three grooves on stem (mild); 2 = multiple grooves that do not coalesce (moderate); 3 = numerous grooves coalesce to form a knotted appearance (severe)

^z Probability value from analysis of variance for differences between treatments

Table 5. Average yearly external stem pitting rates for Marsh grapefruit trees inoculated with various CTV sources showing symptom progression over time.

Treatment ^x	Stem pitting							
	2009	2010	2011	2012	2013	2014	2015	2016
B389-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B389-4	0.0	0.0	0.0	0.4	0.5	0.5	0.4	0.0
B390-3	0.0	0.0	0.4	0.5	0.8	0.8	0.8	0.9
B390-5	0.0	0.0	0.0	0.0	0.6	0.6	0.6	0.7
control	0.0	0.0	0.0	0.3	0.4	0.4	0.0	0.0
GFMS12	0.0	0.0	0.5	1.4	2.3	2.3	2.8	2.8
GFMS12-7	0.0	0.0	0.0	0.3	1.1	1.1	1.3	0.6
GFMS12-9	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.4
GFMS35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
New Venture 41/2	0.0	0.0	0.3	0.3	0.4	0.4	0.7	0.9
ORE8	0.0	0.0	0.6	0.6	0.6	0.6	0.0	0.0
Tambankulu 1	0.0	0.0	0.0	0.0	0.4	0.4	0.9	1.0
Tshipise 19/5	0.0	0.0	0.4	0.5	0.7	0.7	1.2	1.1

^x CTV sources applied and un-inoculated control

Table 6. Averages for four year cumulative yields per tree (kg/tree) for Star Ruby grapefruit inoculated with various CTV sources.

Treatment ^x	Cumulative yield (2013-2016)
GFMS12-7	693 a ^y
B390-5	627 ab
B389-1	607 abc
B389-4	588 abcd
GFMS12-9	563 abcde
New Venture 41/2	560 abcde
B390-3	549 bcde
Tshipise 19/5	520 bcdef
control	519 bcdef
ORE8	469 cdef
Tambankulu 1	468 def
GFMS12	429 ef
GFMS35	399 f
<i>Prob F treat</i> ^z	0.007

^xCTV sources applied and un-inoculated control

^yData presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^zProbability value from analysis of variance for differences between treatments

Table 7. Percentage (%) smaller fruit obtained from 2014 to 2016 from Star Ruby grapefruit trees inoculated with the various CTV sources

Treatment ^x	2014	Treatment	2015	Treatment	2016
GFMS12	69 a ^y	GFMS12	92 a	GFMS12	83 a
Tshipise 19/5	53 ab	Tambankulu 1	77 ab	Tambankulu 1	59 ab
Tambankulu 1	48 abc	GFMS35	67 abc	New Venture 41/2	43 bc
B390-5	40 bcd	GFMS12-7	59 bcd	Tshipise 19/5	42 bc
GFMS12-7	40 bcd	ORE8	55 bcd	ORE8	41 bc
B390-3	37 bcd	B389-1	42 cde	GFMS12-7	39 bc
GFMS12-9	36 bcd	Tshipise 19/5	41 de	GFMS12-9	37 bc
GFMS35	36 bcd	GFMS12-9	38 de	B390-3	37 bc
B389-1	35 bcd	New Venture 41/2	38 de	B389-1	34 bc
New Venture 41/2	33 bcd	B390-3	37 de	GFMS35	27 c
ORE8	30 cd	B390-5	36 de	B390-5	27 c
B389-4	25 cd	B389-4	24 e	B389-4	22 c
control	21 d	control	19 e	control	19 c
<i>Prob F treat</i> ^z	0.021		< 0.0001		0.009

^xCTV sources applied and un-inoculated control

^yData presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^zProbability value from analysis of variance for differences between treatments

Table 8. Averages for four year cumulative yields per tree (kg/tree) for Marsh grapefruit inoculated with various CTV sources

Treatment ^x	Cumulative yield (2013-2016)
B390-5	450 a ^y
B389-1	448 a
GFMS12-7	447 a
B389-4	446 a
control	431 ab
GFMS12-9	419 ab
B390-3	409 ab
New Venture 41/2	401 ab
GFMS35	379 abc
Tambankulu 1	360 abc
GFMS12	354 abc
ORE8	323 bc
Tshipise 19/5	275 c
<i>Prob F treat</i> ^z	0.06

^xCTV sources applied and un-inoculated control

^yData presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^zProbability value from analysis of variance for differences between treatments

Table 9. Percentage (%) smaller fruit obtained in 2015 and 2016 from Marsh grapefruit trees inoculated with the various CTV sources

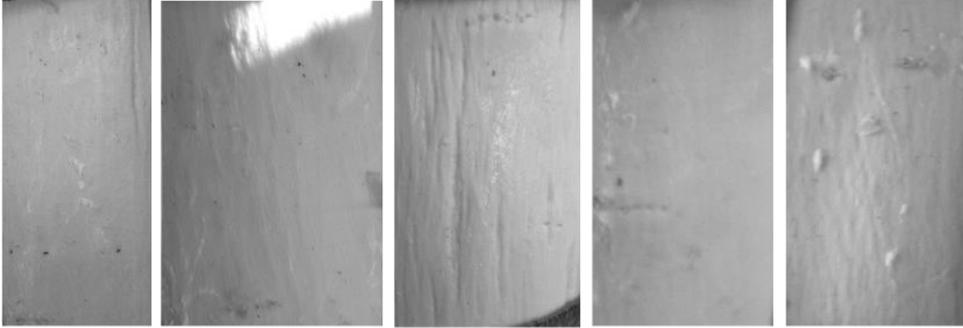
Treatment	2015	Treatment	2016
Tshipise 19/5	32 a	GFMS12	48 a
GFMS12	31 a	Tshipise 19/5	38 ab
Tambankulu 1	27 ab	New Venture 41/2	35 abc
New Venture 41/2	27 ab	GFMS12-7	34 abc
GFMS35	25 abc	B389-4	34 abc
GFMS12-9	24 abc	Tambankulu 1	32 abc
B389-4	22 abc	B390-3	31 bc
B390-5	21 abc	ORE8	30 bc
B390-3	20 abc	control	28 bc
GFMS12-7	19 abc	B389-1	26 bc
ORE8	16 abc	B390-5	25 bc
B389-1	12 bc	GFMS12-9	21 bc
control	9 c	GFMS35	17 c
<i>Prob F treat</i> ^z	0.19		0.09

^xCTV sources applied and un-inoculated control

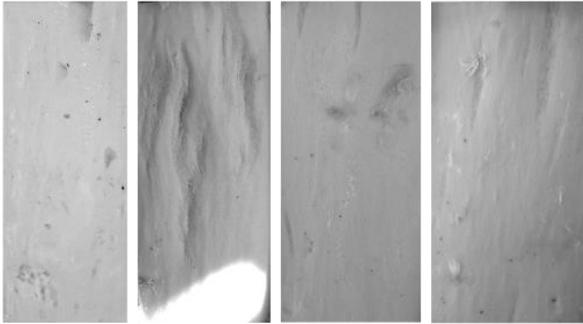
^yData presented are the means of five trees per treatment. Treatments with the same letters for Fischer least square difference do not statistically differ

^zProbability value from analysis of variance for differences between treatments

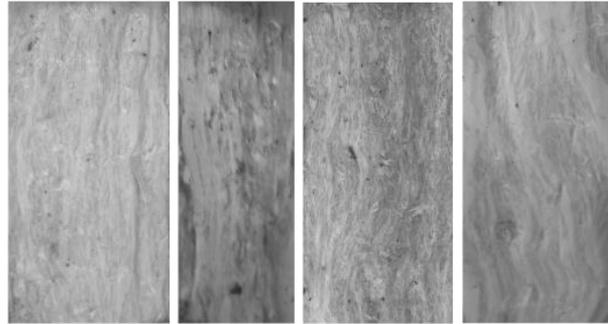
Control



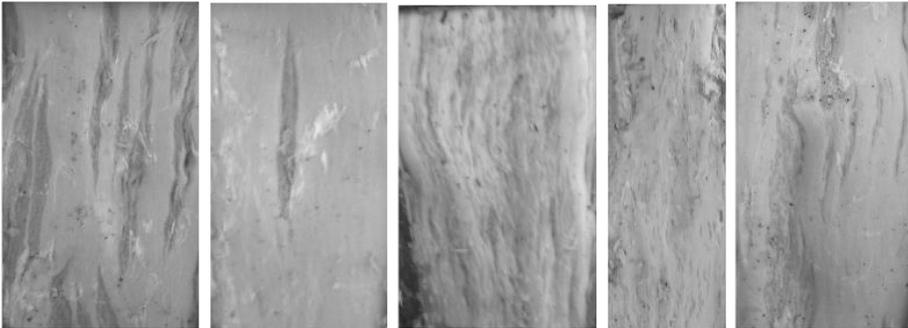
GFMS35



GFMS12



GFMS12-7



GFMS12-9

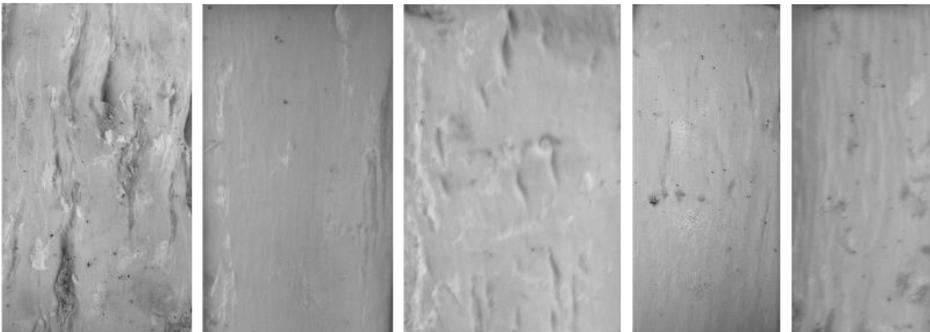
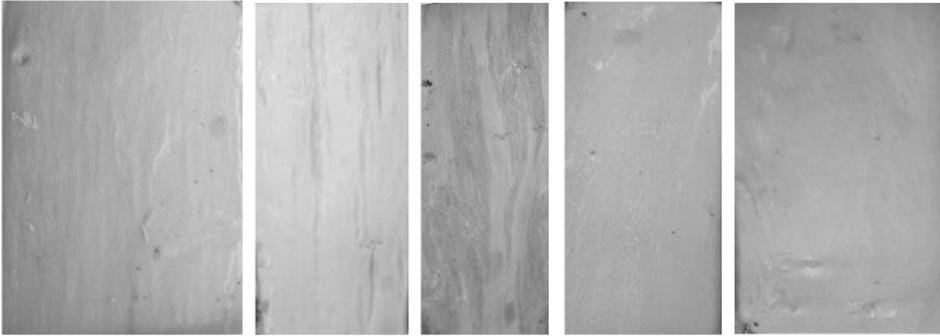
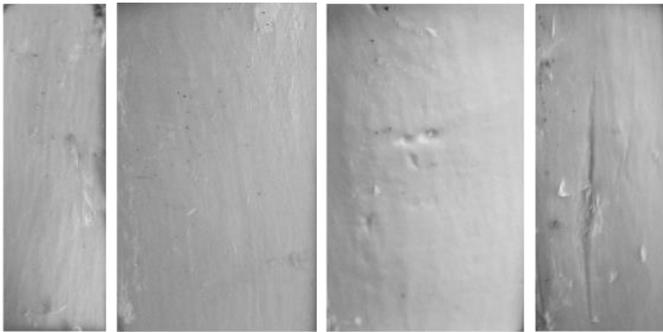


Figure 1. Stem-pitting on Star Ruby grapefruit trees inoculated with CTV sources GFMS35, GFMS12 and two sub-isolates of GFMS12. Control trees were planted without CTV inoculation.

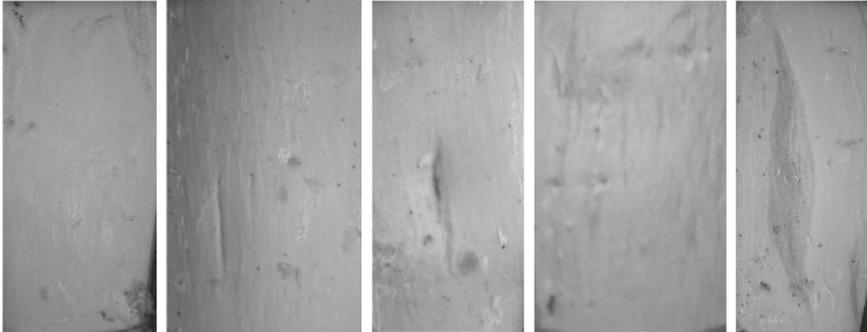
B389-1



B389-4



B390-3



B390-5

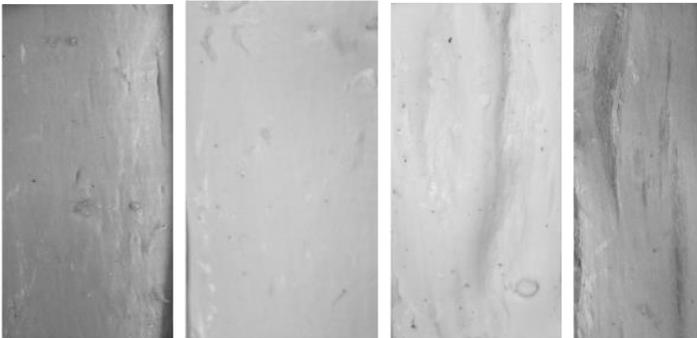
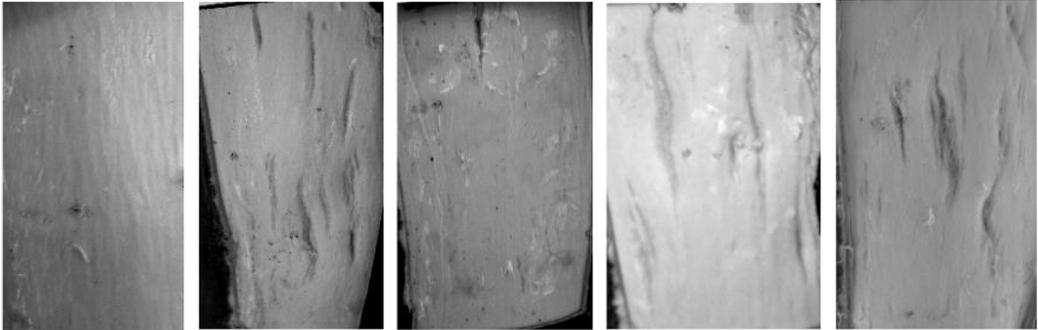


Figure 2. Stem-pitting on Star Ruby grapefruit trees inoculated with the Beltsville CTV sub-isolates.

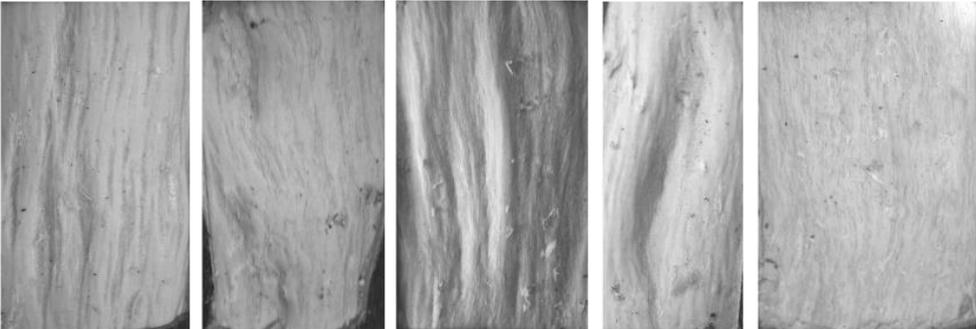
New Venture 41-2



ORE 8



Tambankulu 1



Tshipise 19/5

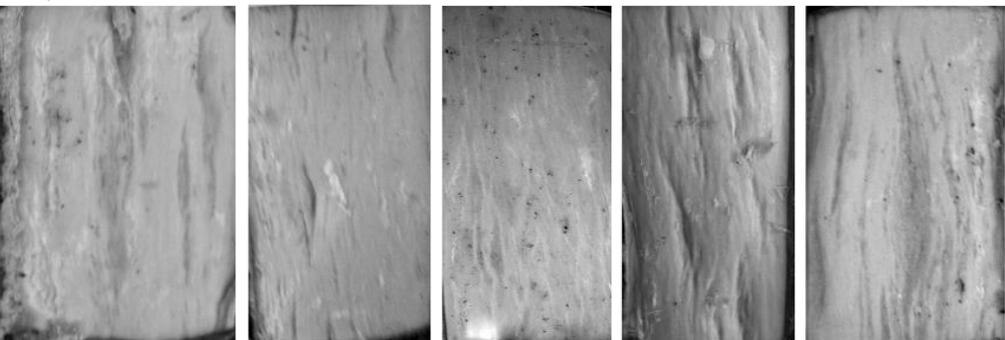


Figure 3. Stem-pitting on Star Ruby grapefruit trees inoculated with four field CTV sources.

Conclusion

Trial trees of both Marsh and Star Ruby, inoculated with GFMS12, developed severe stem pitting early on in both trials indicating that the stem pitting was induced by the CTV components of the source itself and not by field challenge inoculations. Stem pitting of GFMS12 suppressed tree growth in Star Ruby and decreased yield and fruit size. GFMS12 and the two sub-isolates derived from this source, GFMS12-7 and GFMS12-9, were shown to contain the same CTV strain component, T68. However, the symptoms associated with these sources differed in severity. This observation could indicate that variants of the same strain were originally present in the GFMS12 source and different variants were thus sub-isolated. Segregation of strain variants could possibly explain the severe stem pitting later associated with GFMS12, but not initially seen when this source was first used as a cross-protection source in South Africa.

Field transmissions had occurred as demonstrated by the CTV strain analysis of the field trees. CTV strain components not present in the respective sources, used for inoculations, were detected in the field trees. Control trees planted CTV free, did test positive at the final evaluation. However, visual analysis of the stem-pitting found for the various treatments showed that the pitting was similar for trees containing the same CTV sources. This would suggest that the original inoculation sources had the greatest effect on symptom expression, rather than field challenges.

Different phenotypes of stem pitting were observed including large grooves visible on tree trunks and limbs and porous wood pitting, referring to a high density of fine pits. The porous wood pitting was more damaging to the trees, decreasing horticultural performance.

CTV strain analysis showed that the field sources and GFMS35 are strain mixtures, whereas the sub-isolates are single-strain CTV sources. Overall CTV sub-isolates and notably B390-5, performed better than trees inoculated with the field sources and the current grapefruit cross-protection source, GFMS35. GFMS35, although not associated with severe stem-pitting, was associated with poor horticultural performance in Star Ruby and to a lesser degree in Marsh. Consideration should be given to replacing GFMS35 with a single-strain CTV source, based on performance in these trials and the trial reported in Project 679.

Technology transfer

Scientific Publications

Cook, G., van Vuuren, S.P., Breytenbach, J.H.J., Steyn, C. Burger, J.T. and Maree, H.J. 2016. Characterization of *Citrus tristeza virus* single-variant sources in grapefruit in greenhouse and field trials. *Plant Disease*.100:2251-2256.

Oral Presentations at Scientific conferences

Cook, G. Breytenbach, J.H.J. & van Vuuren, S.P. Field performance of various *Citrus tristeza virus* cross-protection sources trialled in grapefruit in different climatic regions. 20th IOCV Conference, Chongqing, China, 10-15 April 2016.

Citrus Symposium presentations

Breytenbach, J.H.J., van Vuuren, S.P. and Cook, G. *Citrus tristeza virus* cross-protection of Star Ruby grapefruit: field trial results. 2012. 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Cook, G., van Vuuren, S.P., Breytenbach, J.H.J., Steyn, C. Burger, J.T. and Maree, H.J. *Citrus tristeza virus*: A journey of discovery. 2016. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

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3.2.3 FINAL REPORT: Detection of '*Candidatus Liberibacter asiaticus*' and biological characterization of *Liberibacter* species from South Africa.

Project 1157 (2016/7 – 2017/18) by R. Roberts (ARC-PPRI) and Glynnis Cook (CRI)

Summary

'*Candidatus Liberibacter asiaticus*' (Las), the bacterium associated with citrus Huanglongbing (HLB), and its vector *Diaphorina citri*, were found to be present on the African continent. The occurrence of both Las and *D. citri*, in Africa is a chief concern to local citrus production as HLB can spread rapidly throughout the continent, unobstructed by temperature fluctuations which has been a limiting factor in the spread of citrus Greening, associated with '*Ca. L. africanus*' (Laf), which affects cooler production areas. To limit the spread of HLB, it is imperative that inoculum sources be removed as soon as possible, a process which is reliant on sensitive and rapid diagnostics, capable of differentiating Las from Laf. Within the current study, a high-resolution melt analysis was developed targeting the ribonucleotide reductase β -subunit (*nrdB*) gene region of *Liberibacter*s associated with citrus. Differentiation between Las and Laf within a single sample is achieved by the dissociation of the amplification products through a steady increase in temperature following PCR. In addition to Laf, various subspecies have been identified from indigenous citrus-related host in South Africa. In this study it was found that a biovar of one of these subspecies i.e. '*Candidatus Liberibacter africanus* subsp. *clausenae*' (LafCl), naturally infected and caused disease symptoms similar to Greening on citrus in East Africa. Attempts were made to transmit LafCl from its indigenous host, *Clausena anisata*, to citrus through grafting. No transmission occurred over a two year period following grafting, suggesting that the transmission of LafCl is either dependant of a vector, or the nucleotide differences observed between LafCl bv citrus and LafCl bv clausena are required for this *Liberibacter* to undergo the host jump observed in east Africa.

Opsomming

'*Candidatus Liberibacter asiaticus*' (Las), die bakterie wat met sitrus Huanglongbing (HLB) geassosieer word, en die vektor verantwoordelik vir die oordraging van hierdie patogeen, *Diaphorina citri*, is beide gevind om teenwoordig te wees op die Afrika kontinent. Hierdie is van groot kommer vir die plaaslike sitrus bedryf aangesien HLB nou sonder enige klimaat beperkenings deur die kontinent kan versprei, anders as Vergroening siekte, geassosieer met '*Ca. L. africanus*' (Laf), wat slegs sitrus bome in koeler areas affekteer. Om die verspreiding van HLB te beperk, is dit belangrik dat innokulum bronne so gou as moontlik verwyder word, 'n proses wat afhanklik is van spoedige en sensitiewe diagnostiese toetse wat tussen Las en Laf kan onderskei. Binne die huidige studie is

’n Hoë-Resolusie Smelt (HSM) analise ontwikkel gebaseer op die ribonukleotied reductase β -subeenheid (*nrdB*) geen van sitrus-geassosieerde Liberibacters. Onderskeid tussen Laf en Las teenwoordig binne ’n enkele monster berus op die dissosiasie van amplifikasie produkte deur ’n stelselmatige verhoging in temperatuur wat volg na ’n polymerase ketting reaksie (PKR). Verskeie subspecies van Laf is bekend in Suid-Afrika en word geassosieer met inheemse sitrus-verwante gasheer. Binne die huidige studie is gevind dat ’n ‘biovar’ van ‘*Ca. L. africanus* subsp. *clausenae*’ (LafCI) sitrus bome in oos Afrika natuurlik infekteer en simptome soortgelyk aan vergroening veroorsaak. ’n Poging is aangewend om LafCI vanaf sy inheemse gasheer, *Clausena anisata*, oor te dra na sitrus deur middel van bas-enting. Na twee jaar was daar geen bewyse dat LafCI oorgedra was na die geïnokuleerde sitrus plante nie en kan dus daarop dui dat die oordrag van LafCI afhanklik is van ’n insek vektor. Alternatief, weens die nukleotied verskille tussen LafCI bv citrus en LafCI bv clausena, was dit moontlik vir LafCI bv sitrus om die gasheer skuif wat waargeneem is in oos Afrika, te maak.

Introduction

Citrus greening disease in South Africa is associated with the heat-sensitive (Schwarz & Green, 1972), insect-transmissible (McClellan & Oberholzer, 1965), fastidious bacterium ‘*Candidatus Liberibacter africanus*’ (Laf) (Jagoueix et al., 1994). Past studies have shown this bacterium to be solely associated with mottling symptoms typical of greening disease on commercial citrus in this country, and that ‘*Ca. L. asiaticus*’ (Las) and ‘*Ca. L. americanus*’, both known to be associated with citrus, are not present (Garnier and Bové, 1996; Garnier et al. 2000a; Korsten et al. 1993; Korsten et al. 1996; Pietersen et al., 2010). Las is associated with citrus Huanglongbing which has caused severe economic losses to Floridian citrus production since being detected from citrus orchards in Florida in 2005 and is considered to currently be one of the most destructive disease of citrus worldwide.

In 2010, Las was detected from citrus in Ethiopia, making this the first report of HLB on the African continent (Saporani et al., 2010). This was followed by a more recent report of Las being identified in Uganda (Kaylebi et al., 2015) and a second report of *Diaphorina citri*, the natural vector of Las, along with an unconfirmed report of Las in Tanzania (Shimwela et al., 2016). The occurrence of both Las and its naturally associated vector from Africa poses a significant threat to African citrus production as Las can now be more effectively spread throughout the continent by *D. citri*, affecting warmer production areas previously unaffected by Greening due to greater heat tolerance of Las and its vector compared to Laf and its vector (Garnier and Bové, 1983). It is therefore important that a pro-active approach is taken to ensure that, if Las should enter the country, it is detected in a timely fashion and infected trees can be removed, preventing further spread of HLB.

A real-time PCR assays developed by Li et al., 2006 is routinely used to detect both Laf and Las. This assay consists of a single probe (HLBp) and reverse primer (HLBr), used in combination with either forward primer HLBas (Las-specific) or HLBAf (Laf-specific) to specifically detect Las or Laf. These primers are directed against 16S rDNA sequences of Las and Laf, which share high sequence homology, often leading to the non-specific amplification of non-target Liberibacters. Additionally, as this assay involves the use of a probe and separate real-time PCR reactions, it is expensive and labour-intensive. High Resolution Melt analysis (HRM) relies on the dissociation of double stranded DNA, influenced by nucleotide differences within a targeted gene region and is conducted following PCR amplification of an intended target. HRM can be used in population studies and, for the purpose of the current study, to differentiate between closely related Liberibacter species. The development of a HRM assay is inexpensive as it merely requires the design of a single primer set within conserved regions between Las, Laf and Lam, spanning a region with different melting temperatures per Liberibacter species. It additionally has the advantage of being more sensitive than conventional PCR and results can be monitored in real-time. The development of such an assay could assist in the rapid identification of Las, should it ever enter the country.

In South Africa, a number of subspecies of Laf has been described, the first of these being ‘*Ca. L. africanus* subspecies *capensis*’ (LafC), which was found to be associated with the indigenous ornamental rutaceous tree, *Calodendrum capense* (Cape Chestnut)(Garnier et al., 2000b). Within the study conducted by Pietersen et al.

2010, it was found that LafC is unlikely to play a role in the epidemiology of Greening disease (Phahladira et al., 2012) despite being identified from *C. capense* sampled in close proximity to citrus orchards.

Subsequent to the discovery of LafC, four additional subspecies of Laf have been described from indigenous rutaceous hosts, namely; ‘*Ca. L. africanus* subspecies *clausenae*’ (LafCI), ‘*Ca. L. africanus* subsp. *vepridis*’ (LafV), ‘*Ca. L. africanus* subsp. *zanthoxyli*’ (LafZ) (Roberts et al., 2015) and ‘*Ca. L. africanus* subsp. *tecleae*’ (LafT) (Roberts and Pietersen, 2017). The three aforementioned subspecies were detected from the known native hosts of *Trioza erytreae* del Guercio (Hemiptera: Triozidae), the triozid vector of Laf (McClellan & Oberholzer, 1965, Moll & Marin, 1973). It is not yet known whether these subspecies of Laf can be transmitted to and cause disease within commercial citrus species.

Stated objectives

A: Design a rapid and sensitive molecular detection method for the simultaneous detection of Laf, Las and Lam from a single sample. This will assist in determining whether Greening/HLB symptoms seen on a tree is due to the presence of Laf or Las, thus surpassing the need to perform two separate reactions and confirming results with a conventional PCR approach.

B: Determine whether Liberibacter species identified from indigenous Rutaceae species are capable of infecting commercial citrus trees

Materials and methods

A: Design a rapid and sensitive molecular detection method for the simultaneous detection of Laf, Las and Lam from a single sample

Various published and newly designed primers (Table 1), spanning different Liberibacter gene regions were assessed to determine whether these primers are capable of differentiating Laf and Las following HRM. Each 25µl HRM reaction consisted of 12.5µl 2X RotorGene SYBR Green Master Mix (Qiagen), 400nM per primer, 200ng of DNA and made up to a final volume with nuclease free water. Reactions were set up as follow; initial denaturation and enzyme activation of 95°C for 5min followed by 40 cycles of denaturation at 95°C for 10s, annealing and acquisition of 58-62°C for 20s. Cycling was followed by a melt curve analysis of which the temperature was increased from 70-90°C in 0.5°C increments. Each primer set was assessed against Laf, Las as well as the known subspecies of Laf (i.e LafC, LafCI, LafT, LafV and LafZ), a healthy citrus control and a buffer control to determine the specificity of the primers.

Table 1. Primers assessed in HRM reactions

Primer Name	Sequence	Reference	Target gene
Las-rpoBF	cgt ctcg tca aga ttg cta t	Anathakrishnan et al., 2013	rpoB
Las-rpoBR	ggt gta gag aag ggc gtc ctt aa	Anathakrishnan et al., 2013	rpoB
Laf-rpoBF	aaa gag gca ggt gtt aag tcg gga	Anathakrishnan et al., 2013	rpoB
Laf-rpoBR	tga gca tgg tag aag gtt cgg ctt	Anathakrishnan et al., 2013	rpoB
RNRf	cat gct cca tga agc tac cc	Zheng et al., 2016	<i>nrdB</i>
RNRr	gga gca ttt aac ccc acg aa	Zheng et al., 2016	<i>nrdB</i>
RNRf_new	ctc cat gaa gct acc ctc ct	This study	<i>nrdB</i>
RNRr_new	tcc tcc cat cga agt acc tc	This study	<i>nrdB</i>
LJ900F	gcc gtt tta aca caa aag atg aat atc	Morgan et al., 2012	Prophage
LJ900R	ata aat caa ttt gtt cta gtt tac gac	Morgan et al., 2012	Prophage

LibSubsp_F	tat cat cgg gag atg aaa g	This study	rplJ
LibSubsp_R	aca act gmy ccr caa gaa	This study	rplJ
LafCI_F	cgg tag tcc tca ctc ttt cgt a	Roberts et al., 2017	omp
LafCI_R	atg aat cac cga aac agc gg	Roberts et al., 2017	omp

To obtain the complete ribonucleotide reductase β -subunit gene (*nrdB*) sequence of Laf, the available protein sequence (Genbank ref: WP_047264385.1) was reverse translated and the obtained sequence was aligned with the target *nrdB* region of Las to determine the number of SNPs between Laf and Las within the primer binding regions for the primers published by Zheng et al., 2016. To verify the Laf *nrdB* gene sequence, primers were designed spanning the length of the sequence (Table 2). End-point PCR was performed using the GoTaq G2 Flexi DNA polymerase system and primers were tested against a positive Laf sample, healthy citrus control and buffer control. Amplification products obtained were bi-directionally sequenced at Inqaba biotechnologies and sequences were assessed by performing a Blast analysis. Additionally, primers spanning 70bp regions across the reverse translated *nrdB* gene were designed and tested within HRM reactions as previously described.

Table 2. Primers designed from Laf *nrdB* sequence

Primer Name	Sequence	PCR type
nrdBaf1F	taa aca tcg tag ccc gag cg	End-point
nrdBaf1R	ttc cgg gtt gct cag atg ac	End-point
nrdBaf2F	gag cat gca ggc gga tat tg	End-point
nrdBaf2R	gca cgc ttt cat cac gca ta	End-point
nrdBaf3F	aaa aac ctg acc agc ccg ag	End-point
nrdBaf3R	gat ccg cgt taa tgc cca ga	End-point
nrdBaf4F	tga aaa ccc gca tct gtg ga	End-point
nrdBaf4R	tgc tgc cct gct gat att cg	End-point
RNRaf_2F	taa aca tcg tag ccc gag cg	HRM
RNRaf_2R	cac atc aat acg ggt gct gc	HRM
RNRaf_3F	aac ccg ttt ccg tgg atg ag	HRM
RNRaf_3R	tgc tgc cct gct gat att cg	HRM
RNRaf_4F	ttt ttg cga gca gcg aaa gc	HRM
RNRaf_4R	ttc cgg gtt gct cag atg ac	HRM
RNRaf_5F	tga tta acg cgc gta gcg at	HRM
RNRaf_5R	gtt gca cgc gct cag ata tt	HRM
RNRaf_6F	aaa aac ctg acc agc ccg ag	HRM
RNRaf_6R	aac gca atc aga tca cgc ag	HRM

B: Determine whether Liberibacter species identified from indigenous Rutaceae species are capable of infecting commercial citrus trees

Bark from LafCI positive *C. anisata* trees, collected throughout Mpumalanga, were grafted onto 40 citrus seedlings at CRI. To verify that any potential symptoms seen on the LafCI inoculated citrus seedlings were due to the transmission of LafCI, bark from a *C. anisata* tree grown from seed and known to be free of LafCI, were grafted onto 20 citrus seedlings, acting as healthy controls. Additionally, 20 *C. anisata* seedlings were graft inoculated with the LafCI positive material to serve as positive controls. One year post-inoculation, these trees were again graft inoculated with LafCI positive bark material.

Additionally, citrus seedlings were grafted with Laf-positive material. This was done to determine the efficiency of the grafting procedure as Laf is known to transmit to citrus following grafting. All citrus seedling were kept at CRI and monitored for graft take and disease expression.

To determine whether LafCI was transmitted to the recipient citrus seedlings, end-point PCR was performed on DNA extracted from these seedlings following the CTAB extraction method by Doyle and Doyle, 1990. The primers and protocol described by Hocquellet et al., 1999 were used for the detection of Liberibacters.

Additional tasks performed to stated objectives:

Detection of Las from citrus in East Africa

Following a report that Las was detected in Uganda and Tanzania, surveys were conducted in Uganda, Kenya and Tanzania by CRI and ICIPE to verify this claim.

Total DNA was extracted from these samples using the CTAB method after which the extracted DNAs from these samples were pre-screened for both Laf and Las using the species-specific real-time PCR assay as described by Li et al., 2007. To verify the results from the real-time PCR assay, positive samples were subjected to PCR amplification of the 16S rDNA. To further identify the nature of the Liberibacter species present from positive samples, amplification of both the ribosomal protein gene J (*rplJ*) using the protocol described by Hocquellet et al. 1999 and the outer membrane protein (*omp*) using primers HPlinv and OMP8inv described by Bastianel et al. 2005 were performed. Amplification products from the three PCR systems were sequenced directionally. Phylogenetic analyses were performed per gene region assessed.

To determine whether a single citrus sample could be co-infected with both Laf and LafCI, Laf and Laf-subspecies specific primers designed in experiment 886B were utilized (Table 3). These primer sets are designed to amplify a region within Liberibacter *omp* sequences, specific to each Liberibacter described from South Africa.

Table 3. Primers used for the detection of Liberibacters associated with citrus in Eastern Africa

Primer	Sequence 5' - 3'	Target gene	Source	PCR product size	Annealing temp (°C)
A2 J5	TATAAAGGTTGACCTTTTCGAGTTT ACAAAAGCAGAAATAGCACGAACAA	rplA/rplJ	Hocquellet et al. (1999)	Las 703; Laf 669	58
OA1_Fw OI2c_Rv	GCGCGTATTTTATACGAGCGGCA GCCTCGCGACTTCGCAACCCAT	16S	Jagoueix et al. (1996)	1160	62
HP1inv OMP8inv	GGGTCACGGGTTTTATGAATTTGTTG CTAAAATCAAGCTCACGACGAATCAC	Omp	Bastianel et al. (2005)	1400	55
Laf F Laf R	TCTCCGACGCGTATCAATCT CGCGATGACACCTTAACTGC	Omp	This study	250	65
LafCI F LafCI R	CGGTAGTCCTCACTCTTTCGTA ATGAATCACCGAAACAGCGG	Omp	This study	199	65

The specificity of the Las-specific real-time PCR primers published by Li et al., 2006 was determined as it appears that the Las-specific primers cross reacts with LafCI, leading to the false-positive detection of Las from citrus samples. Three samples of each Liberibacter known to occur in South Africa (Laf, LafC, LafCI, LafV and LafZ) were included in the study as well as LafCI positive citrus samples from Tanzania and Uganda. Real-time PCR reactions were set up according to KAPA probe-fast kit and was run using Qiagen Rotorgene with annealing

temperature set to 62°C. All of the samples were run in triplicate. A positive/negative threshold of Ct>35 was used as a positive/negative threshold.

Results and discussion

A: Design a rapid and sensitive molecular detection method for the simultaneous detection of Laf, Las and Lam from a single sample

Lam was omitted from the current study as a positive control could not be obtained. Brazilian researchers claim that Lam has been outcompeted by the more pathogenic strain, Las, and is seldom detected in citrus orchards in Brazil anymore. As Lam has not been identified outside of South America, it is of a much lower risk to South African citrus production.

The Las and Laf-specific primers of Anathakrishnan et al., 2013 were found to amplify non-target Liberibacters. Even when these primers were assessed for their capabilities to differentiate between Las and Laf following HRM, the melt peaks did not differ significantly to distinguish Laf from Las. The LibSubsp primer set designed within this study was capable of detecting Las, Laf and Laf-subspecies following PCR amplification with SYBR green. However, this primer was also not capable of differentiating between the various Liberibacter tested following HRM. The failure of these primer sets to differentiate between the Liberibacters tested, lies within the target regions used for HRM. The ability of HRM to differentiate between closely related species, is based on the temperature at which dsDNA dissociates, a process influenced by nucleotide composition. Both the rplJ and rpoB gene regions are highly conserved amongst Liberibacters and are therefore not suitable targets for HRM.

The primers published by Morgan et al., 2012 based on prophage regions within the Las genome was capable of only amplifying Las and none of the other Liberibacters tested. It is, however, not recommended that this primer pair is used as the target is based on prophage regions which can be excised from the genome, leading to a false-negative result.

Primers set LafClf/LafCIR is based on the outer membrane protein region, a gene which shares sequence homologies amongst Liberibacters of >70%. This primer set was initially designed to specifically detect LafCl following end-point PCR with an annealing T of 65°C. When the annealing T is lowered to 58°C within a SYBR green real-time PCR-assay, this primer set was capable of amplifying Las, Laf, LafCl and other Laf-subspecies. Additionally, following melt analysis of 70°C to 90°C, LafCl could be clearly differentiated from Las and Laf based on their dissociation profiles, with LafCl having a dissociation peak at 73°C and Las and Laf having peaks at 81°C. Unfortunately, no differentiation was observed between Laf and Las which is of more concern than LafCl.

Of the primers assessed, the primers published by Zheng et al., 2016 based on the *nrdB* gene of Las, showed the most promise for differentiating between Las and Laf. This primer set amplified both Las and Laf when the annealing T was at 58°C. When the annealing T is increased to >60°C, only Las is amplified within the SYBR green real-time assay. The primer set poorly amplified Laf and Ct values for Laf detection was >30. An additional primer sets was designed against this gene region (Table 1), and it was found that this primer had a higher affinity for Las and Laf than the original primers designed as the Ct value for the detection of these Liberibacters with the new primer set was decreased by Ct of 2 for each of the Liberibacters. Following melt analysis with both primer sets, Las could be distinguished from Laf by the melting peaks, with dissociation of Las amplification products peaking at 79°C and Laf peaking at 84°C.

As the *nrdB* gene was found to best differentiate between Las and Laf, this complete sequence for Laf needed to be confirmed. Only the protein sequence of this gene for Laf is available on Genbank and reverse translation was performed to obtain the sequence. A blast analysis of this sequence confirmed that this sequence in fact originated from the genome of Laf. Of the primers designed to obtain the full gene, only primer set 1 was able to successfully amplify its intended target, the product of which was sequenced. A Blast analysis was performed on the obtained

sequence and the nearest hit was found to be *Citrus sinensis*, despite the healthy citrus control within the PCR reaction not giving any amplification product. None of the other primers successfully amplified their intended targets, despite lowering the stringency of the end-point PCR reaction.

When assessing the Laf-derived HRM primers, primer sets RNRaf_3, RNRaf_4 and RNRaf_6 amplified non-target regions in *Liberibacter* positive and healthy controls. Primer set RNRaf_5 yielded no amplification in any of the controls. Primer set RNRaf_2 amplified only Laf following SYBR green amplification, the products of which dissociated at 85°C. This primer set along with the new RNR primer set against *Las nrdB* can be used within a multiplex SYBR-green reaction at an annealing T at 62°C, followed by a melt analysis as the dissociation peaks of *Las* and Laf can be clearly distinguished (79°C and 85°C respectively). It is, however, proposed that the complete *nrdB* gene for Laf be obtained to confirm the target sequence of the Laf-specific RNR primer.

B: Determine whether Liberibacter species identified from indigenous Rutaceae species are capable of infecting commercial citrus trees

Within the study, only LafCI was graft inoculated onto citrus and none of the other Laf-subspecies described from South Africa were tested. As a biovar of LafCI was found to naturally infect citrus in east Africa, it was decided to focus on this specific subspecies as it is the most likely of the subspecies to be transmitted to citrus through grafting. After two years, LafCI could not be detected from grafted citrus seedlings, despite successful grafts. Citrus seedlings inoculated with Laf became positive for Laf post-grafting, verifying that the grafting technique used was successful. Of the *C. anisata* trees grafted with LafCI, a single sample gave a faint band of the correct size following gel electrophoresis of rplJ amplification, however, this result could not be repeated in a subsequent test.

The lack of transmission of LafCI to citrus through grafting can be explained through two hypotheses. Firstly, it is possible that LafCI requires an insect vector to facilitate transmission of this *Liberibacter* to its host plant. Alternately, LafCI by citrus was shown to contain single nucleotide polymorphisms (SNPs) compared to available sequences for LafCI by clausena. It is likely that these SNPs facilitated the host jump of LafCI from *C. anisata* to citrus, assuming that *C. anisata* is the original host of LafCI. These nucleotide differences would explain why LafCI grafted from *C. anisata* to citrus did not occur within the current study. However, *C. anisata* seedlings inoculated with LafCI, did not tested positive either for LafCI up to this point. As none of the *C. anisata* trees tested positive for LafCI, it is likely that an insect vector might play a vital role in the transmission of LafCI between the rutaceous hosts. It is additionally possible that both a vector and specific mutations are required for LafCI to be transmitted to citrus. Further studies are needed to confirm this.

*Tasks completed in addition to stated objectives:
Detection of Liberibacters from East Africa*

A collective 41 citrus samples showing mottling symptoms were collected during the two surveys in East Africa. Of these samples, 18 originated from Uganda, 7 from Kenya and 16 from high altitude regions (396-668m) in Tanzania.

Of these citrus samples, 11 were putatively positive for Laf, 16 were putatively positive for *Las* and 3 samples from Tanzania gave a positive signal in both Laf and *Las* specific real-time PCR assays, suggesting that these samples were co-infected with multiple *Liberibacter*s. The total number of samples testing positive for a *Liberibacter* spp were 30.

Phylogenetic analysis of 16S rDNA sequences obtained for the *Liberibacter* positive samples showed that 14 of these samples clustered with known Laf sequences sharing 100% nucleotide identities with other Laf 16S rDNA sequences. The remaining 16 samples clustered with 16S rDNA sequences of LafC, LafCI and LafV (Fig. 1). None of the samples clustered with *Las* 16S rDNA sequences. The 16S rDNA sequences spanning 1076bp, which

clustered with the various Laf-subspecies had sequence identities of 99.2%, 98.2% and 94.7% to Laf, Las and Lam, respectively.

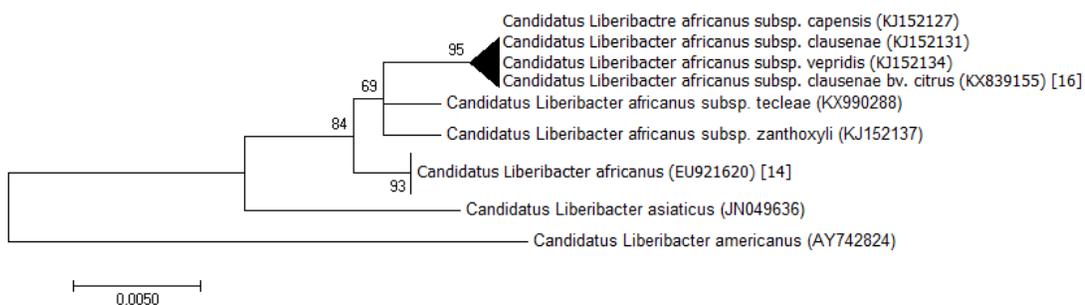


Fig. 1. Maximum-likelihood phylogeny of *Liberibacter* species surveyed based on 16S rDNA sequences.

Based on phylogenetic analysis of *rplJ* sequences however, 13 samples clustered with Laf, sharing 100% nucleotide identities with known Laf sequences, and 17 samples were clustered nearest to LafCI (Fig. 2). These 17 LafCI-type samples shared 98.2% nucleotide identity with LafCI sequences obtained from *Clausena anisata*. Phylogeny based on *omp* sequences supported the grouping of *rplJ* sequences with 13 samples containing sequences identical to Laf and 16 samples showing sequences homologous with LafCI (Fig. 3). LafCI sequences from citrus shared 99% sequence identity with LafCI *omp* sequences from *C. anisata*. A single sample from Tanzania yielding a mixed *omp* sequence, further indicating multiple infection of samples with *Liberibacter* spp.

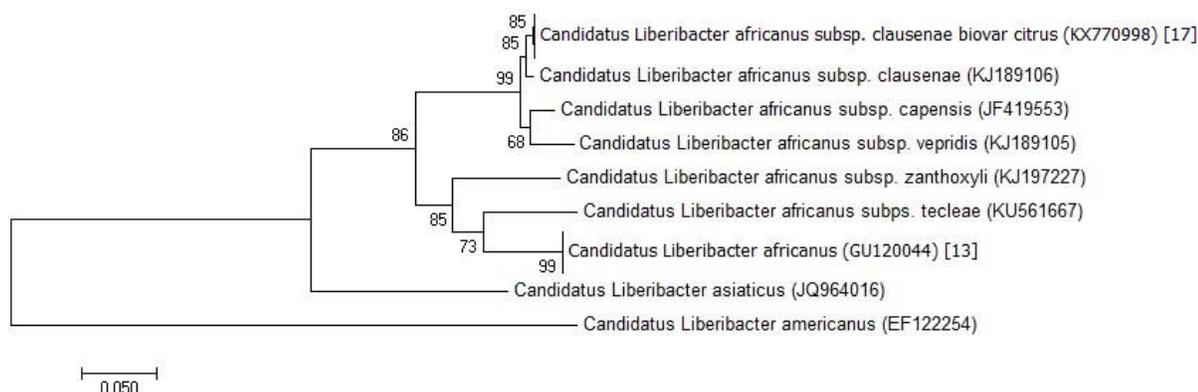


Fig 2. Maximum-likelihood phylogeny of *Liberibacter* species surveyed based on *rplJ* sequences.

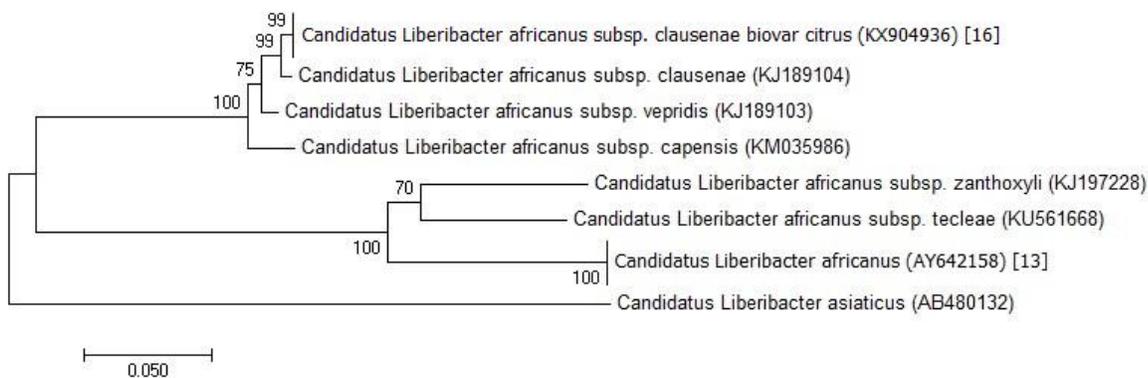


Fig. 3. Maximum-likelihood phylogeny of *Liberibacter* species surveyed based on *omp* sequences.

The LafCI sequences obtained from citrus were not identical to LafCI *rplJ* and *omp* sequences obtained from *Clausena anisata*. Single nucleotide polymorphisms (SNP) are observed between LafCI and LafCI ex citrus

affecting amino acid changes. These SNPs occurred within the same nucleotide positions and were observed for all samples testing positive for LafCI, justifying designating LafCI ex citrus as a separate haplotype to LafCI ex *Clausena*.

The samples containing sequences homologous to LafCI correlated with samples testing positive for Las following Las-specific real-time PCR assays, indicating non-specific amplification of LafCI resulting in the false positive detection of Las. Following the Las-specific Li et al., 2006 real-time PCR assay on the various Laf-subspecies, it was shown that all of the Laf-subspecies, with the exception of a single LafZ sample, yielded strong positive signals within this assay, further demonstrating the non-target amplification of this assay. On average Ct values for the various sub-species were 3 Ct values lower within the Las-specific assay when compared to the Laf-specific assay, demonstrating a higher specificity of the Las specific primers for these subspecies compared to the Laf-primers.

Laf and LafCI-specific PCRs indicated that 10 survey samples were positive for Laf, 15 were positive for LafCI and 5 were positive for both Laf and LafCI. LafCI was further confirmed to be dominant *Liberibacter* associated with citrus showing mottling symptoms in the in Uganda with Laf being the dominant *Liberibacter* present in Tanzania.

Conclusion

It was found that the gene most suited to differentiate between Las and Laf was the *nrdB* gene. Differentiation of the species can be achieved through a multiplex HRM reaction utilizing the primer set derived from the Las primer of Zheng et al., 2016 and the Laf RNR primer set described herein. Further validation of the assay will continue in Project 1200.

LafCI was not transmitted to citrus by grafting from *C. anisata* positive trees, suggesting that either vector transmission is required or that mutations at the nucleotide level are required for the transmission of this *Liberibacter* to occur from the rutaceous host to citrus. LafCI has not yet been identified from citrus in South Africa following the discovery of LafCI by citrus in East Africa. It is the opinion of the authors that LafCI does not pose a severe threat to South African citriculture as LafCI by citrus shares temperature sensitivity traits observed for Laf, thus the current control strategies utilized in controlling citrus greening would apply to the control of LafCI if the same vector is involved.

Future research

Verification of the *nrdB* gene region of Laf and the Laf-subspecies in order to confirm the specificity of the developed diagnostic assay. This will be pursued in project 1200.

It may also be of value to investigate reasons why LafCI by clausena was not transferred to citrus during graft transmission studies. If SNPs observed between LafCI by clausena and LafCI by citrus indicate specific sequence requirements for transmission to the citrus host, genes known to play a role in bacterial host specificity could also be compared between the two LafCI biovars. This could be a target for novel control strategies. Insect transmission studies will also be required to confirm whether *T. erytrae* is the vector of LafCI.

Technology transfer

Peer reviewed Scientific Articles

Roberts, R., Cook, G., Grout, T. G., Khamis, F., Rwomushana, I., Nderitu, P. W., et al (2017). Resolution of the identity of '*Candidatus Liberibacter*' species from Huanglongbing-affected citrus in East Africa. *Plant Disease* 101(8), 1481-1488

Oral Presentations at Scientific conferences

Ronel Roberts, Glynnis Cook, Tim Grout, Ivan Rwomushana, Peterson Nderitu, Zuberi Seguni, Chris Materu, Chanel Steyn, Gerhard Pietersen and Hennie le Roux. Detection of 'Candidatus Liberibacter species' from citrus in Uganda, Kenya and Tanzania. 9th Citrus Research Symposium Champagne Sports Resort, Drakensberg, 21 - 25 August 2016.

Ronel Roberts, Glynnis Cook, Tim Grout, Ivan Rwomushana, Peterson Nderitu, Zuberi Seguni, Chris Materu, Chanel Steyn, Gerhard Pietersen and Hennie le Roux. Detection of 'Candidatus Liberibacter species' from citrus in Eastern Africa. 5th International Research Conference on Huanglongbing. 14-17 March 2017, Orlando, FL, USA.

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

Project 968 (2004 - 2018) by J.H.J. Breytenbach, R. Clase and C. Steyn and G. Cook (CRI)

Summary

CTV sources applied for cross-protection to Clementine and mandarin trees did not propagate or translocate well in a number of cultivars maintained in the Citrus Improvement Scheme and propagation material of certain cultivars were found to be virus free. A glasshouse trial was conducted in 2006 to evaluate additional CTV sources in four different soft citrus cultivars as potential cross-protection sources. The current field trials are extensions of the glasshouse trial. Trees of two Clementine and two mandarin selections on Carrizo citrange rootstocks were prepared and pre-immunized with the CTV sources; CTVSC, SM47, SM48 and SM49. This project aimed to test the influence of the various CTV sources in two climatic regions. After the initial loss of both trials due to frost and poor drainage, they were re-planted. Trees were replanted in Citrusdal in December 2012 and at the Burgersfort site in 2014. Tree canopy volumes were determined at Citrusdal and Burgersfort, five and four years after planting respectively. Tree volumes at both sites differed greatly within treatments and numerous trees showed decline, not associated with the CTV treatments. Soil samples taken at Burgersfort showed *Phytophthora spp.* associated with declining trees. This random decline of trial trees was also observed at Citrusdal. The first harvests were poor

and one mandarin cultivar dropped all fruit at Citrusdal after splitting, but this was related to the cultivar. CTV strain components of the sources applied were determined after the trials were prepared. The CTV sources used were shown to be mixtures of various strains. This diminishes the value of the trials as transmission of strain components to individual trial trees cannot retrospectively be determined and might vary. It is proposed to terminate these trials due to poor tree health at both sites, not associated with the CTV sources and the uncertainty surrounding strain composition of trial trees at time of planting. Any further CTV cross-protection trials should apply single-strain sources.

Opsomming

CTV bronne wat gebruik word, as kruisbeskerming vir Clementine en mandarynbome, het nie goed vermeeder of translokeer in 'n aantal kultivars wat in die Sitrusverbeteringsskema onderhou is nie en voortplantingsmateriaal van sekere kultivars is virusvry bevind. 'n Glashuis proef is gedurende 2006 gedoen om CTV bronne in vier verskillende kultivars te evalueer. Die veldproewe is 'n uitbreiding van die glashuis proef. Twee Clementine en twee mandaryn hibried seleksies is op Carrizo citrange onderstamme ge-okuleer en gepreïmmuniseer met verskillende CTV bronne; CTVSC, SM47, SM48 en SM49. Met hierdie projek was beoog om die invloed van die verskillende CTV bronne in twee klimaatstreke te toets. Na die aanvanklike verlies van beide proewe as gevolg van ryp en swak dreinerings, is hulle weer geplant. Bome is in Desember 2012 in Citrusdal hervestig en op die Burgersfort-perseel in 2014. Boomvolumes is onderskeidelik by Citrusdal en Burgersfort bepaal, vyf en vier jaar na plant. Boomvolumes by albei terreine het grootliks verskil binne behandelings en talle bome het agteruitgang getoon wat nie geassosieer is met die CTV-behandelings nie. *Phytophthora* spp. is in grondmonsters verkry by die Burgersfort perseel. Hierdie lukraak agteruitgang van proefbome is ook by Citrusdal waargeneem. Die eerste oeste was swak en een mandarynkultivar by die Citrusdal perseel het al sy vrugte afgegooi nadat hulle gesplit het voor rypwording. CTV-raskomponente van die bronne wat toegedien is, is bepaal nadat die proewe voorberei is. Daar is gevind dat die CTV bronne gebruik, mengsels van verskillende CTV rasse was. Dit verminder die waarde van die proewe, aangesien die oordrag van raskomponente na individuele proefbome nie terugwerkend bepaal kan word nie en kan wissel. Daar word voorgestel om hierdie proewe te beëindig weens swak boomgesondheid by beide persele en die onsekerheid rondom die samestelling van die CTV komponente van die proefbome met plant. Enige verdere CTV-kruisbeskermingsproewe hoort enkelras CTV bronne te gebruik.

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

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

Summary

To ultimately identify the required *Citrus tristeza virus* (CTV) strain components for cross-protection, it is necessary to genetically and biologically characterise CTV single-strain isolates and evaluate their individual and combined effects on specific citrus hosts. Seven single-strain CTV isolates were identified and biologically characterised on a host range of various citrus types. Complete viral genomes of six CTV strains were determined. Two commercial grapefruit varieties, 'Star Ruby' and 'Marsh', were then used, in a glasshouse trial, to evaluate the ability of specific CTV strains to induce stem-pitting in single or mixed infections. Two isolates, T68 and VT were associated with mild to moderate stem pitting. Evaluation over three years shows that the single-strain isolates, used in combination did not cause more severe stem pitting than when individually inoculated. Relative quantitative analysis of various CTV strains in the 'Marsh' and 'Star Ruby' plants showed that individual strain titres were not significantly affected when in combination with different strains. An additional trial, still in progress, aims to test the 'cross-protection' ability of various CTV strains characterised in the first trial by challenging with a severe stem-pitting strain of grapefruit. Small RNA sequencing was used to investigate the citrus host response to CTV infection. Differential regulation between infected and the healthy samples of three plant miRNAs was shown. Additionally, Next Generation Sequencing (NGS) was applied to diagnostics in citrus viral pathology. A

bioinformatic pipeline for virus diagnostics based on NGS data was optimized and packaged in a user-friendly interface named Truffle (<http://truffle.sourceforge.net>). This work will benefit future viral diagnostics once certain standards and methodologies are in place. A number of objectives are still in progress and a final report will be submitted next year.

Opsomming

Om die geskikte *Citrus tristeza virus* (CTV)-raskomponente vir kruisbeskerming te identifiseer, is genetiese en biologiese karakterisering van CTV enkel-ras isolate nodig asook evaluering van hul individuele en gekombineerde invloed op spesifieke sitrusgashere. Sewe enkel-ras CTV isolate is geïdentifiseer en biologies gekarakteriseer op 'n gasheerreëks van verskillende sitrus tipes. Volledige virus genoom basisvolgordes van ses CTV-rasse is bepaal. Twee kommersiële pomelo variëteite, 'Star Ruby' en 'Marsh', is gebruik in 'n glashuisproef, om die vermoë van sekere CTV-rasse te bepaal om stamgleuf te veroorsaak, alleenlik of in gemengde infeksies. Twee isolate, T68 en VT is geassosieer met ligte tot matige stamgleuf. Evaluering oor drie jaar het gewys dat die enkel-ras isolate wat in kombinasie gebruik is, nie strawwer stamgleuf getoon het as wanneer die isolate individueel geënt was nie. Relatiewe kwantitatiewe analise van verskeie CTV-rasse in die 'Marsh' en 'Star Ruby'-plante het getoon dat individuele rastiters nie beduidend beïnvloed was wanneer in kombinasie met ander rasse voorgekom het nie. 'n Verdere proef is daarop gemik om die kruisbeskerdings-vermoë van verskeie CTV-rasse, gekarakteriseer in die eerste proef, te toets deur inokulasie met 'n strawwe CTV ras van pomelos. Die voorkoms van klein RNA (sRNA) is gebruik om die sitrus gasheer se reaksie op CTV-infeksie te ondersoek. Differensiële uitdrukking van plant-miRNAs is gewys tussen besmette en gesonde monsters. Die bron is egter ook aangetoon om 'n viroid te bevat en differensiaal uitgedrukte sRNA en gene sal verder deur RT-qPCR bevestig word. Verder is metagenomiese volgende-generasie volgordebepaling (NGS) toegepas vir diagnostiese doeleindes in sitrus. 'n Bioinformatiese pyplyn vir virus diagnose, gebaseer op die NGS data is geoptimeer en verpak in 'n gebruikersvriendelike program, genoem Truffle (<http://truffle.sourceforge.net>). Hierdie werk sal tot groot voordeel wees vir toekomstige diagnostiek sodra sekere standaarde in plek gestel is. Daar is 'n aantal doelwitte wat nog onvoltooid is en 'n finale verslag sal volgende jaar ingedien word.

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

Project 1074 (2013 - 2023) by G. Cook, J.H.J. Breytenbach, R. Clase and C. Steyn (CRI)

Summary

Claims that the use of field-cut material of cultivars is more profitable than that supplied by the Citrus Improvement Scheme (CIS) is investigated in both a field and glasshouse trial. Graft transmissible pathogens are removed by shoot tip grafting from accessions submitted to the CIS. Thereafter this material is inoculated with an approved *Citrus tristeza virus* (CTV) source for cross-protection. The objective of this study is to compare tree health, fruit characteristics and production of shoot tip grafted material with that of field-cut material. Three cultivars were selected for this investigation; viz. two navel and one Valencia cultivar. Budwood was collected from original field sources of the cultivars and budded according to normal nursery practices to 'Swingle' citrumelo, 'Carrizo' citrange and 'C35' citrange rootstocks. The same was done with equivalent material obtained from the CFB. Field sources were shown to contain various mixtures of CTV strains as well as citrus viroids. A field trial was planted at Burgersfort in 2016 and the trial trees established well. The second set of trial trees was kept in an insect free tunnel as a duplicate trial as a second trial site could not be found. Each trial tree was tested to determine the pathogen status and transmission success of the various components of each source. Stem-pitting on sweet orange is not commonly observed in South Africa due to the mild CTV pre-immunisation sources applied in the cross-protection programme, however, field-derived sources used for the trial, contained CTV components that caused mild to severe stem-pitting on the young trees. The trial trees have only been in the field for two and a half years and already significant differences in tree growth were observed. Reduced rootstock and scion diameters

were associated with field-cut material of all three cultivars. These results, although preliminary, already demonstrate the value of using budwood supplied by the CFB rather than field-cut material.

Opsomming

Aanname dat veld-gesnyde materiaal meer winsgewend is as Sitrus Verbeteringskema (SVS) materiaal vanaf die Grondvesblok (GVB) word tans ondersoek in beide 'n glashuis en 'n veldproef. GVB materiaal is skoongemaak van alle entoordraagbare siektes deur middel van groeipuntenting en is daarna geïnokuleer met 'n goedgekeurde *Citrus tristeza virus* (CTV) bron vir kruisbeskerming. Die doel van die studie is om boom gesondheid en vrug eienskappe van GVB materiaal met die van veld-gesnyde materiaal te vergelyk. Drie kultivars is betrokke, nl. twee nawels en een Valencia kultivar. Okuleerhout is van oorspronklike bronne van die kultivars versamel en volgens normale kwekery praktyke op 'Swingle' citrumelo, 'Carrizo' citrange en 'C35' citrange onderstamme ge-okuleer. Dieselfde is gedoen met materiaal wat vanaf die GVB ontvang is. Dit was bewys dat die ou kloon bronne verskeie mengsels van CTV rasse en sitrus viroïede bevat. Die veld proef is aangeplant in Burgersfort in 2016 en die proef bome het goed gevestig. Die tweede stel proefbome was in 'n insekvrye tonnel gehou as 'n glashuisproef omdat 'n tweede proefperseel nie gevind kon word nie. Elke proefboom is getoets om die patoogenstatus asook die transmissiesukses van die verskillende komponente van elke bron te bepaal. Stamgleuf op soet lemoene word nie algemeen in Suid-Afrika waargeneem nie as gevolg van die matige CTV- preïmmuniseringsbron wat in die kruisbeskermingsprogram gebruik word, maar die veldbronne bevat CTV-komponente wat ligte tot strawwe stamgleuf veroorsaak het op die jong proefbome. Die proefbome is al twee en 'n half jaar lank in die veld en alreeds is daar aansienlike verskille in boomgroei waargeneem. Verminderde onderstam- en bostam- deursnee is geassosieer met veld-gesnyde materiaal van al drie kultivars. Hierdie resultate toon reeds die waarde van die gebruik van enthout afkomstig van die GVB eerder as ou kloon materiaal.

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

Project 1155 (2016/7 – 2024/5) by G. Cook, J.H.J. Breytenbach, C. Steyn, R. Clase and J. Joubert (CRI)

Summary

The choice of rootstock is a critical factor in establishing any citrus orchard. Apart from the productive advantage imparted by commercial rootstocks, they should be compatible with certain soil and climatic environments as well as demonstrate resistance or tolerance to diseases and pests. Sound rootstock systems also enable growers to top-work an orchard to a different scion, ensuring a quicker return to economic production. *Citrus tristeza virus* (CTV) tolerance is essential for rootstocks used in southern Africa as CTV and its aphid vector are endemic. Due to their CTV sensitivity, sour orange, its hybrids, and *Citrus macrophylla* cannot be used locally. Viroids are graft-transmitted agents that are pathogenic on sensitive scions and rootstocks, but are unlikely to be problematic if disease-free budwood is used. However, given the ease of mechanical transmission with contaminated cutting tools and infected propagation material, viroids are sometimes unintentionally introduced into nurseries and orchards. New rootstocks emerging on the commercial scene over the past two decades include 'C35' citrange and the Minneola x trifoliolate hybrid, 'MxT'. Additional to these, other rootstock selections from the USA were obtained as well as an Australian trifoliolate selection. Apart from their horticultural suitability and performance, it is important to understand the viroid sensitivities of these rootstocks. A field trial is in preparation to test the sensitivity of these newer commercial or potentially commercial rootstocks to *Citrus dwarfing viroid* and *Hop stunt viroid-IIa*. All seed sources were obtained, but release from Post Entry Quarantine was delayed, resulting in a delay in trial preparation. The various seed sources were supplied to a nursery for tree production. All seed sources germinated and scion budding was completed. Bud growth on 3 rootstocks was poor and additional scion buds will be obtained to make up the shortfall. The trial will be inoculated with the viroid sources within 2018 and the planting of the trial is planned for the spring of 2019.

Opsomming

Die keuse van onderstam is belangrik in die vestiging van enige sitrus boord. Afgesien van die produksie voordele wat kommersiële onderstamme bied, moet hulle geskik wees vir sekere grond- en klimaatomgewings, asook weerstand of verdraagsaamheid teenoor siektes en plaë. Gesonde onderstamme stel ook 'n produsente in staat om 'n boord oor te werk na 'n ander kultivar, wat 'n vinniger terugkeer tot ekonomiese produksie moontlik maak. *Citrus tristeza virus* (CTV) verdraagsaamheid is noodsaaklik vir onderstamme wat in Suider-Afrika gebruik word omdat CTV en sy plantluisvektor endemies is. Weens die vatbaarheid vir CTV, kan bitter seville, sy hibriedes asook *Citrus macrophylla* nie plaaslik gebruik word nie. Viroïdes is oordraagbare entiteite wat patogenies is op sensitiewe bo- en onderstamme, en is meestal nie problematies as siektevrye enthout gebruik word nie, maar weens maklike meganiese oordraging deur snoeigereedskap en besmette enthout, word viroïdes soms, per ongeluk, in kwekerye en boorde versprei. Nuwe onderstamme wat die afgelope twee dekades op die kommersiële toneel verskyn het, sluit in 'C35' citrange en die Minneola x trifoliata baster, 'MxT'. Bykomend tot hierdie is ander onderstamme afkomstig van die VSA, asook Australiese trifoliaat bekom. Afgesien van tuinboukundige geskiktheid en prestasie, is dit belangrik om die viroïed sensitiwiteit van hierdie nuwe onderstamme te ondersoek. 'n Veldproef word voorberei om die sensitiwiteit van hierdie nuwe kommersiële of potensieel kommersiële onderstamme teen *Citrus dwarfing viroid* en *Hop stunt viroid*-IIa te toets. Alle saadbronne is verkry, maar vrystelling van die saad uit kwarantyn is vertraag, wat 'n vertraging in die proefvoorbereiding veroorsaak het. Die verskillende saadbronne is aan 'n kwekery vir boomproduksie verskaf. Alle saadbronne het ontkiem, saailinge is uitgeplant en bostamme geokuleer. Ogies op drie onderstamme het sleg gegroei en nuwe enthout sal verkry word om die tekort aan te vul. Die proef sal met die viroid bronne geïnkuleer word in 2018 en die proef sal hopelik in die lente van 2019 geplant word.

3.2.8 **PROGRESS REPORT: Application of CTV infectious clones to combat HLB.**

Project 1160 (2016/17 – 2019/2020) by R. Bester (SU), D. Aldrich (SU), B. Coetzee (SU), G. Cook (CRI), J.H.J Breytenbach (CRI), J. T. Burger (SU), W.O. Dawson (University of Florida, USA), H.J. Maree (SU)

Summary

The confirmed presence of both '*Candidatus*' *Liberibacter asiaticus* (CLAs), and *Diaphorina citri* in East Africa, requires a proactive approach from the South African citrus industry to prepare for the eventual incursion of HLB. The aim of this project is to establish a suite of *Citrus tristeza virus* (CTV) infectious clones with a range of 'payloads' that would form part of a management strategy to contain HLB and limit its impact. Infectious clones of genotype T36 were imported from collaborator Prof W.O. Dawson (Florida University). The plasmids were transfected into *Agrobacterium tumefaciens* and protocols for infiltration into *N. benthamiana* optimised. The efficacy of the infiltrations and the infectious clone was evaluated by GFP expression. The next step will be to use different virus purification techniques and to infect citrus seedlings with virus particles purified from *N. benthamiana*. A strategy was designed to convert these clones from a T36 genotype into the local RB (asymptomatic) and T3 (symptomatic) genotypes. The RB genotype based infectious clone will form the basis for a delivery vector whereas the T3 based infectious clone will be used for research purposes only. A cloning strategy was designed that uses intermediate plasmids that contains large portions of the respective CTV genotype. The next step is to assemble these constructs into complete genomes. A dual reporter infectious clone based on the T36 clone (Dawson) that expresses GFP and also contains a silencing cassette was designed and the intermediate clones constructed. The purpose of this clone is to evaluate the efficacy of a CTV-based vector as an expression and/or a silencing vector. This clone will be used as a test platform to screen different payloads. The intermediate clones for most (7/8) of the ORF deletion and ORF replacement infectious clones, that will be used to study the underlying mechanisms in the formation of stem pits, have also been completed up to the intermediate clones. Additionally, we have employed nano CT scanning technology to view the morphology of stem pits. 3D modelled images of the stem pits could provide leads on how they are formed.

Opsomming

Die bevestigde voorkoms van 'Candidatus' Liberibacter asiaticus (CLas), en *Diaphorina citri* in Oos-Afrika vereis 'n pro-aktiewe strategie van die Suid-Afrikaanse sitrusbedryf om voor te berei vir die uiteindelijke inval van Asiatiese vergroening (HLB). Die doel van hierdie projek is om 'n paneel van CTV infektiewe klone te vestig met 'n verskeidenheid 'payloads' wat sal deel uitmaak van 'n beheer strategie om die impak van HLB te beperk. Infektiewe klone van die T36 genotipe was ingevoer van medewerker Prof W.O. Dawson (Florida University). Die plasmiede was oorgedra na *Agrobacterium tumefaciens* en protokolle vir die infiltrasie in *N. benthamiana* geoptimeer. Die effektiwiteit van die infiltrasies van die infektiewe klone was geëvalueer deur GFP uitdrukking. Die volgende stap sal wees om verskillende virus partikel suiweringsstrategieë te gebruik om virus uit *N. benthamiana* te suiwer en daarmee sitrus saailinge te infekteer. Strategieë was ontwerp om die T36 klone om te skakel in plaaslike RB (asimptomaties) en T3 (simptomaties) genotipes. Die RB gebaseerde genotipe infektiewe kloon sal die basis vorm van die aflewering vektor terwyl die T3 gebaseerde genotipe infektiewe kloon slegs gebruik sal word vir navorsings doeleindes. Die kloneringstrategie wat ontwerp is maak gebruik van intermediêre plasmiede om groot gedeeltes van die CTV genoom te bevat. Die volgende stap is om die gedeeltes bymekaar te sit om die volle genoom te vorm. Daar is ook 'n dubbel rapporteerder infektiewe kloon ontwerp, gebaseer op die T36 kloon (Dawson) wat GFP uitdruk en 'n 'silencing cassette' bevat. Die doel van die kloon is om die doeltreffendheid van 'n CTV gebaseerde vektor te bepaal in terme van proteïen uitdrukking en/of 'silencing'. Hierdie kloon sal kan dien as 'n toetsplatform om die verskillende 'payloads' te sif. Die intermediêre klone vir meeste (7/8) van die ORF uitwissing en ORF vervangings infektiewe klone is voltooi. Hierdie klone sal gebruik word om die onderliggende meganismes van die vorming van stamgleuf te ondersoek. Addisioneel tot die studie was nano CT skandering gedoen om die morfologie van stamgleuwe te bestudeer. 3D beelde kan leidrade verskaf oor die oorsprong en vorming van stamgleuwe.

3.2.9 **PROGRESS REPORT: Field evaluation of three single-strain CTV isolates on Grapefruit, Valencia and Navel cultivars**

Project 1173 (2017/8-2022/3) by G. Cook, J.H.J. Breytenbach, C. Steyn and R. Clase (CRI)

Summary

Single-strain *Citrus tristeza virus* (CTV) isolates were characterized and evaluated for symptom expression in various industry cultivars in a glasshouse trial (Project 1056). The biological trial was done to determine pathogenicity of these CTV isolates in various citrus cultivars. 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 in Project 1160 (Application of CTV infectious clones to combat HLB), 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). Multi-strain CTV sources are difficult to maintain and transmit as stable populations. 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 Kakamas trial has been terminated as numerous trial trees were lost due to a lack of water shortly after planting. Thus far, CTV isolate GFMS12-8, a T68-strain, was shown to translocate poorly in the Mandarin hybrid, Mor26.

Opsomming

Enkel-ras *Citrus tristeza virus* (CTV) isolate is gekarakteriseer en geëvalueer vir simptome uitdrukking in verskeie bedryfskultivars in 'n glashuis proef (Projek 1056). Hierdie biologiese evaluasie is gedoen om die patogenisiteit van sekere CTV isolate in verskeie sitrus tipes te bepaal. 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). Meervoudige-ras bronne is ook moeilik om te handhaaf as stabiele populasies. Dit is dus van waarde om die enkel-ras CTV bronne as potensiële kruisbeskermingsbronne te evalueer. Die pomelo en Valencia-bome is in die Noord-Kaap geplant en Navels in Mpumalanga. 'n Clementine- en 'n Mandaryn is in Limpopo geplant. Die Kakamas-proef is beëindig aangesien talle proefbome gevrek het weens 'n tekort aan water kort na plant. Tot dusver het CTV-isolaat GFMS12-8, 'n T68-ras, swak getranslokeer in die Mandaryn, Mor26.

3.2.10 PROGRESS REPORT: Training of dogs for the detection of African greening and Huanglongbing
Project 1184 (2017/8 – 2018/9) by G. Cook (CRI), P. Olivier (private dog trainer), S. Gillham (private dog trainer), J.H.J. Breytenbach, C. Steyn and R. Clase (CRI).

Summary

A project was initiated to train a sniffer dog for the early detection of the Citrus Greening pathogen, '*Candidatus* Liberibacter africanus (Laf) in citrus following the use of sniffer dogs for early detection of '*Candidatus* Liberibacter asiaticus (Las) in the USA. These dogs could not differentiate Las from the other Liberibacter sp. viz. '*Ca*'. L. americanus (Lam) and Laf. Locally, a suitable dog was acquired and socialization and imprinting of the dog to the African Greening pathogen was achieved. Imprinting of the dog on the scent of infected citrus plants was done by introducing the odour in an indoor environment in order to eliminate other odours and outdoor distractions. Once this imprinting was achieved, the training progressed to an outdoor environment, introducing additional scents and distractions. This process used ground scenting, whereby infected and non-infected material was placed in sterile containers in grassy areas and the dog indicated on positive material. The next stage was to get the dog to wind scent. The dog was required to make use of wind currents to scent in different search patterns. Potted plants, both infected and uninfected, were used for this evaluation. Thereafter, transition to field work was started and a young, uninfected orchard was spiked by planting positive plants in orchard rows. As the dog was initially trained to wind scent, she ran haphazardly through the orchard, often missing positive plants. The training was then adapted to teach the dog directional control in order for her to scent down an orchard row. The possibility of imprinting a second dog on '*Candidatus* Liberibacter asiaticus (Las) was investigated and a request to import infected material to be maintained in a quarantine facility was submitted to DAFF. The request was declined and the training of a second dog will therefore not proceed. The continuation of the project will be focused on investigating the early detection ability of the dog compared to molecular testing by using potted plants, inoculated at various intervals and comparatively tested. Transitioning to field work from pot trials will continue. This is a process of trust building that the trainer needs to develop with the dog. Molecular detection lags the dog's detection ability and a positive field indication cannot always be verified by laboratory tests. Once the trainer has confidence that the dog understands its role, the dog will be used to scout orchards.

Opsomming

'n Projek is geïnisieer om 'n snuffelhond op te lei vir vroeë opsporing van die Vergroenings-patogeen, '*Candidatus* Liberibacter africanus (Laf) in sitrus, wat volg op 'n benadering gebruik in die VSA waar honde vir die vroeë opsporing van '*Ca*'. L. asiaticus (Las) gebruik is. Hierdie honde kon egter nie Las van die ander Liberibacter spp. nl. '*Ca*'. L. americanus (Lam) en Laf, onderskei nie. 'n Geskikte hond is verkry vir die projek en gesosialiseer. Die hond is suksesvol opgelei om die reuk van Laf-besmette sitrusplante uit te ken. Hierdie proses is eers binnenshuis uitgevoer om ander reuke en buitelig afleidings uit te skakel. Die opleiding het daarna gevorder na 'n buiteligomgewing, waar addisionele reuke en afleidings aan haar bekendgestel is. Besmette en nie-geïnfekteerde materiaal is hiervoor in steriele houers in grasagtige gebiede geplaas en die hond het die positiewe materiaal aangedui. Die volgende stadium was om die hond te leer windreuke volg. Die hond moes van windstrome gebruik maak om verskillende soekpatrone te ruik. Potplante, beide besmet en gesond, is vir hierdie evaluering gebruik. Daarna is die oorgang na veldwerk begin en 'n jong, onbesmette boord is gebruik en positiewe plante is in boordrye geplant. Die hond was aanvanklik opgelei om windreuke te volg en hardloop toe lukraak deur die boord en dikwels positiewe plante daardeur gemis. Die opleiding is toe aangepas om rigting beheer toe te pas, sodat die hond die

boomrye sistematies kan ruik. Die moontlikheid om 'n tweede hond op te lei spesifiek vir Las, is ondersoek. 'n Versoek om geïnfekteerde materiaal in 'n kwarantyn fasiliteit in te voer, is aan DAFF voorgelê. Die versoek is afgekeur en die opleiding van 'n tweede hond sal dus nie voortgaan nie. Die voortsetting van die projek sal gefokus wees op die ondersoek van die vroeë opsporingsvermoë van die hond in vergelyking met molekulêre toetse. Hiervoor sal potplante gebruik word wat ingeïnkuleer sal word op verskillende tydperke en opsporing hiervan sal vergelyk word. Oorgang na veldwerk vanaf potproewe sal voortgaan. Dit is 'n proses van vertrouensbou wat die afrigter met die hond moet ontwikkel. Die hond se opsporingsvermoë is meer sensitief in vergelyking met molekulêre opsporing en 'n positiewe veldaanwysing kan nie altyd deur laboratoriumtoetse bevestig word nie. Sodra die afrigter vertrou het dat die hond sy rol verstaan, sal die hond gebruik word om boorde te verken.

3.3 PROGRAMME: SOILBORNE DISEASES

Programme coordinator: Jan van Niekerk (CRI)

3.3.1 Programme summary

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

Projects 762 (3.3.4) and 1030 (3.3.3) are specifically aimed at finding alternative means of control for *Phytophthora* and citrus nematode. Data has been recorded in project 762 since 2011 and the juvenile and female nematode counts still do not clearly show up differences between treatments. However, it is becoming clear that the different pre-plant soil fumigation treatments have caused the trees in these treatments to be taller with thicker trunks compared to the other treatments and the untreated control. In the 2017/2018 season a field trial continued to test for nematode control, OMV-JJ1, a garlic derivative, according to different treatment regimes. Results from nematode counts indicated that OMV-JJ1 does show promise as an alternative, softer option for nematode control. The supplier will be approached to determine if registration will commence. A second season of phosphonate applications to 'Nadorcott' mandarins confirmed the previous results. It was again seen that if full or half dose phosphonate applications are done on these mandarins at colour break or full colour stage of colour development, severe phytotoxicity is seen. It is therefore recommended that for the control of *Phytophthora* brown rot on mandarins, alternative measures such as tree skirting is employed.

Two distinct diseases have recently been observed in orchards in the Kirkwood and Patensie areas of the Eastern Cape province and in orchards in Swaziland and Hoedspruit (3.3.5). Extensive sampling and isolations from diseased material have led to the conclusion that a complex of pathogens are involved in the disease in the Eastern Cape while in the Swaziland/Hoedspruit areas only one pathogen is at work. It was furthermore seen that plants forming the natural vegetation in the valley harbor the same pathogens as was found in the diseased trees. Further characterization of the causal pathogens and the inoculum sources of these pathogens are underway. A pot based rootstock trial was also established to determine if this could indicate a rootstock that have potential to provide a long term solution to this disease.

Following on project 910, project 1092 (3.3.7) was started in April 2015 to further investigate the factors involved in citrus decline. Four orchards, showing various degrees of decline, were selected in the Nelspruit area. In each orchard, 20 trees per decline category (1-3) were selected and marked. In these past three years extensive data collection occurred from these orchards. This collection is now complete and data are in the process of being analysed. A final project report will be submitted in 2019.

In project 1152 (3.3.2) it was found that the basis of citrus replant disease in South Africa is biotic in nature. It was furthermore confirmed *Phytophthora* and *Pythium* spp. along with citrus nematodes, do play a role in citrus replant problems as previously reported. A new finding was that several *Fusarium* spp., including a potential new species, are also present in the replant citrus soils. Further studies, including pathogenicity and fungicide sensitivity work with the *Fusarium* spp. are underway.

The last project in the portfolio (3.3.6), focusses on the soilborne pathogens in citrus nurseries. A large number of *Phytophthora* and *Pythium* isolates were collected during the project. These were characterized using molecular techniques. Ten *Pythium* spp., including a potential new species, were identified to occur in South African citrus nurseries. Mefenoxam sensitivity work furthermore indicated that the isolates within these species vary greatly in their sensitivity to the fungicide. Pathogenicity and chlorine sensitivity studies with the identified ten species are currently underway. Mefenoxam and chlorine sensitivity studies on *Phytophthora nicotianae* and *Phytophthora citrophthora* were completed. It was shown that mefenoxam and chlorine sensitivity between and within species also vary greatly. Some isolates of both species only got eliminated completely from water at a chlorine concentration of 6 ppm and an exposure time of 60 minutes.

Program-opsomming

Die projekte binne die grondgedraagde siekteportefeulje, spreek diverse navorsingsvrae aan wat met grondgedraagde siektes en plaë van sitrus verband hou. Aandag word gegee aan die vind van alternatiewe, sagter en meer volhoubare chemikalieë wat in die bestuur van *Phytophthora* en sitrus aalwurmprobleme gebruik kan word. Hiermee saam word die probleem van sitrus agteruitgang en herplantsiekte ook ondersoek, en word daar veral gekyk na faktore wat gebruik kan word as vroeë indikatore van boom-agteruitgang. Onbekende siektes met onbekende veroorsakende organismes en epidemiologie word verder bestudeer ten einde die oorsake van die waargenome siekte vas te stel, en watter bestuurspraktyke die potensiaal het om die impak van die siekte te verminder.

Projekte 762 (3.3.4) en 1030 (3.3.3) het spesifiek ten doel om alternatiewe te vind vir die beheer van *Phytophthora* en sitrus aalwurm. Data is sedert 2011 in projek 762 aangeteken, en die jong en vroulike aalwurmtellings toon steeds nie duidelike verskille tussen behandelings nie. Dit word egter duidelik dat die verskillende vóór-plant berokingsbehandelings daartoe lei dat bome in hierdie behandelings langer en hul stamme dikker is in vergelyking met die ander behandelings en die onbehandelde kontrole. In die 2017/2018 seisoen is voortgegaan om in 'n veldproef vir aalwurmbeheer te toets, met OMV-JJ1, 'n knoffel-ekstrak, volgens verskillende behandeling regimes. Resultate van aalwurmtellings het aangetoon dat OMV-JJ1 belofte toon as 'n alternatiewe, sagter opsie vir aalwurmbeheer. Die verskaffer gaan genader word om te bepaal of registrasie gaan plaasvind. 'n Tweede seisoen van fosfonaat toedienings op 'Nadorcott' mandaryne het die vorige resultate bevestig. Daar is weer gesien dat indien vol of halwe dosis fosfonaat toedienings op hierdie mandaryne by kleurbreek- of volkleurstadium van kleurontwikkeling gedoen word, ernstige fitotoksiteit gesien word. Daar word dus aanbeveel dat vir die beheer van *Phytophthora* bruinvrot op mandaryne, alternatiewe maatreëls soos boom rompsnoei ("skirting") gedoen moet word.

Twee afsonderlike siektes is onlangs in boorde in die Kirkwood- en Patensie-areas van die Oos-Kaapprovinsie, en in boorde in Swaziland en Hoedspruit waargeneem (3.3.5). Uitgebreide monsterneming en isolasies vanuit siek materiaal het tot die gevolgtrekking laat kom dat 'n kompleks van patogene by die siekte in die Oos-Kaap betrokke is, terwyl in die Swaziland-/Hoedspruit-areas, slegs een patogeen betrokke is. Daar is verder gesien dat plante wat die natuurlike vegetasie in die vallei vorm, dieselde patogene huisves as wat in die siek bome gevind is. Verdere karakterisering van die veroorsakende patogene en die inokulumbronne van hierdie patogene is onderweg. 'n Pot-gebaseerde onderstampoef is ook gevestig ten einde te bepaal of dit op 'n onderstam kan dui wat potensiaal het om 'n langtermyn-oplossing vir hierdie siekte te bied.

Volgende op projek 910, is projek 1092 (3.3.7) in April 2015 begin om verder die faktore betrokke in sitrus agteruitgang te ondersoek. Vier boorde wat verskillende vlakke van agteruitgang getoon het, is in die Nelspruit-area geselekteer. In elke boord is 20 bome per agteruitgang-kategorie (1-3) geselekteer en gemerk. Die afgelope drie jaar het uitgebreide dataversameling vanaf hierdie boorde plaasgevind. Die versameling is nou voltooi en data is in die proses om geanaliseer te word. 'n Finale projekverslag sal in 2019 ingedien word.

In projek 1152 (3.3.2) is gevind dat die basis van sitrus herplantsiekte in Suid-Afrika bioties van aard is. Daar is verder bevestig dat *Phytophthora* en *Pythium* spp., tesame met sitrus aalwurms, 'n rol speel in sitrus herplantprobleme, soos voorheen aangeteken. 'n Verdere bevinding is dat verskeie *Fusarium* spp., insluitende 'n potensieel nuwe spesie, ook in die herplant sitrusgronde teenwoordig is. Verdere studies, insluitende patogenisiteit en fungisied sensitiviteitswerk met die *Fusarium* spp., is onderweg.

Die laaste projek in die portefeulje (3.3.6) fokus op die grondgedraagde patogene in sitruskwekerye. 'n Groot aantal *Phytophthora* en *Pythium* isolate is tydens die projek versamel. Die isolate is deur middel van molekulêre tegnieke gekarakteriseer. Tien *Pythium* spp., insluitende 'n potensieel nuwe spesie, is geïdentifiseer, en kom in Suid-Afrikaanse sitruskwekerye voor. Mefenoxam sensitiviteitswerk het verder getoon dat die isolate binne hierdie spesies grootliks in hul sensitiviteit teenoor die fungisied varieer. Patogenisiteit en chloor sensitiviteitsstudies met die tien geïdentifiseerde spesies, is tans onderweg. Mefenoxam en chloor sensitiviteitsstudies op *Phytophthora nicotianae* en *Phytophthora citrophthora* is voltooi. Daar is getoon dat mefenoxam en chloor sensitiviteit tussen en binne spesies ook grootliks varieer. Sommige isolate van beide spesies is slegs heeltemal in water uitgewis teen 'n chloor konsentrasie van 6 dpm en by 'n blootstellingsperiode van 60 minute.

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

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

Summary

Citrus replant disease occurs when new orchards are established on sites where citrus has been cultivated for many years. The causative agents (fungi, oomycetes and nematodes) involved in citrus replant disease in South Africa, have only been studied to a limited extent. The aim of this study was to investigate the etiology of citrus replant disease in South Africa. Four citrus replant orchard soils were sampled in the Addo, Patensie, Hoedspruit and Letsitele production areas. Analyses of orchard soil samples showed that *Pythium irregulare*, *Phytophthora citrophthora* and *Phytophthora nicotianae* were present in the soil from all four production areas. Infestation levels of juveniles of the citrus nematode *T. semipenetrans* was significantly higher in the soil samples of the Patensie and Hoedspruit orchards, than in the other two orchard soils. The involvement of biological replant agents were further investigated in a soil bioassay trial in the glasshouse, where six treatments were evaluated. Biological agents were involved in three of the orchard soils (Addo, Hoedspruit and Letsitele), since seedling growth (height or weight) was significantly higher for seedlings grown in pasteurized soil versus seedlings grown in the untreated control soil. Furthermore, a dilution of 20% of these replant soils into pasteurized soil resulted in a significant reduction in seedling growth relative to the control and the pasteurized treatments. In the Patensie orchard soil, the involvement of biological agents was unclear. However, dilution of the Patensie soil did result in significantly lower seedling growth than the control. Isolations from the roots of the control bioassay seedlings in all orchards showed that isolates belonging to the '*Fusarium solani*' species complex dominated in all orchards except the Letsitele orchard. *Fusarium oxysporum* species complex isolates and oomycetes (*Phytophthora nicotianae*, *Phytophthora citrophthora* and *Pythium irregulare*) were also associated with the citrus roots at more or less similar frequencies within each orchard.

Opsomming

Sitrus herplantsiekte kom voor wanneer nuwe boorde op areas gevestig word waar sitrus al vir baie jare verbou is. Die veroorsakende agente (swamme, oömisete en aalwurms) betrokke in sitrus herplantsiekte in Suid-Afrika, is net tot 'n beperkte mate al bestudeer. Die doel van hierdie studie was om die etiologie van sitrus herplantsiekte in Suid-Afrika te ondersoek. Vier sitrus herplantboordgronde is in die Addo, Patensie, Hoedspruit en Letsitele produksie-areas versamel. Analises van boordgrondmonsters het getoon dat *Pythium irregulare*, *Phytophthora citrophthora* en *Phytophthora nicotianae* in gronde van al vier produksie-areas teenwoordig was. Infestasievlakke van jong sitrus-aalwurm, *T. Semipenetrans*, was betekenisvol hoër in die grondmonsters van die Patensie en Hoedspruit boorde, as in die ander twee boordgronde. Die betrokkenheid van biologiese herplant-agente is verder in 'n grond bio-analise proef in die glashuis ondersoek, waar ses behandelings geëvalueer is. Biologiese agente was in drie van die boordgronde (Addo, Hoedspruit en Letsitele) betrokke, aangesien saailinggroei (hoogte of gewig) betekenisvol hoër was vir saailinge wat in gepasteuriseerde grond gegroei het teenoor saailinge wat in onbehandelde kontrole grond gegroei het. Verder het 'n verdunning van 20% van hierdie herplantgronde in gepasteuriseerde grond tot 'n betekenisvolle vermindering in saailinggroei relatief tot die kontrole en die gepasteuriseerde behandelings gelei. In die Patensie boordgronde was die betrokkenheid van biologiese agente onduidelik. Verdunning van die Patensie grond het egter tot betekenisvol laer saailinggroei gelei in vergelyking met die kontrole. Isolاسies vanuit die wortels van die kontrole bio-analise saailinge in alle boorde, het getoon dat isolate wat tot die '*Fusarium solani*' spesieskompleks behoort, in alle boorde oorheers het, behalwe in die Letsitele boord. *Fusarium oxysporum* spesieskompleks isolate en oömisete (*Phytophthora nicotianae*, *Phytophthora citrophthora* en *Pythium irregulare*) is ook met die sitruswortels, teen min of meer dieselfde frekwensies binne elke boord, geassosieer.

This project constituted an MSc study at the Department of Plant Pathology, University of Stellenbosch that was concluded in March 2018. The final report is therefore made up of the two research chapters from the thesis.

Chapter 1: Identification and characterization of soilborne pest, diseases and abiotic factors potentially associated with citrus replant disease

Introduction

Replant disease is a phenomenon that is observed in cases where young, healthy nursery trees are planted on old orchard sites, previously planted with the same crop or closely related species. Replant disease symptoms is characterised by newly planted trees that are stunted, with small leaves, exhibiting poor growth (Derrick and Timmer, 2000). The retarded and stunted growth of newly planted trees has been ascribed to the accumulation of phytotoxins in the soil, development of nutrient imbalances, deterioration of soil physical characteristics and the establishment of soil pathogens that damage the root systems of young trees (Cronje *et al.*, 2002). The casual agents associated with citrus replant disease in South Africa according to a previous study done by Le Roux *et al.* (1998) include the citrus nematode, *Tylenchulus semipenetrans* and the oomycete pathogens, *Phytophthora nicotianae* and *Phytophthora citrophthora*. Previous citrus replant studies only focused on the involvement of *Phytophthora* spp. and the citrus nematode. It is therefore important to further investigate any possible pathogens involved in the disease complex instead of focusing only on *Phytophthora* spp. and the citrus nematode.

The biological agents involved in apple replant disease are much more complex than what has been reported for citrus replant disease. Isolation and pathogenicity studies in Italy, South Africa, Australia and the United states have shown that various fungal (*Rhizoctonia* spp.), oomycete (*Pythium*, *Phytophythium* and *Phytophthora*) and nematode (*Pratylenchus* spp.) species are involved. These organisms have been shown to be site specific, and it is therefore important to investigate several replant sites in order to determine the different replant pathogens involved. *Phytophthora cactorum* occurs worldwide in apple replant soils, whereas *P. cambivorum* has been reported less frequently. Many *Pythium* species have been associated with apple replant soils, but only a few are pathogenic. Some of the most virulent and wide-spread species include *P. ultimum*, *P. irregulare* and *P. sylvaticum*. Only a few of bi-nucleate *Rhizoctonia* species are pathogenic, but some isolates within the bi-nucleate

anastomosis groups are non-pathogenic (Dullahide *et al.*, 1994; Mazzola, 1998; Manici *et al.*, 2003; Tewoldemedhin *et al.*, 2011a, b, c; Mazzola and Manici, 2012). *Rhizoctonia solani* AG-5 and AG-6 have only been identified in Italy and Washington State in the USA, where they are known as highly pathogenic species (Mazzola, 1998; Manici *et al.*, 2003). '*Cylindrocarpon*'-like fungi are known to be involved in apple replant disease although isolates in general have low virulence (Dullahide *et al.*, 1994; Mazzola, 1998; Tewoldemedhin *et al.*, 2011a). Several *Fusarium* spp. were also found to be involved with apple replant disease (Verma and Sharma, 1999). However, the species involved are not clear due to recent major taxonomic changes in this group of fungi (Lombard *et al.*, 2014). The *Fusarium* spp., *F. solani*, *F. sambucinum*, *F. tricinctum* and *F. avenaceum* have been identified as apple replant pathogens, but these are considered as unimportant in apple replant disease. This is due to most isolates within a species not being pathogenic, and those isolates that are pathogenic having low virulence (Dullahide *et al.*, 1994; Mazzola, 1998; Manici *et al.*, 2003; Tewoldemedhin *et al.*, 2011 b, c). Some studies have furthermore shown through pathogenicity studies that some of the known apple replant pathogens interact synergistically for example '*Cylindrocarpon*'-like spp. and some *Pythium* spp. (Braun, 1995; Mazzola, 1998; Tewoldemedhin *et al.*, 2011a).

Peach replant is also caused by biological agents, although the agents involved has been studied to a lesser extent than for apple replant. Replant in peach, have been shown to be associated with '*Cylindrocarpon*' *destructans*, '*Cylindrocarpon*' *lucidum*, *Pythium* spp., *Phytophthora cactorum* and *Rhizoctonia solani* (Jaffee *et al.*, 1982; Browne *et al.*, 2006; Bent *et al.*, 2009). In citrus replant, the fumigation of soil with methyl bromide pre-plant significantly increased the net income and the total volume of exportable fruit compared to the untreated replant soils (Le Roux *et al.*, 1998; Duniway, 2002; Schneider *et al.*, 2003). However, this management option is no longer available, since methyl bromide was phased out for developing countries such as South Africa in 2015 (Duniway, 2002; Schneider *et al.*, 2003; Desaegeer *et al.*, 2017). Finding alternatives to methyl bromide for citrus production on old orchard sites are important, since if soilborne pathogens and nematodes are not controlled, this can lead to significant losses in production and fruit quality.

The increase in citrus production in South Africa has necessitated the establishment of new orchards on sites where citrus has previously been cultivated for many years (Burger and Small, 1983). Since our understanding of the biological agents involved in citrus replant disease is limited, it is important to elucidate the biological agents involved. This can ultimately lead to the development of sustainable management strategies that target the complex group of biological agents that might be involved in citrus replant disease. This study was therefore aimed at providing the citrus industry with knowledge regarding whether biological agents were involved and the specific agents involved.

Materials and methods

Soil sampling

Root and soil samples were collected from four citrus orchards, aged between 37 and 47 years, prior to removal of the old trees. The orchards were situated in two of the main citrus growing regions in South Africa, i.e. Eastern Cape (Addo and Patensie areas) and Limpopo provinces (Hoedspruit and Letsitele areas). Sampling was conducted between April and May 2016. The soil samples taken from each orchard, was pooled and mixed and subdivided for use in a glasshouse seedling bioassay trial. A small portion of the pooled soil per orchard were used to determine the physical characteristics of the soil (NviroTek Labs, Hartbeespoortdam) and another portion were used to quantify and identify oomycetes and parasitic nematodes present within the soil (Grimm and Alexander, 1973).

Physical and chemical characterization of soil

The portion of soil per orchard sent away were subjected to standard chemical and physical soil analyses. Soil pH was determined in a KCl solution, while phosphorous content was determined using the Bray I method. The results from the different orchards were compared using analysis of variance (ANOVA) while the means were compared using the Fisher's LSD test at a 95% confidence level (SAS software version 9.4).

Quantification of oomycetes and nematodes from orchard soils

The presence of oomycetes (*Pythium* and *Phytophthora*) in the soils were determined using a leaf disk baiting method to analyse the portion of soil from each orchard subjected to oomycete quantification (Grimm and Alexander, 1973; Linde *et al.*, 1994; Timmer *et al.*, 1988, 1990). The oomycete isolates obtained through leaf baiting was further morphologically identified by sub-culturing onto CMA medium. From these cultures five inoculum plugs (5 mm²) of each oomycete isolate were plated in a sterile petri dish (65 mm) containing non-sterile soil water extract (20 g soil suspension in 1L distilled water and filtered). The plates were incubated at 25°C under cool white fluorescent light for 24h until sporulation was observed (Jeffers, 2006). The plates were examined under a compound light microscope at different magnifications. Identification of *Phytophthora* spp. was identified up to genus level based on sporangia characteristics (Gallegly and Hong, 2008). *Pythium* spp. was identified based on the presence of non-papillated round sporangia (Plaats-Niterink, 1981). From here on representative isolates based on the preliminary identification was used for further molecular identification.

Nematode extraction and quantification

Nematodes were extracted from each of 10 soil samples per orchard. For the extraction of juvenile nematodes, soil samples were subjected to the Baermann pan extraction method (Whitehead and Hemming, 1965). For the extraction of female citrus nematodes the method of Greco and D'Addabbo, 1990 and Galeano *et al.*, 2003 were used. A 1 mL subsample was taken from the 100 mL nematode suspension and pipetted into a Peters' counting slide, and observed under a microscope (Luc *et al.*, 2005; Fourie *et al.*, 2017).

Glasshouse seedling bioassay trial

Plant material

Carrizo citrange seeds were collected from the Citrus Foundation block near Uitenhage in the Eastern Cape province. The seeds were sown in March 2016 into seedling pots containing a steam pasteurized perlite and peat moss (50:50) mixture. After 3 months, the seedlings were selected for uniformity before being transplanted in treated or untreated soil.

Trial treatments

The soil from each of the four orchards were divided into six equal portions that each received one of six different treatments. Treatments included (i) steam pasteurization, (ii) 20% dilution of each orchard soil into the corresponding steam pasteurized soil, and the application of (iii) mefenoxam, (iv) difenoconazole, (v) cadusafos and (vi) untreated control. After the soils received the different treatments, the treated soil of each treatment was dispensed into six 500 mL plastic pots. Prior to planting two Carrizo citrange rootstock seedlings per pot, the weight of each seedling was recorded. The height of each seedling was also recorded after planting. The potted Carrizo citrange seedlings were placed in a randomize block design in a glasshouse, located in Nelspruit at Citrus Research International (CRI). The temperature of the glasshouse was at ambient temperature and if temperature raised above 30°C, fans automatically switch on and decrease the temperature. The seedlings were watered as needed. The trial was repeated two weeks apart and left to grow for a period of 7 months.

Trial evaluation

Seedlings growth measurements

After seven months of growth, the glasshouse seedling trial was terminated. The number of dead seedlings per treatment was noted. The remaining seedlings were removed from the soil. The root systems were washed with sterile distilled water, removing excess soil, and seedlings were then left to air-dry on sterile tissue paper. The length of the above ground growth was measured using an electronic calliper and data logger. The total seedling mass (combined root and above ground mass) of each seedling were measured and compared to data taken at planting. Subsequently, the increase in seedling weight and height was calculated.

Isolation for fungi and oomycetes from roots

The roots of the untreated control seedling were used for isolations to determine which fungi and oomycetes colonized the roots of seedlings. After seedling weight measurements at trial termination, root systems were removed using a pruning knife (blades spray-sterilised with 70% ethanol) and washed with sterile distilled water and left to air-dry on sterile tissue paper in the laminar flow cabinet. Thirty-six root pieces per pot, each approximately 10 mm in length, were removed from the root systems using a flame sterilised scalpel and plated out onto four different mediums in 90 mm Petri dishes, (i) 2% potato dextrose agar (Difco, Becton, Dickinson and Company) amended with 1 mL streptomycin sulphate solution (40 mg/L, Calbiochem, Merck) (PDA+s), (ii) 1.5% bacteriological agar (Difco, Becton, Dickson and Company) amended with streptomycin sulphate (40 mg/L, Calbiochem, Merck) and metalaxyl (250 a.i. g/L) (WA+s+m), as well as (iii) PARPH amended with benomyl (500 a.i. g/kg) (PARPH+B) and (iv) PARP amended with benomyl (0.2g/100mL) (PARP+B). Nine root pieces were plated onto each medium, thus eight 90mm Petri dishes per pot. Plates were incubated at 29°C for 2 to 3 days. When fungal growth emerging from the roots was observed, subcultures representing each fungal colony was transferred to PDA+s Petri dishes (65 mm). The subcultured plates were incubated under the same conditions as mentioned previously. Growth that was observed on PARPH+B and PARP+B were purified by hyphal-tipping to WA and sub-cultured onto corn meal agar (CMA) Petri dishes (65 mm) and incubated at 29°C for further identification.

Five CMA inoculum plugs (5 mm²) of each oomycete isolate were plated in a sterile petri dish (65 mm) containing non-sterile soil water extract (20 g soil suspension in 1L distilled water and filtered). The plates were incubated at 25°C under cool white fluorescent light for 24h until sporulation was observed (Jeffers, 2006). The plates were examined under a compound light microscope at different magnifications. *Phytophthora nicotianae* was identified by the sporangia being papillated and more ovoid in shape. The sporangia of *Phytophthora citrophthora* is also papillated but more asymmetrically in shape, and often with more than one apex. The preliminary identification of *P. citrophthora* at this stage was based on the presence of papillated sporangia with two apices (Gallegly and Hong, 2008). *Pythium* spp. was identified based on the presence of non-papillated round sporangia (Plaats-Niterink, 1981). The sub-cultured fungi and oomycetes were identified to the species level as described in the section below under "Molecular identification of isolated fungal species".

Isolation of nematodes

No isolations of the female citrus nematode were made after seven months due to the lack of root development. There were not enough roots to extract female nematodes, and therefore not meaningful to compare the data before and after seven months. The isolation of juveniles from the soil would also not have given any meaningful information due to the females influencing the growth of the roots.

Statistical analysis

Two experimental trials were conducted. For each trial, the experimental design was a randomised block with the 24 treatment combinations (six treatments applied to soil from four production areas) replicated at random in four blocks. An experimental unit consisted of 10 seedlings in total (five pots each containing two seedlings). The average increase in weight and length per seedling were calculated for each experimental unit.

For each trial, the increase in weight and length per seedling were subjected to analyses of variance (ANOVA) using GLM (General Linear Models) Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Trial results were also combined in one analysis of variance (John and Quenouille, 1977) after confirmation of trial homogeneity of variance using Levene's test (Levene, 1960). Shapiro-Wilk test was performed on to test for deviation from normality (Shapiro and Wilk, 1965). Square root transformation was applied to improve weight increase deviation from normality (Snedecor and Cochran, 1980). Fisher's least significant difference was calculated at the 95% level to compare means for significant effects (Ott, 1998). A probability level of 95% was considered significant for all significance tests.

Molecular species identification of isolated fungal and oomycete isolates

The sub-cultured oomycete (soil and root) and fungal (roots) isolates were classified into different groups based on their cultural and morphological characteristics on PDA+s for fungal and CMA media for oomycetes. Cultural characteristics used included colony size, texture, shape and colour. Oomycetes were further identified by observing the morphology of sporangia in non-sterile soil water extract under a compound light microscope as previously described in the section "Quantification of oomycetes and nematodes from orchard soils". The *Fusarium* isolates were grouped according to the colour of the mycelia and growth pattern on PDA+s. Microscopic slides of five representative isolates from each group were prepared to observe the conidial shape, size and colour using a bright-field microscopy (Leslie and Summerell, 2006). The oomycete and fungal groups were identified as belonging to the families Peronosporaceae, Pythiaceae and Nectriaceae. All genera that have not been previously identified as being involved in replant disease associated with apples, peach and citrus were discarded. These genera included *Penicillium*, *Alternaria* and *Trichoderma*. The isolates of importance were stored for further identification. Oomycete isolates were stored in sterile distilled H₂O with lemon leaf pieces, and the fungi was stored on ½ strength PDA slants and in sterile distilled H₂O.

DNA extraction

DNA was extracted from 3-week-old fungal cultures growing on PDA+s and oomycete cultures growing on CMA+s. The DNA extraction protocol of Osmundson *et al.* (2013) was used with minor adjustments.

Polymerase chain reaction (PCR) and electrophoresis

The ITS regions and 5.8S gene for the oomycete isolates were amplified using universal primers ITS-6 (5'-GAAGGTGAAGTCGTAACAAGG-3') (Cooke and Duncan, 1997; Cooke *et al.*, 2000) and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990). In a total reaction volume of 25 µl, the PCR reaction contained 2 µl of DNA, 2X Promega G2 GoTaq Master Mix (Promega Madison, WI USA), 0.3µM of each primer and 9 µl PCR H₂O. The PCR reaction conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 32 cycles of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C, and a final extension step at 72°C for 5 min.

The putative *Fusarium* spp. isolates were identified by amplifying the translation elongation factor 1-alpha (EF 1-α) gene using primers EF1 (5'-ATGGGTAAGGARGACAAGAC-3') and EF2 (5'-GGARGTACCAGTSATCATGTT-3') (O'Donnell *et al.*, 1998). A fragment length of 700 bp were amplified for the TEF-1α gene region. The PCR reactions contained 2 µl of DNA, 2X Promega G2 GoTaq Master Mix (Promega Madison, WI USA), 0.32µM of

each primer per PCR reaction as described above and 9 µl PCR H₂O. Reaction conditions consisted of an initial denaturation step at 95°C for 3 min followed by 30 cycles of 1 min at 95°C and 45 s at 55°C, 1 min at 72°C with a final extension of 3 min at 72°C. All PCR reactions were performed in an Applied Biosystems 2700 PCR machine (Carlsbad, California, USA). A non-template water control was also included in each PCR run.

PCR products were separated by electrophoresis on a 1.5% (w/v) SeaKem® LE agarose gel (Lorenza Rockland, ME USA) in TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.5) after ethidium bromide staining. The GeneGenius Gel Documentation and Analysis System (Syngene, UK) were used to visualize the gel under ultraviolet (UV) light alongside a 100-bp DNA ladder (GeneRuler, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Restriction fragment length polymorphism (RFLP) of oomycete species

The ITS-PCR products of the oomycete isolates were used to obtain restriction fragment length polymorphism banding profile patterns, for the classification and identification of the organisms by using a single and double-restriction enzyme RFLP. In a total reaction volume of 20 µl the ITS-RFLP double-enzyme reaction contained 8 µl ITS-PCR product, 2 µl CutSmart buffer (BioLabs NEB, New England), 1 µl *HhaI* and 1 µl *Hinfi* restriction enzymes (BioLabs NEB, New England) and 8 µl nuclease free water (Promega Madison, WI USA). For the single reaction, 1 µl *HhaI* enzyme was used and 9 µl nuclease free water instead of 8 µl mentioned in the double restriction enzyme reaction. The restriction enzyme reactions were all incubated at 37 °C for 15 min. PCR-RFLP products were separated by electrophoresis on a 3% (w/v) SeaKem® LE agarose gel (containing ethidium bromide) in TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.5) for 90 min at 75 V/cm. The GeneGenius Gel Documentation and Analysis System (Syngene, UK) were used to visualize the gel under ultraviolet (UV) light alongside a 100-bp DNA ladder (Promega, Madison, WI USA). Isolate that contained the same banding patterns were grouped into the same PCR-RFLP group.

Sequencing of PCR products

The PCR products were purified using the MSB Spin PCRapase Kit (Invitex, Berlin, Germany) according to manufacturer's instructions. In the final step, DNA was eluted from the column using 15 µl water. The cleaned PCR products were then sent for sequencing to the DNA Sequencing Unit at the Central Analytical Facility (CAF) of Stellenbosch University. Sequencing was conducted using the forward and reverse primers used in the initial PCRs. Sequencing was conducted using the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, United States) and an ABI 3130xl DNA sequencer (Applied Biosystems, Foster City, California, United States)

Results

Chemical and physical soil analyses

Results of the physical and chemical analyses of the sampled soils in the different orchards showed that the different soils varied greatly with regards to their physical and chemical characteristics. This variation is illustrated in Table 1, where it is clear that in many cases there were significant differences ($P \leq 0.05$; ANOVA not shown) in the levels of pH, different minerals and the clay, silt and sand percentages contained in the different soils from the different areas. There was no significant difference in soil pH between the Addo (6.36), Hoedspruit (6.60) and the Letsitele (6.50) soils. The soil pH of Patensie differed significantly from the other three areas, with a lower soil pH of 5.82 (Table 1). The pH of the soil should be adjusted according to the citrus needs during the period of planting and establishing new orchards. In this study, the pH of the soil was not adjusted prior to planting Carrizo citrange seedlings into the pots. There was a significant difference ($P < 0.0001$; Table 1) in P levels (mg/kg) between Patensie (138.62 mg/kg), Addo (30.21 mg/kg) and Hoedspruit (4.57 mg/kg) soil, and no significance was observed between Hoedspruit (4.57 mg/kg) and Letsitele (7.52 mg/kg; Table 1). There was a significant difference in soil

Calcium (Ca) levels per area ($P < 0.0001$; Table 1). Patensie soil had the highest Ca level (2260.21 mg/kg), statistically higher than any of the other areas. Hoedspruit (1532.42 mg/kg) and Addo (1181.83 mg/kg) had statistically similar Ca levels, but significantly differed from the Ca level in Letsitele (685.50 mg/kg; Table 1). In terms of magnesium (Mg) content significant differences were also observed between the different areas. Patensie (248.51 mg/kg) and Addo (299.12 mg/kg) soils had statistically similar Mg content. Hoedspruit at 420.57 mg/kg had significantly the highest Mg content of all the areas. Letsitele was the area with the lowest Mg content in the soil of 180.59 mg/kg (Table 1). The clay percentage of the different soils showed significant variation ($P < 0.0001$) where the percentage of clay in Patensie (21.70%) and Addo (21.20%) was statistically similar (Table 1). The clay percentage of Hoedspruit (16.50%) and Letsitele (13.10%) was significantly different from one another and that of Patensie and Addo (Table 1). Patensie soil had a significantly higher ($P < 0.0001$) silt percentage of 26.17% than Addo (17.59%). The silt content for both Patensie and Addo soils were also significantly higher than Hoedspruit (9.84%) and Letsitele (7.67%; Table 1). There was a significant difference ($P = <0.0001$) in the percentage sand of the soil from all four areas. Letsitele had significantly the highest sand percentage of 79.23% followed by Hoedspruit (73.66%), Addo (61.21%) and Patensie (52.13%; Table 1).

Quantification of oomycetes and nematodes from orchard soils

Semi-quantitative analyses of oomycetes

Oomycete isolates were obtained from all four orchard soils and in total consisted of 91, 132, 110 and 140 isolates from the Addo, Patensie, Hoedspruit and Letsitele orchards respectively. *Pythium* spp. were the most frequently isolated oomycete in all four orchards (Figure 1). Orchard soils from Letsitele and Patensie had the highest mean percentage (>90%) leaf discs colonized by *Pythium* spp. *Phytophthora* spp. were also isolated from the soil through soil baiting. Letsitele had the highest mean percentage ($\geq 90\%$) leaf discs colonized by *Phytophthora* spp., whereas Patensie, Hoedspruit and Addo had a lower mean percentage ($\leq 40\%$) *Phytophthora* spp. colonized leaf discs (Figure 1).

RFLP analyses of oomycetes

Representative isolates obtained from soilbaiting were identified through Restriction fragment length polymorphism (RFLP) of ITS-PCR (Figure 2) amplicons and sequencing. Ten *Phytophthora* and ten *Pythium* isolates from each of the four orchards was selected for further identification based on morphology observed in non-sterile soil water extract. A total of three different RFLP groups were identified and from each RFLP group, three isolates were sequenced (Figure 2). Nucleotide sequence analyses of isolates representing the three-different oomycete PCR-RFLP groups resulted in RFLP group 1 being identified as *P. nicotianae*, RFLP group 2 as *P. citrophthora* and RFLP group 3 as *P. irregulare*. Representative oomycete isolates, obtained from soilbaiting was identified through RFLP of ITS-PCR (Figure 2) amplicons and sequencing. *Phytophthora nicotianae* (lanes 6 and 12), *Pythium irregulare* (lanes 2, 3 and 8) and *Phytophthora citrophthora* (lanes 1, 4, 5, 7, 9 to 11) was identified as the oomycetes colonising citrus leaf disks from soilbaiting (Figure 2).

Nematode extraction and quantification

Citrus nematodes *Tylenchulus semipenetrans*, were isolated from all four orchard soils. Analyses of variance (ANOVA) of mean juvenile and female counts obtained from soil and root samples from the different orchards, indicated a significant difference in mean juvenile counts between areas ($P < 0.001$; Table 2). With regards to the female counts, the ANOVA indicated no significant difference in female counts between areas ($P = 0.788$; Table 2). In Addo no analysis for females could be done due to the trees in the orchard being very small without sufficient root mass (Table 3). Between the other three areas, Patensie, Hoedspruit and Letsitele, there were no significant difference between the female counts. The counts ranged from 1720 females per 10 g roots to 2140 females per 10 g roots (Table 3). With regards to juvenile counts, the mean juvenile counts in soils from Patensie (4290) and Hoedspruit (4940) were statistically similar but significantly higher than the mean counts recorded for the Addo

(75) and Letsitele (1040) soils. The last two means were statistically similar but numerically there were a marked difference between the mean juvenile counts in the Addo and Letsitele soils (Table 3).

Glasshouse seedling bioassay trial

Seedling growth measurements

A total of 18 seedlings died during the seven-month period in the glasshouse. These seedlings were excluded from all analyses. The analyses of variance of the seedling weight and length increase indicated no significant differences between trials ($P = 0.5524$). As a result, the data from the two trial repetitions could be combined in all the analyses. Further analysis showed that there was a significant production area x treatment interaction for mean weight increase ($P < 0.0001$) and mean seedling length increase ($P = < 0.0001$; Table 4). Therefore, the data of each area was considered separately.

In Patensie, the mean seedling weight increase (1.76 g) for seedlings grown in steam pasteurized soil was statistically similar to the weight increase observed in the untreated soil (1.69 g; Table 5). However, the mean weight increase of seedlings in the soil dilution was significantly lower (0.68g) compared to the weight increases observed in both the steam pasteurized and untreated soils (Table 5). The biocide treatments performed, in terms of mean weight increase, statistically similar (mefenoxam and difenoconazole) or poorer (cadusafos) compared to the steam pasteurized treatment and the untreated soil (Table 5). The mean seedling length increase for Patensie seedlings grown in steam pasteurized soil, was 8.74 mm. This value was statistically lower than that observed for the seedlings in the untreated control (10.38 mm) but significantly better compared to the soil dilution treatment (5.51 mm; Table 5). For this measurement, mefenoxam was the only biocide that caused a mean seedling length increase that was statistically similar to the untreated control (Table 5). The mean weight increase of seedlings grown in steamed soil from Addo was 1.94 g, and was statistically different from the untreated control (1.52 g) and soil dilution (1.20 g) treatments (Table 5). Both the cadusafos (1.44.g) and difenoconazole (1.41 g) treatments performed for this measurement statistically similar to the untreated control. The mefenoxam treatment caused a significantly lower mean weight increase compared to the untreated control (Table 5). In terms of the mean length increase of seedlings grown in the Addo soil, the mean length increase for the untreated soil was 8.81 mm, statistically similar to that observed in the steam pasteurized (8.65 mm) soil and soil dilution (7.89 mm) treatments (Table 5). All three the biocides performed in terms of the mean length increase significantly poorer than the untreated control treatment (Table 5).

In the steam pasteurized soil of Hoedspruit, the mean increase in seedling weight and length (2.02g and 10.02 mm) was statistically better compared to the untreated control (1.34 g and 7.46 mm) and soil dilution (0.78 g and 6.30 mm) treatments (Table 5), respectively. In terms of mean weight increase all three biocides caused significantly lower increases compared to the untreated control (Table 5). The mean length increase caused by the mefenoxam (6.51 mm) and difenoconazole (6.21 mm) treatments were statistically similar to the untreated control while the increase seen for the cadusafos treatment (4.30 mm) was significantly lower than the untreated control (Table 5). In the steam pasteurized treated soil of Letsitele the mean seedling weight and length increases (2.12 g and 10.96 mm) was statistically higher than the untreated soil (0.86 g and 5.03 mm) and soil dilution (0.53 g and 6.52 mm) treatment (Table 5). The mean weight increase seen in seedlings from the soil dilution treatment (0.53 g) were statistically similar to that of the untreated control (0.86 g). However, in terms of mean length increase of seedlings, the soil dilution treatment had a mean (6.52 mm) length significantly better than the untreated control (5.03 mm; Table 5). In terms of mean weight increase the different biocides had statistically the same effect as the untreated control treatment (Table 5). However, the mean length increase results showed that mefenoxam (7.23 mm) had a significantly better mean length increase compared to the untreated control. The cadusafos and difenoconazole (5.32 mm) treatment had a mean length increase (4.64 mm) that was statistically the same as the untreated control (Table 5).

Isolation of fungi and oomycetes from roots

Root isolations from untreated control seedlings showed that *Fusarium solani* was the most predominant genus isolated in all four areas followed by oomycetes (*P. citrophthora*, *P. nicotianae* and *P. irregulare*) and *Fusarium oxysporum* (Figure 3). In three of the orchards (Patensie, Addo and Hoedspruit) isolates belonging to the *Fusarium solani* species complex was the most abundant species isolated (Figure 3). BLAST analyses of these isolates, showed that the isolates had 99.8% sequence identity to several Genbank accession belonging to the FSSC complex (Sandoval-Denis *et al.*, 2017) including *F. solani* (LT746338), *Neocosmospora* spp. (LT746330) (Sandoval-Denis *et al.*, 2017), *Fusarium falciformis* (KF255514) and *Fusarium* spp. (EF469980; O'Donnell *et al.*, 2007). The *F. oxysporum* isolates was confirmed with a BLAST analyses (LT746314; Sandoval-Denis *et al.*, 2017 and LT841210; Brankovics *et al.*, 2016) of 99.8% identical sites.

Nucleotide sequence analyses of isolates representing the three-different oomycete PCR-RFLP groups resulted in RFLP group 1 being identified as *P. nicotianae*, RFLP group 2 as *P. citrophthora* and RFLP group 3 as *P. irregulare*. Representative oomycete isolates, obtained from root isolations was identified through RFLP of ITS-PCR (Figure 4) amplicons and sequencing. *Phytophthora nicotianae* (lanes 1 to 16 and 20 to 36), *Pythium irregulare* (lanes 17 and 19) and *Phytophthora citrophthora* (lane 18) was identified as the oomycetes colonising citrus roots (Figure 4). The RFLP group 1 *P. nicotianae* sequences (769 to 877 bp) had 99.3% sequence similarity to published *P. nicotianae* sequences in Genbank (KT455619, KT337714, Yang and Hong, 2015; Sanahuja *et al.*, 2016). The PCR-RFLP group 2 *P. citrophthora* sequences (785 to 825 bp) had 99.7% identity to published Genbank sequences in BLAST analyses (KU877816, Das *et al.*, 2016). The PCR-RFLP group 3 *P. irregulare* isolate sequences (830 to 931 bp) from citrus roots had 99.7 % identity to *P. irregulare* (KC855076, Bahramisharif *et al.*, 2014).

Discussion

In this study, the etiology of citrus replant disease was investigated in four citrus replant orchard soils, one from each of four production areas in South Africa. The disease was shown to be caused by biological agents in three of the orchards, but not in the fourth orchard. The application of biocides (mefenoxam, cadusafos, difenoconazole) to the orchard soils was unable to conclusively show the involvement of specific groups of biological agents (oomycetes, nematodes or fungi). The exception was in the Letsitele orchard where oomycetes were likely involved. Nonetheless, several known and putative replant pathogens were found associated with citrus roots in a seedling bioassay for all four orchards. These agents included the oomycetes *P. nicotianae*, *P. irregulare* and *P. citrophthora* and fungi belonging to the FSSC and FOSC. Soil analyses of the orchards supported the presence of the three oomycetes. Juveniles of the citrus nematode *T. semipenetrans* were furthermore also identified in soil analysed from all four orchards, and thus likely also play a role in citrus replant disease in South Africa.

In the current study, the growth response (weight and length increases) of the seedlings in the bioassay (glasshouse trial) indicated that, as with apple replant (ARD) disease (Tewoldemedhin *et al.*, 2011a; Mazzola and Manici, 2012), the cause of citrus replant disease is biological in nature. This was true for three (Addo, Hoedspruit and Letsitele) orchards, but not the Patensie orchard. In the Hoedspruit and Letsitele orchards, the pasteurized control seedlings had a significantly higher increase in weight and length than the untreated control, whereas as for the Addo orchard this was only true for the weight increase. Hoestra (1968) and Sewell *et al.* (1992) indicated that weight rather than length increase is a better indication of apple replant disease severity, but that there is always a variation in severity between sites as also seen in the current study. Further support for the biological nature of citrus replant disease in South Africa is provided by the fact that for all four orchards adding 20% of the untreated soil to steam sterilised soil resulted in significantly lower increase in weight compared to the steam and untreated control treatments. This can be attributed to the re-inoculation of the pathogens and nematode containing orchard soils into the biological vacuum created by the 80% steam sterilised soil. This probably resulted in rapid growth and proliferation of the biological agents and consequently severe infection of seedling roots. For apple replant disease, a significant reduction in growth (length and weight) of the soil dilution treatment relative to the pasteurized treatment has also been reported for most replant soils. However, a significant reduction in growth of the soil dilution treatment relative to the untreated control has not been reported (Tewoldemedhin *et al.*, 2011c).

This might be due to a higher percentage of untreated soil (20%) used in the current study, than the 15% used by Tewoldemedhin *et al.* (2011c).

In the current study on citrus in South Africa, the effect on seedling growth of the biocide treatments consisting of mefenoxam, cadusafos and difenoconazole provided no support for the involvement of biological agents of citrus replant disease, with the exception of the Letsitele orchard. The Letsitele orchard was the only orchard where seedlings grown in one of the biocide treatments had a significant higher growth (length or weight) than the untreated soil. In the Letsitele soil, oomycetes were likely involved since the mefenoxam treatment seedlings had a significantly higher increase in seedling length than the untreated control. The fact that none of the biocide treatments resulted in an increase in seedling growth relative to the untreated control was unexpected, considering the association of several known citrus pathogens and parasitic nematodes with the roots and soil of all four orchards, as discussed below. A few reasons for the lack of response in improved seedling growth to the two biocide applications could firstly be that the biocide dosages were too high resulting in damage of the seedlings. For example, the cadusafos and difenoconazole treatments resulted in significantly lower height or weight increases of seedlings compared to the untreated in three of the orchards. Alternatively, it could be that the application of only one biocide resulted in the specific targeted group of pathogens being suppressed, but then resulted in the excessive proliferation of another group of pathogens that caused severe seedling damage. This has sometimes been reported in apple replant disease when management practices are applied that do not suppresses all of the biological agents involved (Mazzola and Manici, 2012). In citrus, the negative effect of some biological agents on others have been reported, and could thus result in an increase in some groups when one specific group is suppressed. For example, the suppression of nematodes by cadusafos could have resulted in an increase and more aggressive infections by *Fusarium solani*. Chandel and Sharma (1989) and El-Borai *et al.* (2002b) found that *Tylenchulus semipenetrans* suppressed the growth of *F. solani* while it also reduced host infection by this pathogen in dual inoculations. It has furthermore also been found that citrus root infections by *Phytophthora nicotianae* were reduced by root infection by *Tylenchulus semipenetrans* (Chandel and Sharma, 1989; El-Borai *et al.*, 2002a, b).

The current study did not only focus on the pathogens previously identified to be associated with citrus replant disease, but investigated the involvement of other possible pathogens. In previous studies on this topic in South Africa (Le Roux *et al.*, 1991, 1998; Cronje *et al.*, 2002) soil from fewer orchards in one production area were evaluated, and only the presence of nematodes and *Phytophthora* spp. were investigated. In the current study, *P. irregulare*, *P. nicotianae* and *P. citrophthora* together with *F. solani*, *F. oxysporum* and the citrus nematode *T. semipenetrans* was the predominant pathogen species found to be associated with citrus replant disease. In previous studies that focused on citrus replant disease, it was shown that the citrus nematode, *T. semipenetrans*, two *Phytophthora* spp. (*P. nicotianae* and *P. citrophthora*) and *F. solani* were associated with the disease complex (Baines *et al.*, 1978; Nemeček *et al.*, 1978, 1980; Labuschagne *et al.*, 1987; Le Roux *et al.*, 1998). In the current study, it is clear that *Pythium* spp. also might play a role in citrus replant disease which was not previously indicated. However, pathogenicity studies will have to be conducted. In apple replant disease, *Pythium* species are known to play an important role (Mazzola, 1998). Other apple replant pathogens such as 'Cylindrocarpon'-like fungi and *Rhizoctonia* spp. were not found to be associated with citrus replant disease in South Africa. It is possible that some of the FSSC and *Phytophthora* spp. identified in the current study, could interact in causing replant disease. Dandurand and Menge (1992) reported that the severity of citrus root rot caused by *Phytophthora nicotianae* and *P. citrophthora* were increased when co-inoculation with *Fusarium solani* was done.

This indicates that the citrus- and apple replant disease complex differs from one another but that there are similarities. Methyl bromide was previously used to fumigate soils in replant situations as it had a broad-spectrum effect on oomycetes, soilborne fungi and nematodes (Le Roux *et al.*, 1998). However, as this is no longer available, abovementioned soil survey prior to replanting gains major importance as it will determine which soil fumigants or mixture of fumigants to employ as chloropicrin and 1,3-dichloropropene does not have the same broad-spectrum efficacy (Jhala *et al.*, 2011). Ultimately the effective prevention of citrus replant disease using non-methyl bromide

fumigation is dependent on knowing what is present in the soil and making the correct decisions based on this knowledge.

Objectives

1. Identification of replant sites and the sampling of soil and roots in these orchards. At least two orchards will be identified in the Limpopo and Eastern Cape citrus producing areas of South Africa.
2. Determine if the basis for citrus replant disease is biotic or abiotic.
3. Physical and chemical analyses of replant soils.
4. Molecular and morphological characterisation of fungi and oomycetes associated with citrus replant soils.
5. Finalisation of results and trials. Writing up of research papers.

Conclusion

This study indicated that the basis of replant disease in citrus in South Africa is also biological in nature, similar to apple and peach replant disease. It was furthermore found that apart from *Pythium* and *Phytophthora* spp. and the citrus nematode, several *Fusarium* spp. are also involved in the pathogen complex associated with citrus replant disease in South Africa. This is of great relevance as it indicates that future research on replant management in citrus should focus on managing a broader complex of pathogens than previously thought.

Future research

The *Fusarium* and *Neocosmospora* spp. identified in this study represents several new reports on citrus. Pathogenicity studies with these species on citrus are therefore needed along with further characterization of the *Fusarium* and *Neocosmospora* spp. occurring in old citrus soils in other production areas.

Technology transfer

Results of this study have been presented at the SASPP 2017 congress and will also be presented at the CRI Research Symposium in 2018.

Two peer reviewed papers and one SAFJ paper will be published from this study.

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TABLES AND FIGURES

Table 1. Mean pH, phosphorous (P), calcium (Ca), magnesium (Mg), clay, silt and sand levels of sampled top soil (0-30 cm) from the four citrus producing areas of Patensie, Addo, Hoedspruit and Letsitele.

Area	pH	P (mg/kg)	Ca	Mg	Clay	Silt	Sand
		Bray 1	(mg/kg)	(mg/kg)	(%)	(%)	(%)
Patensie	5.82 b	138.62 a	2260.21 a	248.51 b	21.70 a	26.17 a	52.13 d
Addo	6.36 a	30.21 b	1181.83 b	299.12 b	21.20 a	17.59 b	61.21 c
Hoedspruit	6.60 a	4.57 c	1532.42 b	420.57 a	16.50 b	9.84 c	73.66 b
Letsitele	6.50 a	7.52 c	685.50 c	180.59 c	13.10 c	7.67 c	79.23 a
LSD	0.467	24.356	89.723	55.64	1.858	2.556	3.718
<i>P</i> -value	0.010	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in a column followed by the same letter are not significantly different ($P < 0.05$) according to Fisher's least significant difference test.

Table 2. Analysis of variance of mean juvenile and female *Tylenchulus semipenetrans* counts in sampled soil (juveniles) and roots (adult females) from the four citrus producing areas of Patensie, Addo, Hoedspruit and Letsitele.

Source	Juveniles				Females			
	DF	SS	MS	SL	DF	SS	MS	SL
Area	3	171401687,500	57133895,833	<0.001	2	924666,667	462333,333	0.788
Error	36	275353250,000	7648701,389			51845000,000	1920185,185	
Corrected Total	39	446754937,500				52769666,667		

DF Degrees of freedom

SS Sum of Squares

MS Mean Square

SL Significance level

Table 3. Mean juvenile and female citrus nematode counts from ten soil and root samples collected from four old orchards in the citrus producing areas of Patensie, Addo, Hoedspruit and Letsitele.

Citrus producing areas	<i>Tylenchulus semipenetrans</i>	
	Juvenile ^a	Females ^b
Patensie	4290.0 a ^c	2140.0
Addo	75.0 b	*
Hoedspruit	4940.0 a	1850.0
Letsitele	1040.0 b	1720.0
LSD	2508.0	1272.0

^a Mean count per 250 cm³ sampled soil

^b Mean count per 5 grams of roots

^c Means in a column followed by the same letter are not significantly different ($P < 0.05$) according to Fisher's least significant difference test.

* No females were extracted from the roots

Table 4. Analysis of variance of mean weight- and length increase of citrus seedlings grown in four putative citrus replant orchard soils subjected to six different treatments and grown for seven months under glasshouse conditions.

Source	DF	Weight increase			Length increase		
		SS	MS	SL	SS	MS	SL
Trial	1	0.1119	0.1119	0.0717	0.2751	0.2751	0.5524
Trial (Block)	2	0.1862	0.0931	0.0696	10.1700	5.0850	0.0030
Area	3	3.3769	1.1256	<0.0001	54.1685	18.0561	<0.0001
Treatment (TRT)	5	12.0491	2.4098	<0.0001	122.2909	24.4581	<0.0001
Area x TRT	15	3.9281	0.2618	<0.0001	127.7945	8.5196	<0.0001
Trial x Area	3	0.2295	0.0765	0.0876	1.5441	0.5147	0.5745
Trial x TRT	5	0.06528	0.0130	0.8486	3.0483	0.6096	0.5594
Trial x Area x TRT	15	0.3977	0.0265	0.6658	14.1776	0.9451	0.2849
Error	45	1.4815	0.0329		34.5352	0.7674	
Corrected Total	94	21.8266			368.0045		

DF Degrees of freedom
SS Sum of Squares
MS Mean Square
SL Significance level

Table 5. Mean seedling weight (g) - and length (mm) increases of citrus seedlings in response to six treatments applied to four different citrus replant orchard soils.

Treatment	Patensie		Addo		Hoedspruit		Letsitele	
	Weight (g)	Length (mm)						
Pasteurized	1.76 bc	8.74 st	1.94 ab	8.65 stu	2.02 a	10.02 qr	2.12 a	10.96 q
Untreated control	1.69 bcd	10.38 q	1.52 cdef	8.81 rst	1.34 fgh	7.46 uvw	0.86 jk	5.03 yz
Mefenoxam	1.62 cde	9.76 qrs	1.16 hi	7.25 vw	1.03 ij	6.51 wx	1.03 ij	7.23 vw
Cadusafos	1.26 ghi	8.66 stu	1.44 defg	7.41 uvw	1.03 ij	4.30 z	0.69 kl	4.64 z
Difenoconazole	1.55 cdef	7.35 vw	1.41 efgh	7.99 tuv	0.76 kl	6.21 wxy	0.61 kl	5.32 xyz
Soil dilution ^a	0.68 kl	5.51 xyz	1.20 ghi	7.89 tuv	0.78 jkl	6.30 wx	0.53 l	6.52 wx
LSD ^b	0.2602	1.2563						
P-value	<0.0001	<0.0001						

^a The soil dilution treatment consisted of diluting 20% of the untreated control soil into pastuerized soil.

^b t-LSD (least significant difference) was calculated at a 95% significance level.

Mean seedling weight (g) and length (mm) increase of Carrozo citrange rootstock seedlings were determined 7 months after trial establishment in the four citrus replant soils receiving the different treatments. The data is the average of two experiment, with each treatment containing twenty replicates. Means in columns and rows followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

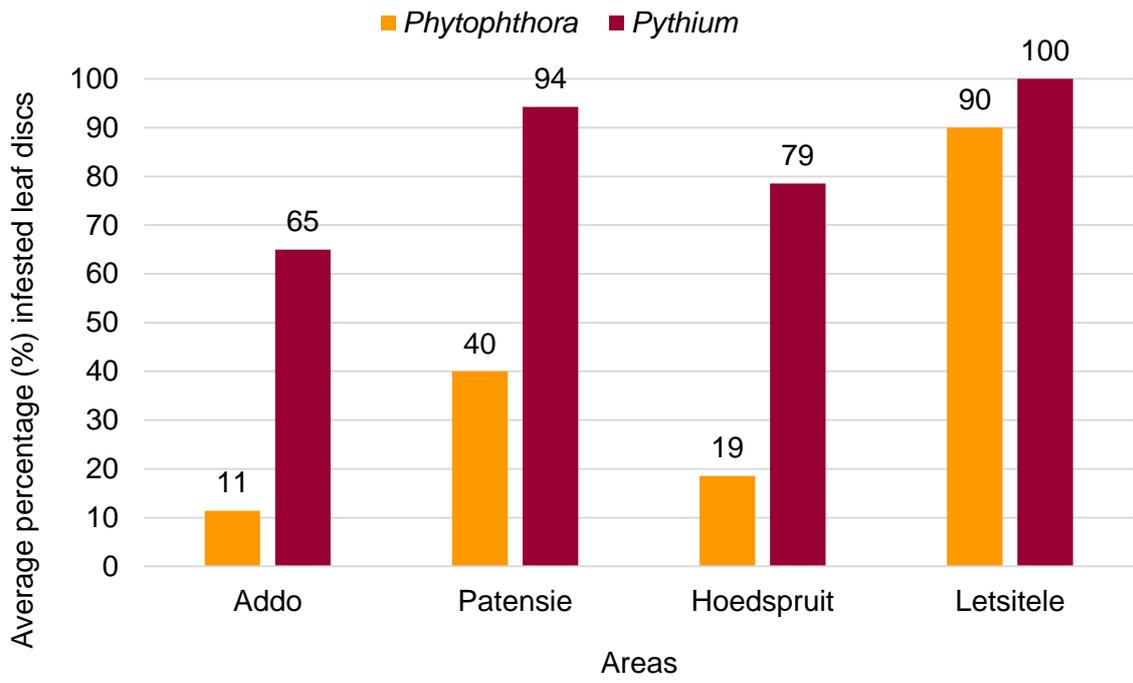
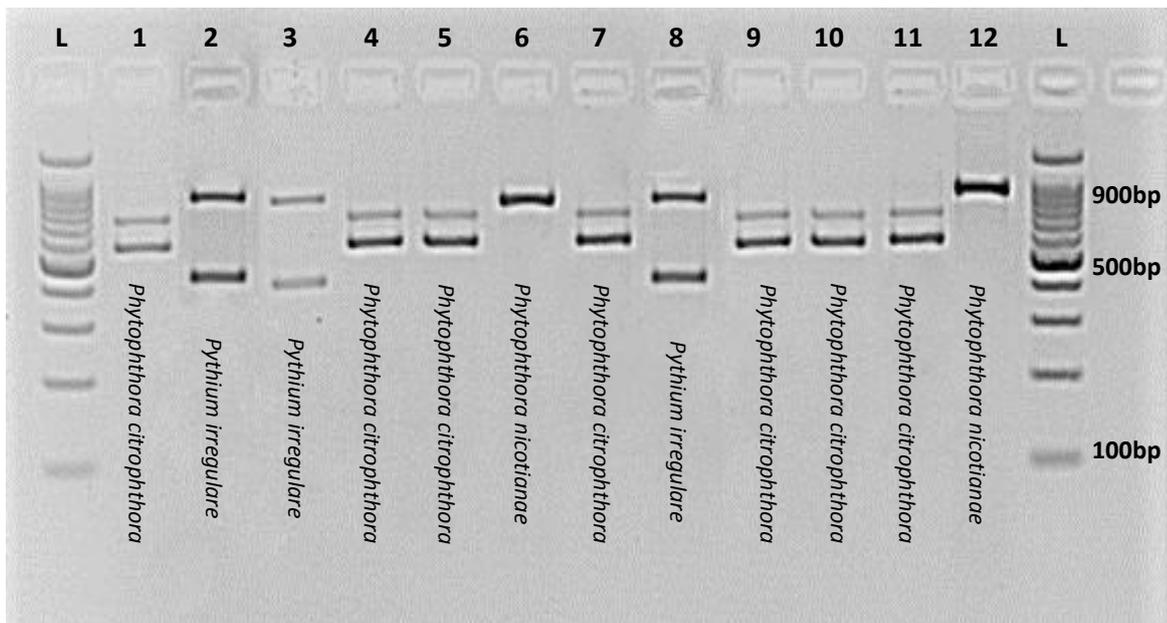


Figure 1. Average percentage (%) infested leaf discs following soilbaiting of citrus replant orchard soils collected from four major citrus producing areas.



Figure

2. Restriction fragment length polymorphism (RFLP) analysis of the ITS PCR amplicons obtained from oomycete isolates from soilbaiting. Lanes 1, 4, 5, 7, 9 to 11 are *Phytophthora citrophthora* (two DNA fragments, 812-bp and 608-bp). Lanes 2, 3 and 8 represent *Pythium irregulare* with two DNA fragments one 920-bp and one 480-bp. *Phytophthora nicotianae* are represented by lane 6 and 12 with only one DNA fragment of 980-bp in size. The size fragments of a 100-bp DNA ladder are shown on the right and left side of the figure (lanes marked L).

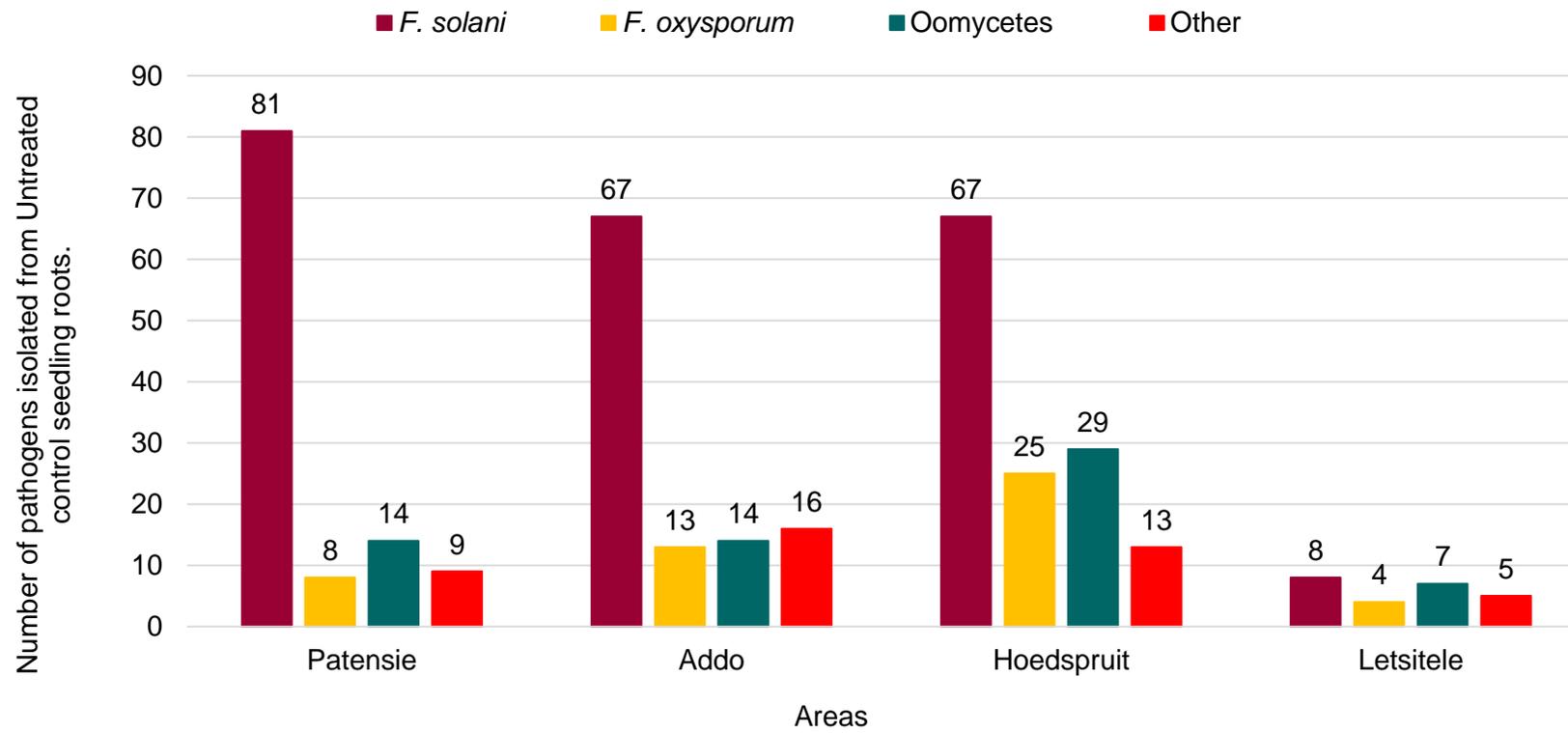


Figure 3. The number of isolates of oomycete and fungal pathogens isolated from citrus seedling roots grown in untreated control replant soil for seven months.

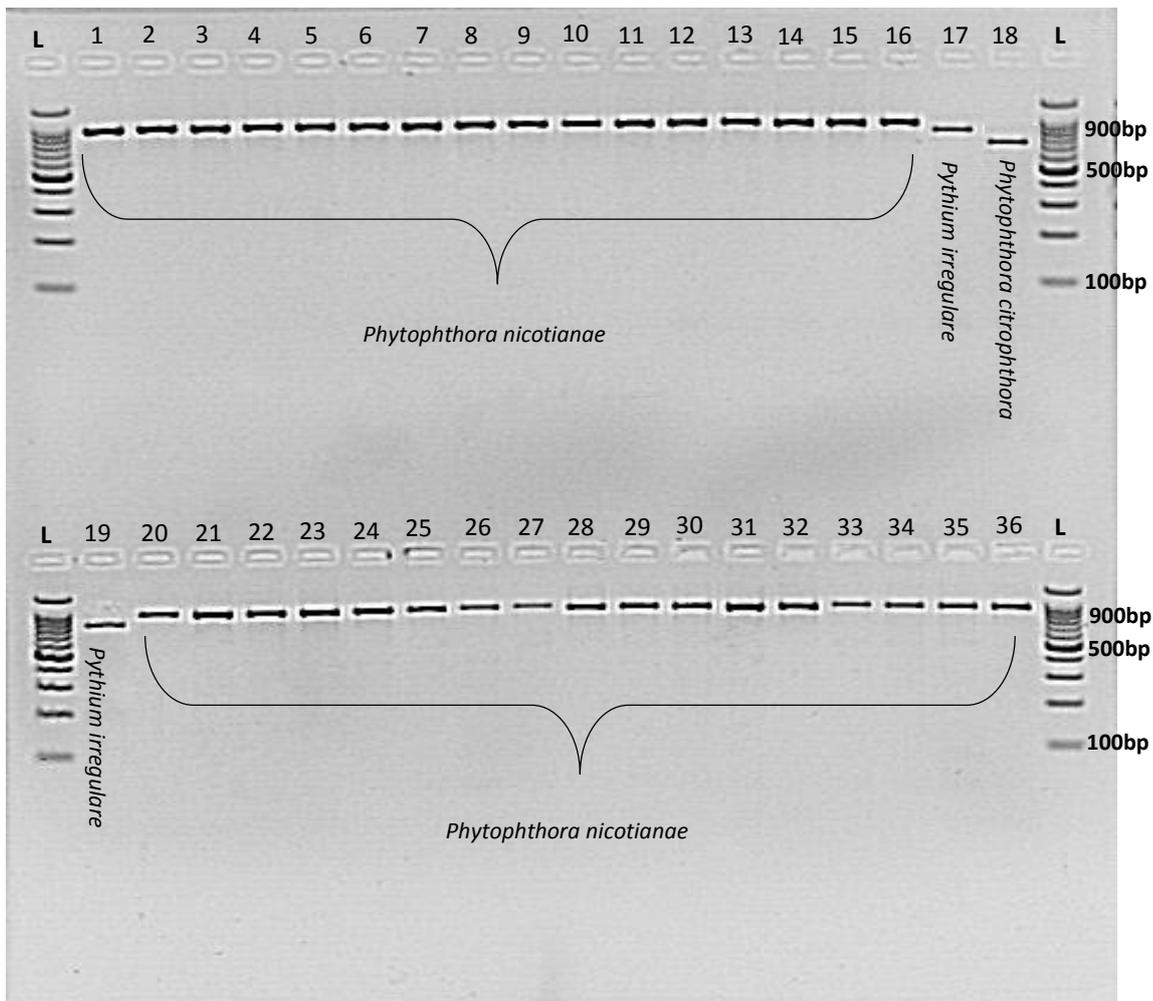


Figure 4. Restriction fragment length polymorphism (RFLP) analysis of the ITS PCR amplicons obtained from oomycete isolates from root isolations. Lanes 1 to 16 and 20 to 36 are *Phytophthora nicotianae* (one DNA fragment, 1000-bp). Lanes 17 and 19 represent *Pythium irregulare* with one DNA fragments 910-bp. *Phytophthora citrophthora* are represented by lane 18 with only one DNA fragment of 815-bp in size. The size fragments of a 100-bp DNA ladder are shown on the right and left side of the figure (lanes marked L).

Summary

Phylogenetic analyses were used in this study to determine the phylogenetic species identity and genetic diversity of '*Fusarium solani*' Species Complex (FSSC) and the *Fusarium oxysporum* Species Complex (FOSC) isolates from four different citrus production areas (Addo, Patensie, Hoedspruit and Letsitele) in South Africa. The isolates (13 *F. oxysporum* and 39 '*F. solani*') were obtained from a previous citrus replant study (Chapter 1), which preliminarily identified the isolates as belonging to these two species complexes. The phylogenetic species identity of the isolates were determined separately for each species complex, using a concatenated multi-gene phylogeny of the translation elongation factor 1-alpha (TEF) and RNA polymerase II second largest subunit (RPB2) gene regions. Phylogenetic analyses of only the TEF region, which has traditionally been used for identification of these fungal groups, were also conducted for each species complex. The multi-gene phylogeny of the FSSC isolates showed that the citrus isolates grouped into four clades including a *Neocosmospora solani* clade, *Neocosmospora croci* clade, an unnamed *Fusarium* spp. clade with *F. falciformis* as the most related known *Fusarium* spp., and another clade containing an unnamed *Fusarium* species. The citrus *Fusarium* spp. isolates that were related to *F. falciformis* also represent a putative new species. The citrus *N. croci* isolate was only obtained from Addo production area. The most widely distributed FSSC species from citrus was *N. solani*, which occurred in all four production areas. The TEF phylogeny of the FOSC isolates resulted in a better resolution and support of clades than the multi-gene phylogeny. According to the TEF phylogeny, all the citrus FOSC isolates grouped within the *F. oxysporum* phylogenetic species II. The FOSC citrus isolates were furthermore distributed among two subclades, previously designated as Clade 3 and Clade 4 by O'Donnell et al. (1998, 2004). Both clades contained isolates from Patensie, Addo, Hoedspruit and Letsitele citrus production areas.

Introduction

The Ascomycota is a large and important group of fungi, characterised and distinguished from other fungi by a saclike ascus carrying haploid ascospores (Alexopoulos *et al.*, 1996). These fungi consist of over 32 000 species and form symbiotic, parasitic and saprobic relationships with both animals and plants (Hawksworth *et al.*, 1995; Alexopoulos *et al.*, 1996). The genus names of some members within the Ascomycota have recently changed. One of these include a change of some members of the genus *Fusarium* to the genus *Neocosmospora*. The genus name change was due to the fact that many genera within the *Nectriaceae* family were previously poorly characterised due to a lack of DNA sequence data and were thus solely identified based on phenotypic characters. These characters included, uniloculate ascomata that are yellow, orange-red to purple, with phialidic asexual morphs (Rossman *et al.*, 1999). The identification of *Fusarium* species at the morphological level is based on distinctive characters such as the shape and size of the macro- and microconidia (Leslie and Summerell, 2006). The genus name change of some members of *Fusarium* to *Neocosmospora* were based on a multi-gene phylogenetic analyses [translation elongation factor (TEF), internal transcribed spacer gene (ITS), RNA polymerase II second largest subunit (RPB1 and RPB2), the large subunit of the ATP citrate lyase (*acl1*), α -actin (*act*), β -tubulin (*tub2*), calmodulin (*cmdA*), histone H3 (*his3*) and the nuclear large subunit 28S rDNA (NLSU) gene region] conducted for all available type and authentic strains of the known genera in *Nectriaceae*, as well as for genera of which no sequence data were previously available. These studies showed that the genus *Neocosmospora* contained members of some *Fusarium* spp., including some '*F. solani*' isolates, which were distinct from the genus *Fusarium* (Geiser *et al.*, 2013; O'Donnell *et al.*, 2013; Lombard *et al.*, 2014, 2015)

Members of the '*Fusarium solani*' Species Complex (FSSC) are known as plant, human and animal pathogens (O'Donnell *et al.*, 2008) and are frequently isolated from soil and mainly acts as decomposers. Some species act as parasites on plants, insects, humans and animals (Booth, 1971). '*Fusarium solani*' is known to be associated with the roots of symptomless as well as declining citrus trees and are commonly found in citrus soils (Labuschagne *et al.*, 1987; Smith *et al.*, 1988). Characteristic symptoms of infection on citrus includes colonisation and discoloration of the cortical tissue of feeder roots (Adesemoye *et al.*, 2011). Aboveground

symptoms are evident as leaves that turn yellow, dieback and wilting of branches and the overall weakening of the tree with reduced fruit quality (Adesemoye *et al.*, 2011). '*Fusarium solani*' was classified into the section Martiella by Booth, (1971) and can be divided into 50 sub-specific lineages based on the molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex (O'Donnell, 2000a).

At the phylogenetic level, there are many differences among the members of the FSSC. Three clades have been identified in the FSSC based on translation elongation factor (TEF), internal transcribed spacer gene (ITS) and nuclear large subunit 28S rDNA (NLSU) phylogenies (O'Donnell *et al.*, 1998, 2008; O'Donnell, 2000b; Nalim *et al.*, 2011). Clade 1 includes two known species, *Fusarium illudens* and *Nectria plagianthi*, members of Clade 2 consists of pathogens that cause sudden death syndrome on soy-bean (Aoki *et al.*, 2003, 2005, 2012). A study done by Nalim *et al.*, (2011) showed that members of Clade 2 are paraphyletic. Clade 3 is known to contain the most common *Fusarium* spp. associated with plant diseases and include *Fusarium falciformis* and *Fusarium keratoplasticum* (O'Donnell, 2000a; Zhang *et al.*, 2006; O'Donnell *et al.*, 2008; Short *et al.*, 2013, 2014). The most haplotype-diverse species were also placed within Clade 3 (O'Donnell *et al.*, 2008; Nalim *et al.*, 2011; Short *et al.*, 2014).

The *Fusarium oxysporum* Species Complex (FOSC) is known as an anamorphic species and is a widespread fungus found world-wide (Kistler, 1997; Leslie and Summerell, 2006). It contains both pathogenic and non-pathogenic isolates (Gordon and Martyn, 1997). Pathogenic isolates of *F. oxysporum* usually cause Fusarium wilt on several agricultural crops and are divided into *formae speciales* (f. sp.) based on their host range, and may be further subdivided into pathogenic races (Hawksworth *et al.*, 1995; O'Donnell and Cigelnik, 1999). The close association with plant roots, and the ability of pathogenic isolates to colonise the root cortex and xylem vessels leads to characteristic wilting symptoms by limiting water movement through the plant (Beckman and Roberts, 1995). Pathogenic and non-pathogenic strains of *F. oxysporum* can be found in many native plant groups, in soils that have never been cultivated as well as in agricultural soils throughout the world (Gordon and Martyn, 1997; Gordon *et al.*, 1992). *F. oxysporum* isolates from uncultivated soil and native plants are known to be closely associated with plant roots, but are most often non-pathogenic to plants in native soils, even when high populations are present in some areas (Booth, 1971; Armstrong and Armstrong, 1978). *Fusarium oxysporum* is considered as a minor disease of citrus and only pathogenetic toward woody hosts under adverse conditions or when the plant is stressed by environmental conditions in South Africa (Labuschagne *et al.*, 1987).

The taxonomy of *F. oxysporum* was previously based on the morphology of the asexual reproductive structures. The limited variability of these characters led to a broad description of *F. oxysporum* (Snyder and Hansen, 1940), which did not reflect the inherent variability within the species complex (Kistler, 1997). There are to date more than 70 described *formae speciales* (f. sp) causing vascular wilt in over 100 plant species (Gordon and Martyn, 1997). Phylogenetic analyses have shown that many *forma speciales* are polyphyletic or paraphyletic, meaning that it is derived from more than one common evolutionary ancestor or ancestral group (O'Donnell *et al.*, 1998; Skovgaard *et al.*, 2001). Previous phylogenetic studies, based on the translation elongation factor 1- α (TEF) and the mitochondrial small subunit rDNA (*mtSSU*) loci showed that *F. oxysporum* consist of three clades, designated as Clades 1, 2, and 3 (O'Donnell *et al.*, 1998; 2004). Subsequently, a fourth clade was defined within the FOSC, with the addition of clinical isolates found by O'Donnell *et al.* (2004). The four clades were further divided into two phylogenetic species, PS I and PS II (Laurence *et al.*, 2014). Phylogenetic species II (PS II) consists of Clades 2 to 4, and Phylogenetic species I (PS I) only consists of Clade 1 (Laurence *et al.*, 2014).

This study focused on the phylogenetic analyses of FOSC and FSSC isolates found in citrus orchard soils in South Africa, using DNA sequence data of the TEF and RNA polymerase second largest subunit (RPB2) gene regions. The isolates used in this study were collected from citrus root isolations in a previous study, where fungi associated with citrus replant disease were investigated. Preliminary Blast analyses of TEF sequences of the isolates showed that the isolates likely belonged to the FOSC and FSSC (Chapter 1).

Materials and methods

Isolate collection

An isolate collection of 13 *F. oxysporum* and 39 '*F. solani*' isolates (Table 1) from a previous study (Chapter 1) were used in this study. The isolates were obtained through a seedling bioassay with Carrizo citrange seedlings using soil from four different citrus orchards located in the Patensie, Addo, Hoedspruit and Letsitele citrus production areas in South Africa (Chapter 1).

DNA extraction

DNA of the selected *Fusarium* isolates was extracted from 3-week-old fungal cultures grown on 2% potato dextrose agar (Difco, Becton, Dickinson and Company) amended with 1 mL streptomycin sulphate solution (40 mg/L, Calbiochem, Merck) (PDA+s). The DNA extraction protocol of Osmundson *et al.* (2013) was used, with some modifications as described in a previous study (Chapter 1).

Polymerase chain reaction (PCR) and electrophoresis

Two gene regions, including the TEF and RNA polymerase II second largest subunit (RPB2) regions, were amplified and sequenced for the selected *Fusarium* isolates. The TEF 1- α and RPB2 sequences of the *Fusarium* isolates were obtained in a previous study (Chapter 1). A fragment length of approximately 700 bp was amplified for the TEF gene region. For the RPB2 region, two separate but adjacent and overlapping regions within the RPB2 gene were amplified, using two primer pairs in separate PCR reactions. The first primer pair consisted of the RPB2-5F2 primer (O'Donnell *et al.*, 2008; O'Donnell *et al.*, 2010) and the fRPB2-7R primer (Lui *et al.*, 1999). The second primer pair consisted of primers fRPB2-7F and fRPB2-11aR (Lui *et al.*, 1999). The length of the fragment amplified with the first primer pair (RPB2-5F; fRPB2-7R) was 1186 to 2336 bp and for the second primer pair (fRPB2-7F; fRPB2-a11R) it was 2317 to 3314 bp (primers indicated in Table 2). This resulted in a total RPB2 fragment length of approximately 3841 bp. The PCR reaction and conditions was followed in the same manner as described previously (Chapter 1). The PCR products were separated by gel electrophoresis on a 1.5% (w/v) SeaKem® LE agarose gel (Lorenza Rockland, ME USA) in TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.5).

Sequencing of PCR products

The PCR products were purified using the MSB Spin PCRapase Kit (Invitex, Berlin, Germany), according to manufacturer's instructions. The PCR products sequenced (both directions) by the Central Analytical Facility (CAF) of Stellenbosch University. The sequencing reactions were conducted using the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, United States) according to manufacturer's protocol. The sequencing primers were the same as those described in the previous section for amplifying each of the gene regions. The sequence products were treated with sodium dodecyl sulfate (SDS) and transferred onto Sephadex columns (Princeton Scientific) using a Tecan Freedom EVO 150 (Biorad, Germany) and centrifuged. The nucleotide order of samples was read in an ABI 3130xl DNA sequencer (Applied Biosystems, Foster City, California, United States) using a 50cm capillary array and POP-7 (Applied Biosystems, Foster City, California, United States).

For each gene region (TEF and RBP2), forward and reverse sequences for each of the FSSC and FOSC isolates were aligned and edited in Geneious R 9.1.8 (Biomatters Ltd., Auckland, New Zealand) and a consensus sequence was constructed. Consensus sequences were run through the Basic Local Alignment Search Tool (BLAST) of the National Centre of Biotechnology Information's nucleotide database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the *Fusarium* ID database (Geiser *et al.*, 2004) to confirm identity. Several reference sequences representing different '*F. solani*', *F. oxysporum* and closely related species downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>), were selected based on previous published phylogenetic trees. Several '*F. solani*' sequences from a phylogenetic study by Sandoval-Denis *et al.* (2017) related to *Fusarium* spp. on citrus were also used in this study. Sequences from each phylogenetic species within the FOSC representing the four clades were also included in the phylogenetic dataset.

Phylogenetic analysis of FSSC and FOSC isolates

Separate phylogenetic analyses were conducted for the FSSC and FOSC isolates. For each species complex, a single gene phylogeny of the TEF region was conducted using maximum parsimony (MP) analysis and maximum likelihood (ML) analyses. A concatenated phylogeny consisting of the combined datasets of TEF and RPB2 was conducted using only ML analysis.

Phylogenetic analyses of FSSC isolates

The FSSC phylogenetic analysis of only the TEF data set was carried out by first aligning the TEF sequence data set in MAFFT v7.017 (Kato *et al.*, 2002; Kato and Standley, 2013; <http://mafft.cbrc.jp/alignment/server/>), followed by editing in MEGA v7.0 (Kumar *et al.*, 2016). A MP analysis was conducted in PAUP v4. The heuristic search option with ten random taxon additions, tree bisections and reconstruction (TBR) was used as branch swapping algorithm. All characters were unordered and of equal weight, and gaps were treated as missing data. A bootstrap analysis of 1 000 heuristic search replicates was performed to estimate the reliability of inferred phylogenies. A ML analysis for the same dataset was conducted using PhyML v3.0 (Guindon *et al.*, 2010; <http://www.atgc-montpellier.fr/phyml/>). The settings used for bootstrap were the same as that used for the MP analysis. The best-fit model was inferred using the Smart Model Selection (SMS) model test program (v1.8.1). The general time reversible model with gamma distribution and proportion invariable sites (GTR+G+I) was selected as the best-fit model for nucleotide substitution in MP analyses. Both the gamma distribution parameter and proportion of invariable sites were estimated. Bootstrap values were based on 1 000 repetitions and clades with bootstrap support $\geq 60\%$ were considered significant with good support (Hillis and Bull, 1993; Figure 1).

The concatenated phylogenetic tree of the TEF and RPB2 regions for FSSC was conducted by first aligning and editing the data in the same way as described for the TEF data set. A ML analysis was conducted using PhyML v3.0 (Guindon *et al.*, 2010; <http://www.atgc-montpellier.fr/phyml/>) and bootstrap values were based on 1 000 repetitions and clades with bootstrap support $\geq 60\%$ were considered significant (Hillis and Bull, 1993; Figure 2). The outgroup for both the concatenated and single gene phylogenetic tree analysis consisted of two isolates, *Nectria illudens* (NRRL 22090; O'Donnell, 2000a) and *Nectria plagianthi* (NRRL 22632; O'Donnell, 2000a) (Sandoval-Denis *et al.*, 2017).

Phylogenetic analysis of FOSC

The alignment, MP and ML analyses for the FOSC isolates were conducted as described for FSSC. The TEF dataset for FOSC isolates used the Tamura-Nei (TN93+G) substitution model, where the gamma distribution parameter was estimated and the proportion of invariable sites were fixed. The conditions used for bootstrapping were the same as those used for MP analysis and clades with bootstrap support $\geq 60\%$ were considered significant and highly supported (Hillis and Bull, 1993; Figure 3). The outgroups for the TEF dataset consisted of two isolates, *Fusarium commune* (NRRL 22903; Skovgaard *et al.*, 2001) and *Fusarium circinatum* (NRRL 25331; O'Donnell, 2000b). A multi-locus concatenated phylogenetic tree for the FOSC isolates was prepared in the same way as described for *F. solani*, using ML analysis. The outgroup consisted of two isolates, *Fusarium polyphialidicum* (NRRL 13459; O'Donnell *et al.*, 2007) and *Fusarium concolor* (NRRL 25728; O'Donnell *et al.*, 2010; Figure 4).

Results

Phylogenetic analyses

The MP and ML analyses of the TEF datasets yielded similar phylogenetic tree topologies for both the FSSC and FOSC isolates and the concatenated multi-gene phylogenies were only analysed using ML. Bootstrap values for both analyses are indicated on the TEF trees (Figure 2 and 4).

Phylogenetic analyses of FSSC isolates

The multi-gene phylogeny of the FSSC isolates grouped into four clades including a *N. solani* clade (25 isolates), *N. croci* clade (one isolate), an unnamed *Fusarium* spp. clade (13 isolates) with *F. falciformis* as the most related known *Fusarium* spp., and another clade (one isolate) containing an unnamed *Fusarium* species (Figure 1). The latter citrus isolate (STEU 8454) was obtained from Patensie, and may represent a putative new species. The citrus *Fusarium* spp. (13 isolates; 91% bootstrap support) that were related to *F. falciformis* also represent a putative new species and were obtained from Hoedspruit and Letsitele production regions. Isolate STEU 8462 obtained from Addo, grouped with the ex-type *N. croci* sequence. The most widely distributed FSSC species from citrus, which occurred in all four production areas grouped with the ex-epitype strain (100% bootstrap support) of *N. solani* (NRRL 66304^{ET}) (Sandoval-Denis *et al.*, 2017).

The TEF tree differed from the concatenated tree in a few instances. In the TEF tree, the clade containing the 13 FSSC isolates closely related to the *F. falciformis* reference sequences also had relative high bootstrap support (91%), but the *F. falciformis* reference sequences were unresolved (Figure 2). The sequences of STEU 8462 (Addo orchard) and the ex-type *N. croci* sequences were unresolved in the TEF phylogeny, but not in the multi-gene phylogeny. The clade containing the *N. solani* isolates differed in the TEF and concatenated trees, since in the TEF tree isolate STEU 8455 (Addo orchard) grouped with low bootstrap support (87%) with an unknown *Fusarium* spp. (NRRL46703), and not with the *N. solani* clade as in the concatenated tree. Furthermore, in the TEF tree STEU 8454 (Patensie orchard) grouped with the *N. solani* clade, unlike in the multi-gene tree (Figure 1 and 2).

Phylogenetic analysis of FOSC

The 13 FOSC citrus isolates all grouped into phylogenetic species PS II within the FOSC, which was previously described by Laurence *et al.* (2014) (Figure 3 and 4). The isolates furthermore grouped into two of the four subclades described by O'Donnell *et al.* (1998; 2004). The concatenated tree of FOSC did not provide bootstrap support for most of the clades and sub-clades as described by Laurence *et al.* (2014) and O'Donnell *et al.* (1998, 2004) (Figure 4). This can be since several gene regions were used to define the four clades described by Laurence *et al.* (2014). Due to the availability of TEF sequence data, a single gene phylogeny was conducted to compare the data with the multi-gene phylogeny for variability. For example, the clade 3 isolates within PSS II of the multi-gene phylogeny had low bootstrap support (81%) and included several sequences that did not group with this clade in the TEF phylogeny. Therefore, the TEF tree will be discussed in detail (Figure 3).

In the TEF phylogeny, two of the 13 citrus FOSC isolates (STEU 8492 and STEU 8508) grouped into clade 4 (99% bootstrap) within *F. oxysporum* PS II clade (99% bootstrap); both isolates were from Hoedspruit. The most closely related species to these two citrus isolates was *F. oxysporum* f. sp. *passiflorae* (BRIP28044) (Rooney-Latham and Blomquist, 2001; Cizislawski *et al.*, 2017). The remaining 11 of the 13 FOSC citrus isolates grouped into a well-supported (86% bootstrap) clade known as Clade 3 (88% bootstrap; O'Donnell, 1998, 2004) within the PS II clade (73% bootstrap) (Figure 3). A subclade with 65% bootstrap support contained three citrus STEU isolates all from Hoedspruit (STEU 8499, STEU 8512 and STEU 8494), along with some *Fusarium* spp. and *F. oxysporum* f.sp. sequences. There are good bootstrap support values (91%) that citrus isolates grouped within clade 3 of the FOSC and low bootstrap values was observed within clade 3 between the citrus isolates and representative isolates. The STEU 8516 (Letsitele orchard) isolate and STEU 8491 (Addo orchard), also within clade 3, grouped in two sub-clades that were distinct from the other citrus FOSC isolates within clade 3 (Figure 3).

Discussion

Citrus is an important agricultural crop and is affected by a range of fungal pathogens, including *Fusarium* spp. that are known to be associated with a variety of symptoms, including dry root rot. Phylogenetic analyses were used in this study to evaluate the diversity and determine the phylogenetic species identity of *Fusarium* spp. isolates from the roots of citrus seedlings grown in soils obtained from old citrus orchards in South Africa. To our knowledge this is the first study to investigate the phylogenetic diversity of both the FSSC and FOSC associated with citrus in South Africa. The citrus seedlings from which isolations were made showed symptoms of citrus replant disease. Preliminary identification of the isolates in a previous study (Chapter 1) indicated that the isolates grouped within the FSSC and FOSC.

Phylogenetic analyses of the citrus associated FSSC and FOSC isolates from South Africa, confirmed that the isolates belonged to these two species complexes. The isolates furthermore represented some known and putative new species in the genera *Fusarium* and *Neocosmospora*. A concatenated multi-gene phylogeny of the FSSC isolates provided a better resolution of clades than the TEF phylogeny. In the multi-gene FSSC isolates showed citrus isolates (23 isolates) mostly belonged to *N. solani* s.s. The second largest group of FSSC citrus isolates (13 isolates) may represent a putative new species, with the most closely related known species being *F. falciformis*. The STEU 8454 sequence grouped with high bootstrap support in a clade related, but distinct from *N. croci*. The TEF phylogeny of the FOSC isolates provided a better resolution of clades than the multi-gene phylogeny. The 13 FOSC isolates from citrus all belonged to *F. oxysporum* PS II previously described by Laurence *et al.* (2014). Most of the citrus isolates (11), furthermore, belonged to FOSC Clade 3 described by O'Donnell *et al.* (1998, 2004), whereas only two isolates belonged to Clade 4 of O'Donnell *et al.* (1998, 2004).

The thirteen citrus FSSC isolates from South Africa most closely related to *F. falciformis*, may represent a putative new species. The isolates had a restricted occurrence and were mainly found in the Hoedspruit area (12 isolates), whereas only one isolate was obtained was from the Letsitele production area. The *F. falciformis* sequences (DQ247075, DQ24713) to which the citrus isolates were related to were from isolates that were isolated from sand and from a human in the USA. *Fusarium falciformis* has been associated with infections in both humans and in plants, but the severity and pathogenicity toward plants are unknown (Zhang *et al.*, 2006). *Fusarium falciformis* has never before been associated with citrus roots.

Neocosmospora croci is a recently described species (Sandoval-Denis *et al.*, 2017). This species along with FSSC isolates related to it, is associated with citrus in South Africa and Italy. In Italy, *N. croci* was also associated with citrus in Catania, and specifically orchards with dry root rot symptoms (Sandoval-Denis *et al.*, 2017). *Neocosmospora croci* belongs to FSSC Clade 3, which was described by O'Donnell *et al.* (1998, 2008). FSSC Clade 3, contains a group of important plant pathogens and human and animal opportunistic parasites (O'Donnell *et al.*, 2008; Schroers *et al.*, 2016). The morphological characteristics of *N. croci* are similar to those of the FSSC isolates. It can be distinguished from *N. solani* by the presence of a saffron diffusible pigment at 36°C and slower growth on artificial media (Schroers *et al.*, 2016).

Most of the citrus associated FSSC isolates (23) from South Africa belonged to *N. solani*. These isolates were widely distributed and occurred in all four of the investigated production areas. This is the first study, subsequent to the transfer of '*F. solani*' to *Neocosmospora* to report this specie associated with citrus. *Neocosmospora solani* belongs to the FSSC clade 3 (O'Donnell, 2000a; O'Donnell *et al.*, 2008; Sandoval-Denis *et al.*, 2017). FSSC clade 3 is known to contain *Fusarium falciformis* and *Fusarium keratoplasticum* (Zhang *et al.*, 2006; O'Donnell *et al.*, 2008; Short *et al.*, 2013, 2014). The two citrus FOSC isolates from South Africa in Clade 4 (O'Donnell *et al.*, 1998, 2004) was only obtained from the Hoedspruit and Letsitele areas, whereas the other 11 FOSC citrus isolates from Clade 3 were from all four production regions. The two clades indicate that the isolates are polyphyletic and define evolutionary events. The clades are not informative in terms of pathogenicity towards citrus. The citrus FOSC isolates were related to several *Fusarium oxysporum* and *F. oxysporum* f.sp. sequences in the TEF phylogeny. It is known that housekeeping genes such as TEF, cannot differentiate between non-pathogenic *F. oxysporum* isolates and pathogenic *F. oxysporum formae speciales*. Therefore, the grouping of some of the citrus isolates with specific *F. oxysporum formae speciales* sequences, does not provide an indication of their pathogenicity (Leslie and Summerell, 2006).

The results from this study indicated that the *Fusarium* isolates collected from citrus soils in South Africa were phylogenetically much more diverse than previously thought. The thirteen isolates representing putative new species within the FSSC and closely related to *Fusarium falciformis* should be further investigated. The *N. croci*, *N. solani* isolate and the FSSC isolate related to it, will be of particular interest in pathogenicity assays, since it has been associated with citrus dry rot in Italy (Sandoval-Denis *et al.*, 2017). Morphological studies of all of the citrus isolates and pathogenicity testing should be done to determine whether the isolates are pathogenic to citrus. Future studies should furthermore focus on other citrus production areas such as the

Western Cape and Mpumalanga to identify species in these areas and compare it to the findings in this study. The current study only included isolates from the Eastern Cape and Limpopo province.

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Tables and figures

Table 1. *Fusarium* and *Neocosmospora* isolates originating from four citrus production areas (Patensie, Addo, Hoedspruit and Letsitele) used in phylogenetic studies.

Strain number	Species name	Province	Production area
STEU 8448 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8449 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8450 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8451 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8452 x	<i>Fusarium</i> sp.	Limpopo	Letsitele
STEU 8453 *	<i>Fusarium</i> sp.	Eastern Cape	Patensie
STEU 8454 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8455 #	<i>Fusarium</i> sp.	Eastern Cape	Addo
STEU 8456 ^	<i>Neocosmospora solani</i>	Limpopo	Hoedspruit
STEU 8457 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8458 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8459 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8460 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8461 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8462 #	<i>Neocosmospora croci</i>	Eastern Cape	Addo
STEU 8463 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8464 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8465 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8466 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8467 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8468 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8469 ^	<i>Neocosmospora solani</i>	Limpopo	Hoedspruit
STEU 8470 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8471 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit

STEU 8472 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8473 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8474 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8476 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8477 ^	<i>Fusarium</i> sp.	Limpopo	Hoedspruit
STEU 8478 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8479 *	<i>Neocosmospora solani</i>	Eastern Cape	Patensie
STEU 8480 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8481 ^	<i>Neocosmospora solani</i>	Limpopo	Hoedspruit
STEU 8482 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8483 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8484 #	<i>Neocosmospora solani</i>	Eastern Cape	Addo
STEU 8486 x	<i>Neocosmospora solani</i>	Limpopo	Letsitele
STEU 8487 ^	<i>Neocosmospora solani</i>	Limpopo	Hoedspruit
STEU 8488 ^	<i>Neocosmospora solani</i>	Limpopo	Hoedspruit
STEU 8489 #	<i>Fusarium oxysporum</i>	Eastern Cape	Addo
STEU 8491 #	<i>Fusarium oxysporum</i>	Eastern Cape	Addo
STEU 8492 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8494 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8499 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8508 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8510 *	<i>Fusarium oxysporum</i>	Eastern Cape	Patensie
STEU 8511 #	<i>Fusarium oxysporum</i>	Eastern Cape	Addo
STEU 8512 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8514 x	<i>Fusarium oxysporum</i>	Limpopo	Letsitele
STEU 8515 x	<i>Fusarium oxysporum</i>	Limpopo	Letsitele
STEU 8516 ^	<i>Fusarium oxysporum</i>	Limpopo	Hoedspruit
STEU 8517 x	<i>Fusarium oxysporum</i>	Limpopo	Letsitele

-
- * Representing STEU isolates from Patensie production area
 - # Representing STEU isolates from Addo production area
 - x Representing STEU isolates from Letsitele production area
 - ^ Representing STEU isolates from Hoedspruit production area

Table 2. Primers, primer sequences and annealing temperatures used as putative molecular markers for the identification of *Fusarium* and *Neocosmospora* species.

Target area	Primers	Sequence	Annealing Temp (°C)	References
TEF	EF1 / EF2	ATGGGTAAGGARGACAAGAC / GGARGTACCAGTSATCATGTT	55	O'Donnell <i>et al.</i> , 1998
RPB2	RPB2-5F2 / RPB2-7R	GGGGTGACCAGAAGAAGGC / CCCATRGCTTGYTTRCCCAT	55	O'Donnell <i>et al.</i> , 2008; 2010 Lui <i>et al.</i> , 1999
	RPB2-7F / RPB2-11aR	ATGGGYAARCAAGCYATGGG / GCRTGGATCTTRTCRTCSACC	55	Lui <i>et al.</i> , 1999



Figure 2. Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic tree of *Fusarium* and *Neocosmospora* species which was based on translation elongation factor 1-alpha (TEF) sequence data. Bootstrap support values were calculated from 1000 replicates and bootstrap support of 60% and higher are shown. *Nectria illudens* and *Nectria plangianthi* was used as the outgroups. Isolates obtained in this study are indicated in bold.

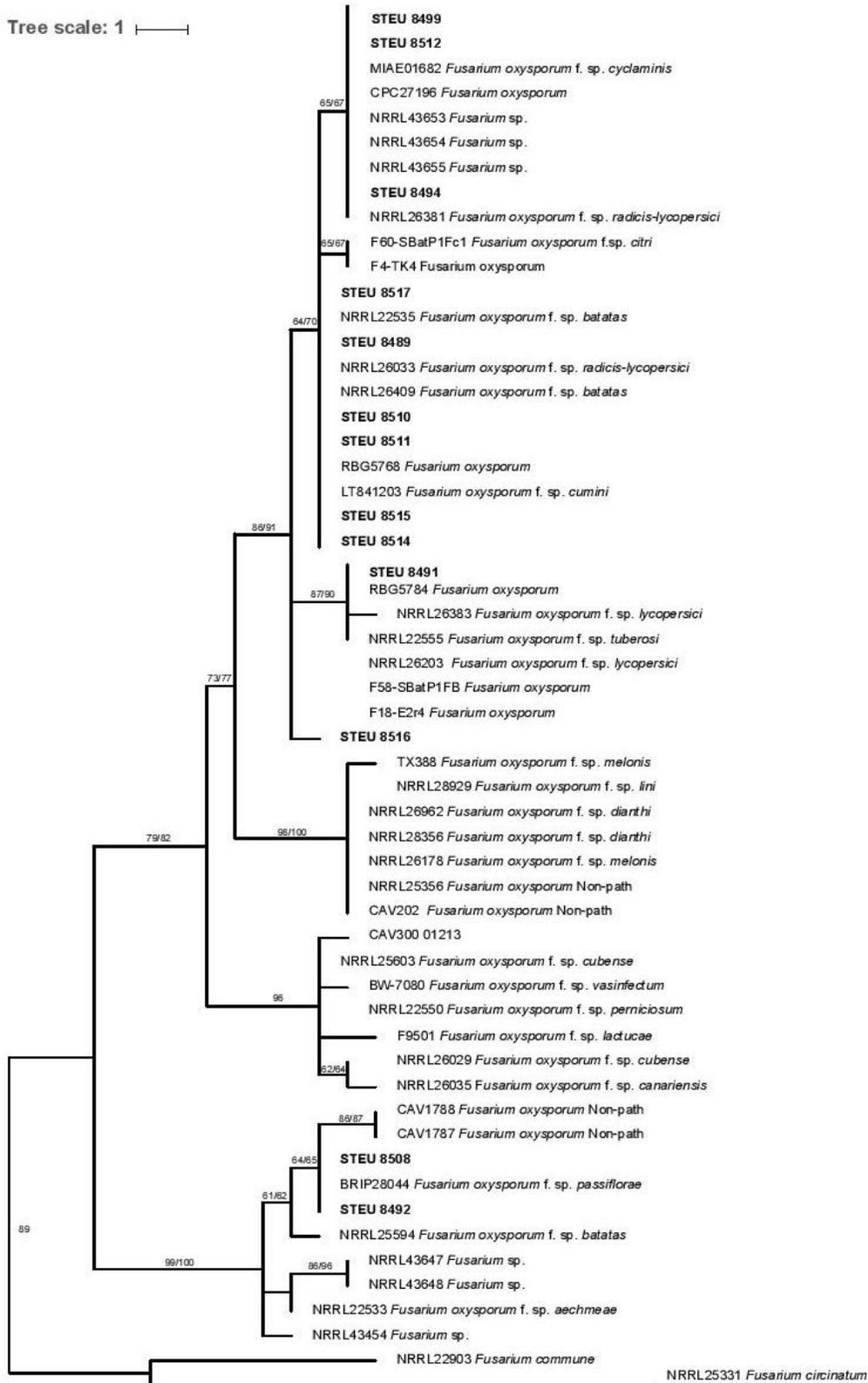


Figure 3. Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic tree of *Fusarium* species which was based on translation elongation factor 1-alpha (TEF) sequence data. Bootstrap support values were calculated from 1000 replicates and bootstrap support of 60% and higher are shown. *Fusarium circinatum* and *Fusarium commune* was used as the outgroups. Isolates obtained in this study are indicated in bold

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

Project 1030 (2008 – Ongoing) by JM van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

In the 2017/2018 season a nematicide based on garlic extract was tested for the third year. As in the other years, OMV-JJ1 performed well in comparison with the standard cadusafos treatment. In the past season, the application regimes consisting of 4 applications at different dosages and timings, performed similarly or better compared to the 3 cadusafos applications in terms of reducing of juvenile or female nematode numbers. Discussions will be initiated with the supplier to investigate the possibility of registration on citrus. Trials evaluating phytotoxicity of phosphonate foliar applications on “Nadorcott” mandarin fruit were again repeated to confirm the results of the previous season. Results indicated that full and half dose phosphonate applications for *Phytophthora* brown rot control caused phytotoxic damage on the fruit if the applications were done after color break stage of fruit development. On “Nadorcott” mandarin fruit late season control of *Phytophthora* should therefore be based on cultural practices such as the skirting of trees.

Opsomming

In die 2017/2018 seisoen is 'n aalwurmdoder, gebaseer op 'n knoffel-ekstrak, vir die derde jaar getoets. Soos gevind in die vorige jare, het OMV-JJ1 goed gedoen in vergelyking met die standaard cadusafos behandeling. Gedurende die afgelope seisoen het die toediening regimes, bestaande uit vier toedienings teen verskillende dosisse en tydsbepalings, dieselfde of beter gevaar, in vergelyking met die drie cadusafos toedienings, in terme van die vermindering van jong of vroulike aalwurmgetalle. Besprekings sal met die verskaffer geïnisieer word om die moontlikheid van registrasie op sitrus te ondersoek. Proewe wat die fitotoksiteit van fosfonaat blaartoedienings op “Nadorcott” mandarynvrugte evalueer, is herhaal ten einde die resultate van die vorige seisoen te bevestig. Resultate het aangedui dat vol en halwe dosis fosfonaat toedienings vir *Phytophthora* bruinvrotbeheer fitotoksiteit skade op die vrugte veroorsaak het, indien die toedienings ná die kleurbreekstadium van vrug-ontwikkeling gedoen is. Op “Nadorcott” mandarynvrugte moet láát seisoen beheer van *Phytophthora* dus op verbouingspraktyke soos die rompsnoei (“skirting”) van bome gebaseer word.

Introduction

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

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

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

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

In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used in the irrigation water against this nematode with great success. The nematode was effectively controlled while yields were also substantially increased (O'Bannon *et al.*, 1963; Philis, 1969). The activity on eggs is the most important difference between the soil fumigants used to control nematodes earlier this century, and today's non-fumigant chemicals. Following the withdrawal of DBCP, non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) were introduced. These chemicals, however, could not eliminate, or greatly reduce, nematode populations even if applied every year. Fenamiphos is translocated systemically in the vascular system of plants, whilst the other nematicides are non-systemic and reduce nematode populations through their initial contact action only. This explains the quick recovery of nematode populations once the nematicide has been degraded in soil and emphasizes the adverse effect of enhanced degradation, as eggs hatching after the nematicide has been degraded can continue the nematode's life-cycle. The following nematicides are currently registered on citrus in South Africa: aldicarb, cadusafos, fenamiphos, terbufos, ethoprophos, fosthiazate and furfural (Nel *et al.*, 2002). When multiple nematicide applications were introduced on a commercial scale to citrus orchards in South Africa, situations occurred where growers were not successful in disrupting the nematode's life cycle despite adhering strictly to prescribed procedures. In an investigation to determine the efficacy of cadusafos in soil where aldicarb and fenamiphos failed as a result of accelerated degradation, it was found that in the absence of sufficient irrigation water none of the nematicides were distributed thoroughly through the soil profile and they consequently failed to eliminate the citrus nematode (Le Roux *et al.*, 1998).

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

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

Objectives 2017/2018

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

Materials and methods

Nematicide evaluation

A nematode infested citrus orchard with nematode female counts in excess of 5000 females per 10 g of roots was identified in 2016. This was regarded as a suitable trial site, as the standard threshold value of 1000 females per 10 g of roots was exceeded. The 17-year-old Late Valencia on Rough Lemon citrus orchard with a 10 m² drip zone is situated east of Nelspruit at Crocodile Valley Citrus Co. Single tree plots were randomly selected and replicated eight times for each treatment. The product tested was OMV-JJ1 which was based on a garlic extract. The different treatment regimes and application times are given in Table 1. Soil and root sampling for nematode analyses was done in January, March, May and July 2017. The nematode analyses were done using the normal procedures at the Diagnostic Centre in Nelspruit.

The liquid formulated products were applied by means of a 10 litre watering can to ensure an even distribution of the products under the drip zone of the trees. Cadusafos served as the standard chemical control. Protective clothing was worn to protect the researcher and assisting staff during application of these products. All the applications were executed in good weather conditions with an average day temperature of 29 °C.

Table 1. Dosages and application programmes of treatments for the OMV-JJ1 trial during 2016/2017 at Crocodile Valley Estate, Nelspruit.

Treatment no.	Product	Rate per hectare (L/ha)	Rate/m ²						
				Nov 2015 (A)	Dec 2015 (B)	Jan 2016 (C)	Feb 2016 (D)	Mar 2016 (E)	May 2016 (G)
V 1	Untreated	-	-	-	-	-	-	-	-
V 2	OMV-JJ1	5 L	0.5 ml	X					
V 3	OMV-JJ1	5 L	0.5 ml	X	X	X	X		
V 4	OMV-JJ1	5 L	0.5 ml	X		X		X	X
V 5	OMV-JJ1	10 L	1.0 ml	X					
V 6	OMV-JJ1	10 L	1.0 ml	X	X	X	X		
V 7	OMV-JJ1	10 L	1.0 ml	X		X		X	X
V 8	OMV-JJ1	20 L	2.0 ml	X					
V 9	OMV-JJ1	20 L	2.0 ml	X	X	X	X		
V 10	OMV-JJ1	20 L	2.0 ml	X		X		X	X
V 11	Rugby 10 ME	150 L	15.0 ml	X		X		X	

*Tree canopy size 10 m²

Evaluation of phosphonate phytotoxicity on “Nadorcott” mandarin fruit

The first trial site (2016 and 2017) was located outside Nelspruit in the Mpumalanga province of South Africa, which is characterized by little or no rain during the ‘Nadorcott’ harvest period in winter. The trees in this orchard were 10 years old and planted on Carrizo citrange (*C. sinensis* (L.) Osbeck cv. Washington sweet orange x *Poncirus trifoliata* (L.) Raf.) rootstock.

In 2016, the second trial site was located at Riviersonderend in the Western Cape province of South Africa. This area is prone to rain, and therefore at a high risk for brown rot development, during the ‘Nadorcott’ harvest period, making this an area with a high citrus brown rot risk. This orchard was 12 years old and planted on Carrizo citrange

rootstock. The second trial site in 2017, near Riebeeck-Kasteel, was a 10 year old orchard on Carrizo citrange rootstock.

In the first season (2016), potassium phosphite (555 g/L a.i. = 350 g/L phosphorous acid equivalent) [Fighter, Agchem, South Africa] and ammonium phosphite (386 g/L a.i. = 300 g/L phosphorous acid equivalent) [Brilliant, Arysta LifeScience, South Africa] were applied according to label rates to treated trees. In the case of the potassium phosphite it was 570 ml/100 L water and for ammonium phosphite the rate was 666 ml/100 L water with trees sprayed from both sides to just before the point of run-off, approximately 10 L per tree. A Stihl® SR 420 motorized backpack mist blower was used in all applications in order to only spray single trees. The application times coincided with the immature green, color break and full color stages of fruit development. At both trial sites the treatments were the same while a single buffer tree was left between treatments.

In the second season (2017) the treatments at both sites were the same as applied in 2016. However, 2 additional treatments were added. Apart from the label dosages, both potassium phosphite and ammonium phosphite were also applied at half the recommended dosages, i.e. 285 ml/100 L water for potassium phosphite and 333 ml/100 L water for ammonium phosphite. These additional treatments were replicated in the same manner as the full rate treatments.

In 2016 the experimental layout at both sites was a randomized block split plot design. The main plot factor was chemical treatment (potassium phosphite, ammonium phosphite and unsprayed control) replicated in three blocks and the subplot factor was application time (different fruit color development stages - immature green, color break and full color stage). An experimental unit consisted of 40 fruit that were harvested from the two trees of each treatment x application time combination within each block replicate. The 2017 layout was the same except for two chemical treatments (half dosages for potassium and ammonium phosphite) that were added to the main plot.

The incidence of the phytotoxicity was documented as both percentage incidence as well as a phytotoxicity Index (PI). This dual recording resulted in a better understanding of not only incidence of the damage per treatment, as expressed by mean percentage damaged fruit, but also the severity of the damage on the individual fruit as expressed as an index. The effects on the fruit were recorded as follows:

At commercial harvest, 40 fruit were picked from two trees representing a treatment x application time combination. In the laboratory, the fruit were evaluated for phytotoxic damage according to a 0 – 3 severity index which was quantified as a rating; 0 being fruit with no damage, rating 1 fruit with <10% of fruit surface damaged, rating 2 fruit having 11% – 30% surface damaged and a rating 3 where fruit with >30% of the fruit surface displayed damage. $PI = \sum (\text{rind damage severity (0-3)} \times \text{number of fruit within each class}) / \text{total number of fruit}$.

At each application time, a sample of 20 fruit was collected. The fruit rind color of these fruit was measured, to determine the fruit color at the different application times and to indicate the change in rind color development between application times. The measurements were done with a Chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) on the sun-exposed side of each fruit and expressed by means of the Hunter *a/b* ratio (Figure 2).

For each experimental unit the percentage fruit within each phytotoxic damage rating class was calculated out of 40. This data were subjected to analysis of variance (ANOVA) according to the experimental design using SAS (SAS Institute Inc. NC, USA). Experimental results from the two sites were also combined after confirmation of site homogeneity of variance. Where site variances were unequal (2017 data) a weighted ANOVA was conducted. Fisher's least significant difference was calculated at a 5% significance level to compare means.

For each experimental unit of 40 fruit, a mean phytotoxic rating was calculated to use as an indication of damage severity. These ratings were not subjected to any further statistical analysis.

Results and discussion

Objective / Milestone	Achievement
Apr –Jun 2017	

<ol style="list-style-type: none"> 1. Annual report 2. Complete trial applications and evaluations. 	<ol style="list-style-type: none"> 1. Annual report was written and submitted. 2. Final trial applications was done as well as trial evaluations.
<p>Jul – Sept 2017</p> <ol style="list-style-type: none"> 1. Trial planning 2. First applications according to trial layout 3. Finalization of phosphonate results and research paper. 	<ol style="list-style-type: none"> 1. Trial was planned and products obtained. 2. The applications were done according to the trial layout in Table 1. 3. Phosphonate paper was completed.
<p>Oct – Dec 2017</p> <ol style="list-style-type: none"> 1. Do applications according to trial layout. 2. Collect soil and root samples. 	<ol style="list-style-type: none"> 1. Applications were done according to trial layout. 2. Soil and root samples were collected.
<p>Jan – Mar 2018</p> <ol style="list-style-type: none"> 1. Do applications according to trial layout. 2. Collect soil and root samples. 3. Submit phosphonate research paper 	<ol style="list-style-type: none"> 1. Applications were done according to trial layout. 2. Soil and root samples were collected. 3. Phosphonate research paper was completed and submitted to HortTechnology for publication. Pending revision, the paper was accepted.

Nematicide evaluation

Juveniles

Based on the juvenile counts from July 2017, that followed after the completion of the trial, the treatments that are standing out from the rest is numbers 5, 7, 8 and 9 (Table 2). All these treatments significantly reduced the juvenile counts by 75% or more in comparison to the untreated control. These treatments also performed statistically similar to the standard Rugby treatment, which is also a good result.

Females

Based on the July 2017 female count results, the best performing treatments were 3, 5, 6, 7 and 10 (Table 3). Although these treatments did not perform significantly better than the untreated control, all of them reduced the counts by more than 40%. The performance of these treatments were also similar to the performance of the Rugby treatment (Table 3).

Table 2. Mean citrus nematode juvenile (J2) counts in soil samples collected during four sampling periods in 2017 from trees subjected to different OMV-JJ1 dosages and application programmes.

No	Treatments	Rate prod/10m ²	January 2017		March 2017		May 2017		July 2017	
			L2/ 250cc soil	% Increase(+)/ Decrease(-) vs control						
1	Untreated control		731 bc	–	494 ab		2750 a		3644 ab	
2	Vigga	0.5 ml	850 a-c	16	481 ab	-3	644 b-d	-77	1475 c-e	-60
3	Vigga	0.5 ml (x 4 consecutive)	1513 ab	107	381 ab	-22	1344 b-d	-51	4081 a	12
4	Vigga	0.5 ml (x 4 every 2nd month))	1088 a-c	49	188 ab	-62	750 b-d	-73	1300 c-e	-64
5	Vigga	1,0 ml	619 c	-15	250 ab	-49	1119 b-d	-59	838 de	-77
6	Vigga	1,0 ml (x 4 consecutive)	1250 a-c	71	513 ab	4	863 b-d	-69	1969 b-e	-46
7	Vigga	1,0 ml (x 4 every 2nd month))	1119 a-c	53	456 ab	-8	1038 b-d	-62	706 de	-81
8	Vigga	2,0 ml	1000 a-c	36	463 ab	6	1450 b-d	-47	725 de	-80
9	Vigga	2,0 ml (x 4 consecutive)	756 a-c	3	338 ab	-32	288 cd	-90	750 de	-79
11	Vigga	2,0 ml (x 4 every 2nd month))	763 a-c	4	469 ab	-5	169 d	-94	1125 c-e	-69
12	Rugby	150ml	450 c	-38	156 ab	-68	306 cd	-89	338 e	-91

¹Means followed by the same letter is not significantly different based on a 95% confidence level.

Table 3. Mean citrus nematode female counts in root samples collected during three sampling periods from trees subjected to different OMV-JJ1 dosages and application programmes.

No	Treatments	Rate prod/m ²	January 2017		March 2017		May 2017		July 2017	
			♀ / 10g roots	% Increase(+)/ Decrease(-) vs control	♀ / 10g roots	% Increase(+)/ Decrease(-) vs control	♀ / 10g roots	% Increase(+)/ Decrease(-) vs control	♀ / 10g roots	% Increase(+)/ Decrease(-) vs control
1	Untreated control		650 b	–	800 c-g	–	888 a-e		850 b-d	
2	OMV-JJ	0.5 ml	1000	54	488 fg	-39	525 c-f	-41	688 b-d	-19
3	OMV-JJ	0.5 ml (x 4 consecutive)	2700 a	315	1425 a-e	78	863 a-f	-3	425 d	-50
4	OMV-JJ	0.5 ml (x 4 every 2nd month))	1075 b	65	1613	102	575 b-f	-35	800 b-d	-6
5	OMV-JJ	1,0 ml	838 b	29	800 c-g	0	400 ef	-55	488 cd	-43
6	OMV-JJ	1,0 ml (x 4 consecutive)	788 b	21	863 b-g	8	425 d-f	-52	425 d	-50
7	OMV-JJ	1,0 ml (x 4 every 2nd month))	863 b	33	1388 a-e	73	525 c-f	-41	463 cd	-46
8	OMV-JJ	2,0 ml	675 b	4	1713 ab	114	550 b-f	-38	600 cd	-29
9	OMV-JJ	2,0 ml (x 4 consecutive)	1113 b	71	988 a-g	23	525 c-f	-41	813 b-d	-4
10	OMV-JJ	2,0 ml (x 4 every 2nd month))	763 b	17	1788 a	123	288 f	-68	375 d	-56
11	Rugby	150ml	900 b	38	588 e-g	-27	488 c-f	-45	375 d	-56

¹Means followed by the same letter is not significantly different based on a 95% confidence level.

Phosphonate phytotoxicity on mandarins

At trial evaluation in both years similar symptoms of phytotoxic damage were observed on fruit sprayed with either potassium or ammonium phosphite. On fruit that was sprayed with these chemicals at color break phase, the phytotoxic damage on the fruit rind manifested as dark brown lesions of variable sizes (Figure 1 a, b). These lesions consisted of an area where the flavedo was damaged to such an extent that the white, underlying albedo was exposed. A green margin was furthermore observed to occur around the lesions (Figure 1 a, b). On fruit that was sprayed with the abovementioned chemicals at the full color developmental phase (Figure 1 c-d), much more severe damage was observed of the fruit rind compared to fruit sprayed at the green stage (not shown). Again the lesions were of variable sizes but had the appearance of dark brown, sunken areas where the flavedo was damaged and the underlying albedo had a reddish brown color (Figure 1 c-d).

Analysis of variance (ANOVA) of the mean percentage fruit with phytotoxic damage in 2016 indicated a significant ($P < 0.0001$; ANOVA not shown) area x treatment x color phase interaction. At both trial sites no damaged fruit was observed at harvest when potassium phosphite was sprayed at green color stage (Table 4; Figure 2). Compared to this, 0.2% damaged fruit was observed at harvest, at both trial sites, on fruit sprayed with ammonium phosphite at the green color stage. However, this was not significantly more than the unsprayed control (Table 4). For this application, the damage severity on the 0.2% damaged fruit, was only 0.002 indicating that very slight damage had occurred (Table 4).

However, for fruit sprayed at the color break stage, significant differences compared to the control were observed between the trial sites, irrespective of the chemical applied (Table 1). At the Riviersonderend trial site, no fruit were damaged by either of the chemicals when applied at the color break stage. Compared to this, at the Nelspruit trial site, a mean percentage of 44.2% was damaged by potassium phosphite applications at color break stage. The mean PI of this fruit was 1.23 (Table 4). A mean percentage of 40% was damaged by ammonium phosphite applications at the same stage resulting in a PI of 1.12 (Table 4). For the respective chemicals these mean percentages were statistically similar but significantly more than the unsprayed treatment where no damaged fruit was observed (Table 4).

The results obtained with full color applications again indicated significant differences between the areas and the two chemicals tested. At the Riviersonderend trial site 100.0% of fruit sprayed with potassium phosphite at this stage showed damage with a high PI of 2.36 (Table 4). This application in Nelspruit caused significantly less damage at 85.0% although the PI of 2.33 did not differ much from that observed at Riviersonderend (Table 4). The ammonium phosphite applications at the two sites resulted in statistically similar incidences of 93.3% in Riviersonderend and 95.0% in Nelspruit. Furthermore the PI of this application at the different sites were similar at 2.69 and 2.58 respectively and not in all cases notably more than the unsprayed control (Table 4).

Analysis of the 2017 data indicated only a significant ($P = 0.0006$) treatment x color phase interaction (ANOVA not shown). In terms of the incidence of damage observed, ammonium phosphite applications at half dose during the green phase, caused 1.7% fruit to show phytotoxic damage at harvest. However, the PI of this fruit was only 0.03 (Table 5) and the percentage damaged fruit was not significantly higher than the other chemical treatments or the unsprayed control where no damage was observed (Table 5).

At the color break phase the potassium phosphite and ammonium phosphite applications, at full and half dosages, caused phytotoxic damage that was significantly more than the unsprayed control (Table 5). The full dose potassium phosphite application caused a mean percentage of damaged fruit of 59.2% that was, although not significant, more than potassium phosphite applied at half dose (40.0 %) and ammonium phosphite (39.2%). The application with half dose ammonium phosphite caused a mean percentage of damaged fruit of 21.7% that was statistically similar to the other treatments, except the full dose potassium phosphite application (Table 5). In terms of the severity, the most severe damage (PI of 1.07) was also caused by the full dose potassium phosphite

application. This was similar to the half dose potassium phosphite application (PI of 0.70) and full dose ammonium phosphite application (PI of 0.70). The latter two treatments did, however, not cause notably more severe damage than the half dose ammonium phosphite application (PI of 0.33; Table 5).

Significant increased incidence of damage occurred in fruit in all treatments applied at the full color stage (Table 2). The highest incidence of damage was seen in the full dose of potassium phosphite applications, i.e. 63.3% and PI of 1.11. This percentage damage was statistically similar to the full dose ammonium phosphite applications (52.5%) with a severity rating of 1.11, which was markedly higher than any of the other treatments (Table 5). The half dose ammonium phosphite application caused an incidence of 41.7% with a severity of 0.85. The least amount of damage was caused at colour break by the half dose potassium phosphite application with an incidence of 20.8% and severity of 0.37, both significantly the lowest (Table 5).

Table 4. Mean percentage damaged fruit observed at harvest after applications of potassium and ammonium phosphite treatments at the green, color break and full color stages of Nadorcott fruit development in 2016 at the Riviersonderend and Nelspruit trial sites.

Treatment	Riviersonderend			Nelspruit		
	Green	Color break	Full color	Green	Color break	Full color
potassium phosphite	0.0 e ¹ (0.000) ²	0.0 e (0.000)	100a (2.358)	0.0 e (0.000)	44.2 d (1.229)	85 c (2.325)
ammonium phosphite	0.2e (0.002)	0.0 e (0.000)	93.3 b (2.692)	0.2 e (0.002)	40 d (1.121)	95 ab (2.575)
Unsprayed	0.0 e (0.000)	0.0 e (0.000)	0.0 e (0.000)	0.0 e (0.000)	0.0 e (0.000)	0.0 e (0.000)
<i>LSD</i> value			5.207			

¹Means followed by the same letter are not significantly different at the 95% confidence level.

²Mean phytotoxic rating calculated per experimental unit of 40 fruit.

Table 5. The incidence (% phytotoxicity) and severity (PI) in fruit observed at harvest, caused by full and half dose potassium and ammonium phosphite treatments applied at the green, color break and full color stages of Nadorcott fruit development in 2017.

Treatment	Color phase		
	Green	Color break	Full color
potassium phosphite (full dose)	0.00 f ¹ (0.000) ²	59.17 ab (1.067)	63.33 a (1.108)
potassium phosphite (half dose)	0.00 f (0.000)	40.00 bcd (0.696)	20.83 de (0.367)

ammonium phosphite (full dose)	0.00 f (0.000)	39.17 bcd (0.704)	52.50 ab (0.917)
ammonium phosphite (half dose)	1.67 ef (0.033)	21.67 cde (0.333)	41.67 bc (0.850)
Unsprayed	0.00 f (0.000)	0.00 f (0.000)	0.00 f (0.000)
<i>LSD</i> value	20.41		

¹Means followed by the same letter are not significantly different at a 95% confidence level.

²Mean phytotoxic rating calculated per experimental unit of 40 fruit.

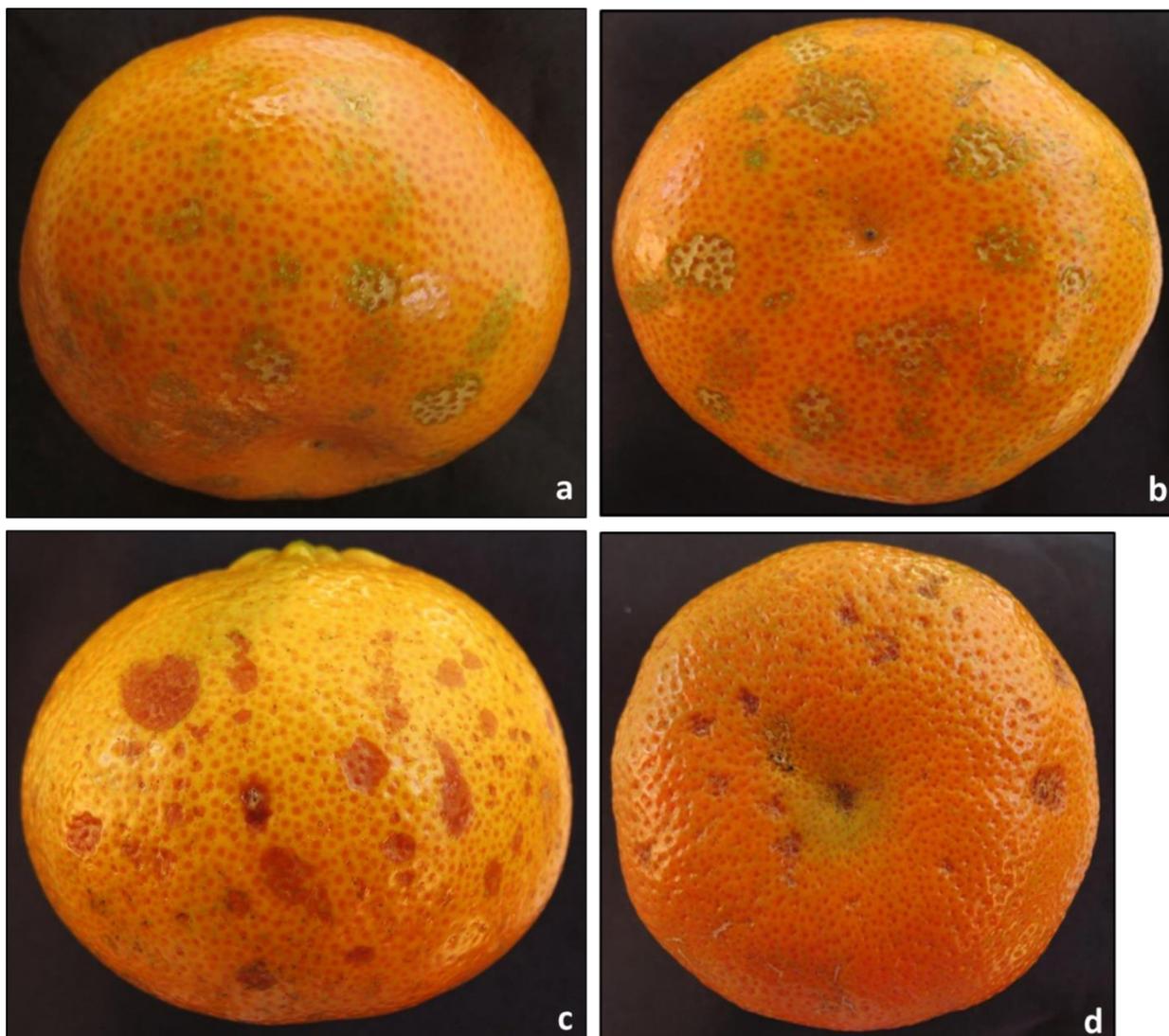


Figure 1. Phytotoxic damage caused on the rind of 'Nadorcott' mandarin fruit at harvest after potassium or ammonium phosphite applications at color break (a, b) or full color (c, d) stage of fruit development.

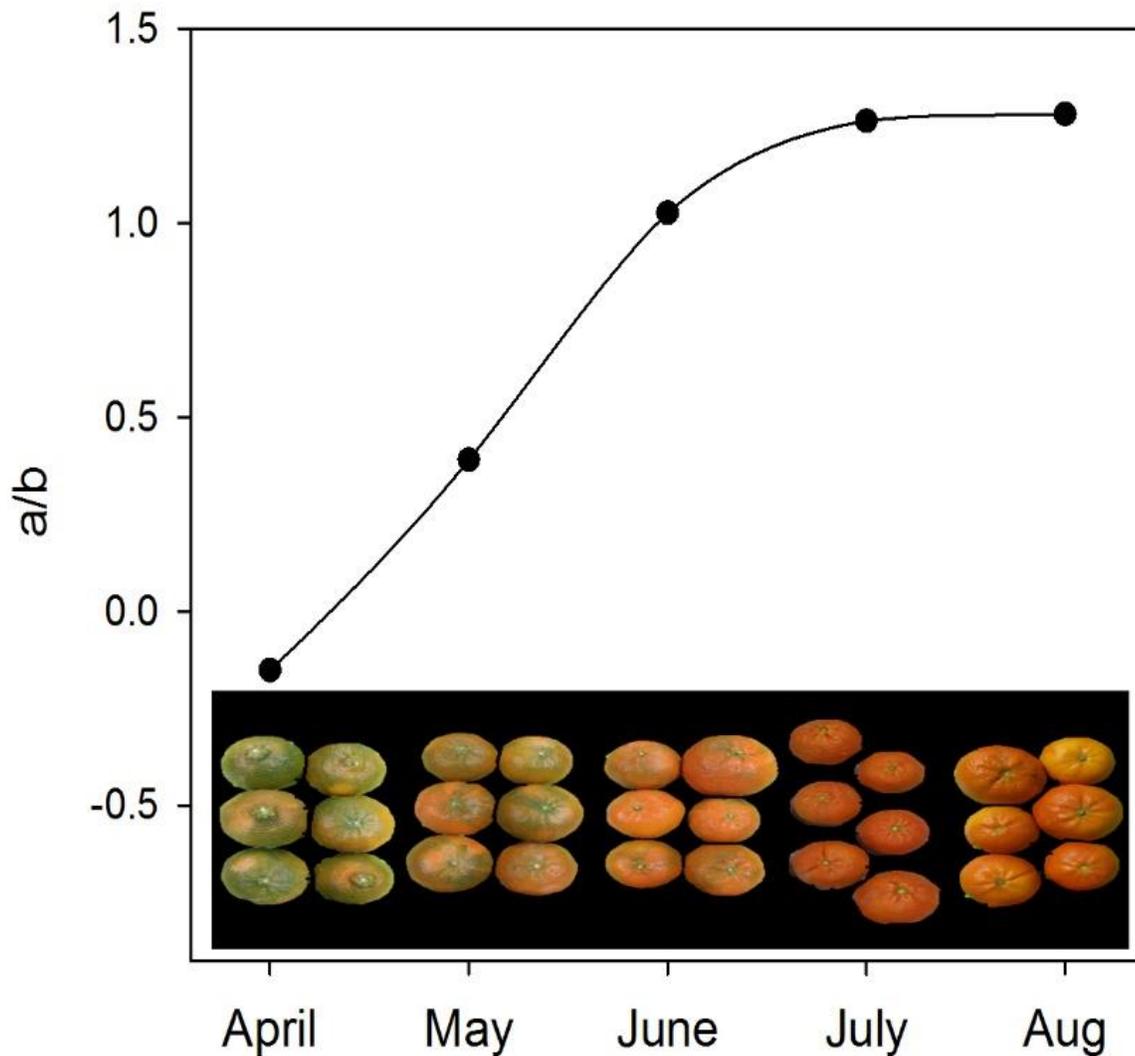


Figure 2. Graphic representations of the colour change in the 'Nadorcott' mandarin at the various treatment application dates. Colour change is expressed as the Hunter a/b ratio.

Conclusions to date

1. The OMV-JJ1 nematicide applications are showing great promise and are performing at levels similar to that achieved by cadusafos applications.
2. Applications of phosphonates, at full or half dosages, to mandarin fruit past colour break phase, leads to phytotoxic damage to the rind. Late season phosphonates for brown rot control are therefore not recommended for mandarins. These findings were recorded in a scientific publication that was accepted for publication.

Technology transfer

Cutting Edge 218 on the phytotoxic damage of phosphonate applications on Nadorcott mandarins. Scientific publication accepted for publication in HortTechnology.

Data will be presented at the biennial CRI Symposium in August 2018.

Further objectives and work plan

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

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3.3.4 **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 C Kotze (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 all caused the trees in these treatments to be taller with thicker trunks compared to the other treatments. It is therefore becoming clear that pre-plant soil fumigation in a replant situation does improve tree growth in comparison to no treatment or post-plant treatments.

Opsomming

Die doel van hierdie projek is om vóór-plant behandelings te vind wat effektief is om boordgronde vry van sitrus aalwurm en *Phytophthora* spp. te hou vir so lank as moontlik ná plant. Die proef is al sedert Januarie 2010 aan die gang. Die verskeie behandelings is vóór plant in Januarie 2010 toegedien, terwyl sommige behandelings steeds jaarliks in Januarie en November toegedien word. Boomstamdeursnit, boomhoogte, aalwurm grond- en wortel-analise, *Phytophthora* status in die grond, en 'n visuele boomgradering, is die parameters wat jaarliks sedert die begin van die proef gemonitor word. Tot op datum het geen behandeling in terme van aalwurmbheerresultate uitgestaan nie. Gebaseer op boomhoogte en stamdeursnitmetings, het die vóór-plant berokingsbehandelings egter almal veroorsaak dat die bome in hierdie behandelings langer was met dikker stamme in vergelyking met die ander behandelings. Dit word dus duidelik dat vóór-plant grondberoking in 'n herplant situasie boomgroei verbeter in vergelyking met geen behandeling of ná-plant behandelings.

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

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

Summary

A decline and death of citrus trees have been reported from Swaziland, Hoedspruit and the Gamtoos and Sunday's River valleys for a number of years. Several fungal genera were isolated from diseased material. Molecular identification of the isolated fungi identified *Kretzschmaria deusta* as being the only pathogen associated with the symptoms observed in Swaziland and Hoedspruit. In the two Eastern Cape areas a complex of pathogens were found to be associated with the observed symptoms. In this case the dominant ones were *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* and *Eutypella* sp. *Phaeoacremonium parasiticum* and *Schizophyllum commune* were also isolated but at levels much lower than the above-mentioned group. Work done in the past year indicated that plant species forming part of the natural vegetation in the Sunday's River Valley are probably the source of the pathogens involved. This is especially since *Fusarium* spp. were often isolated from the roots of these plants and the soil they were growing in. It also became evident that a number of factors are potentially involved as stress factors predisposing citrus trees to infection by above-mentioned pathogens. Further work will include further studies on the inoculum sources, characterization of the pathogens, testing of rootstocks for tolerance and then the identification of orchard treatments that can maintain or improve tree health of affected trees.

Opsomming

'n Agteruitgang en afsterwing van sitrusbome is vir 'n aantal jare vanuit Swaziland, Hoedspruit en die Gamtoos- en Sondagsriviervalleie aangeteken. Verskeie swamgenera is vanuit siek materiaal geïsoleer. Molekulêre identifikasie van die geïsoleerde swamme het *Kretzschmaria deusta* geïdentifiseer as die enigste patogeen wat met die simptome, waargeneem in Swaziland en Hoedspruit, geassosieer is. In die twee Oos-Kaap areas is gevind dat 'n kompleks van patogene met die waargenome simptome geassosieer is. In hierdie geval was die dominerende patogene *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* en *Eutypella* sp.. *Phaeoacremonium parasiticum* en *Schizophyllum commune* is ook geïsoleer maar teen baie laer vlakke as die bogenoemde groep. Werk wat die afgelope jaar gedoen is, het aangedui dat plantspesies wat deel uitmaak van die natuurlike vegetasie in die Sondagsriviervallei, moontlik die bron van die betrokke patogene is. Dit is veral moontlik aangesien *Fusarium* spp. dikwels vanuit die wortels van hierdie plante en die grond waarin hulle groei, geïsoleer is. Dit het ook duidelik geword dat 'n aantal faktore moontlik as stresfaktore betrokke is wat die sitrusbome vatbaar maak vir infeksie deur bogenoemde patogene. Verdere werk sal verdere studies insluit oor die inokulumbronne, karakterisering van die patogene, toets van onderstamme vir bestandheid, en dan die identifikasie van boordbehandelings wat die boomgesondheid van geaffekteerde bome kan handhaaf of verbeter.

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

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

Summary

During 2017/2018 the characterization of *Pythium* spp. occurring in South African citrus nurseries was completed. Ten species was identified of which one is a potential new species. Mefenoxam sensitivity testing of these ten species indicated that they vary greatly within and between species with regards to their mefenoxam sensitivity and that some isolates are even at a 100 ppm of mefenoxam not completely inhibited. Currently the pathogenicity testing and chlorine sensitivity testing of these 10 *Pythium* spp. are underway. With regards to *Phytophthora nicotianae* and *P. citrophthora* results were obtained indicating that within these two species the variation in mefenoxam sensitivity is also great, again with some isolates not being totally inhibited *in vitro* by 100 ppm mefenoxam. It was furthermore shown that different isolates within the two species also varies in their chlorine sensitivity with a group of isolates only being totally inhibited at a chlorine concentration of 6 ppm and an exposure time of 60 minutes. These findings and future results will greatly improve soilborne disease management practices in South African citrus nurseries.

Opsomming

Die karakterisering van *Pythium* spp. wat in Suid-Afrikaanse sitruskwekerie voorkom, is gedurende 2017/2018 voltooi. Tien spesies is geïdentifiseer, waarvan een 'n potensiële nuwe spesie is. Mefenoxam sensitiviteitstoetsing van hierdie tien spesies het aangedui dat hulle grootliks binne en tussen spesies met betrekking tot hulle mefenoxam sensitiviteit varieer, en dat sommige isolate selfs teen 'n 100 dpm mefenoxam nie ten volle geïnhibeer is nie. Die patogenisiteitstoetsing en chloor sensitiviteitstoetsing van hierdie 10 *Pythium* spp. is tans onderweg. Resultate is met betrekking tot *Phytophthora nicotianae* en *P. citrophthora* verkry wat daarop dui dat die variasie in mefenoxam sensitiviteit binne hierdie twee spesies ook groot is. Sommige isolate is weereens *in vitro* nie heeltemal geïnhibeer teen 100 dpm mefenoxam nie. Verder is aangetoon dat die verskillende isolate binne die twee spesies ook in hulle chloor sensitiviteit varieer, met 'n groep isolate wat slegs totaal geïnhibeer word teen 'n chloor konsentrasie van 6 dpm en 'n blootstellingsperiode van 60 minute. Hierdie bevindinge en toekomstige resultate sal grondgedraagde siektebestuurspraktyke in Suid-Afrikaanse sitruskwekerie grootliks verbeter.

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

Summary

'Slow decline' of citrus trees in older orchards has been known for decades, but the causal factors have not been fully elucidated. The aim of the study was to identify parameters associated with the syndrome, and their interactions that could help in determining the origin of the decline. Two declining orchards in the Mpumalanga Province of South Africa were selected and trees were classified into three categories based on the visual decline of the tree canopies. For each tree category, yield, soil and leaf nutrient characteristics, soil-borne pathogens and root disease associated symptoms were measured at two occasions with one-year interval, and averaged to produce the best representative picture of the orchard situation and avoid accidental variations. Principal component analysis (PCA) performed on all parameters collected on the healthy trees indicated that the two orchards were very different from each other. Multivariate analyses (principal component and classification) of the measured factors were conducted separately for the two orchards to determine which parameters had the strongest tendencies to be associated with specific tree categories. The measured parameters were shown to be weakly associated with the decline. However, on the factorial plan, the clouds corresponding to each tree category moved in a similar direction in numerical order from the lowest to the highest category for both orchards, despite the fact that the orchards are very different, which tend to prove that the same interactions are at the origin of the process. Healthy trees were associated with clay. This parameter was furthermore shown through orchard mapping to be randomly distributed within the orchards, but when mapped to specific tree categories, it was evident that clay mapped to the healthy trees despite low differences. On the other hand, silt, percentage P and Mo were associated with the declining trees although this trend was clearer in orchard 1 than in orchard 2. From this study, it would seem that K levels in soil and Mn and B levels in leaves could be linked to citrus decline and by manipulating these levels, reduced susceptibility could be provided, but this should be further tested.

Opsomming

Stadige agteruitgang van sitrus bome in ouer boorde is al vir dekades bekend, maar die veroorsakende faktore is nog nooit ten volle bepaal nie. Die doel van hierdie studie was om die parameters geassosieer met die sindroom, en hul interaksies, te bepaal ten einde die oorsprong te bepaal. Twee boorde wat agteruitgang simptome toon is in die Mpumalanga provinsie van Suid-Afrika geïdentifiseer en die bome is in drie kategorieë verdeel op grond van die visuele agteruitgang van die boomlower. Vir elke kategorie is opbrengs, grond en blaar minerale eienskappe, grondgedraagde patogene en wortelsiekte simptome twee keer, een jaar uitmekaar, bepaal. Die gemiddeld van hierdie metings is bepaal om 'n verteenwoordigende beeld van die boorde te skep. Hoofkomponent analise ("PCA") van al die versamelde parameters het aangedui dat die twee boorde baie van mekaar verskil. Multi-faktor analises van die gemete faktore is apart gedoen vir die twee boorde om te bepaal watter parameters die sterkste geassosieer is met die verskillende boom kategorieë. Die gemete parameters is bevind om swak geassosieer te wees met die agteruitgang. Dit is egter deur verdere ontledings bevind dat dieselfde interaksies die oorsprong is van die agteruitgangproses. Gesonde bome was geassosieer met klei. Hierdie faktor is bevind om lukraak versprei te wees in die boorde, maar was gereeld geassosieer met gesonde bome. Slik, % P en Mo is aan die ander kant geassosieer met die simptomatiese bome. Hierdie assosiasie was sterker in boord 1 as in boord 2. Uit hierdie studie wil dit voorkom dat K vlakke in die grond en Mn en B vlakke in die blare verbind kan word met agteruitgang. Bestuur van hierdie faktore kan dus moontlik die probleem verminder en moet verder ondersoek word.

3.4 PROGRAMME: FRUIT AND FOLIAR DISEASES (WITH CBS)

Programme coordinator: Providence Moyo (CRI)

3.4.1 Programme summary

Citrus black spot (CBS) and Alternaria brown spot (ABS) are two major fruit and foliar diseases that hamper the export of citrus fruit to fresh markets by South African producers. The focus of the Fruit and Foliar (with CBS) programme is to study the epidemiology and control aspects of these diseases. The epidemiology of

CBS is not fully understood and strict regulations have been implemented by certain of our existing export markets, on the use of certain products registered for the control of ABS. Citrus black spot, caused by *Phyllosticta citricarpa* McAlp van der Aa, is the subject of an intense scientific and regulatory debate. The European Union (EU) which has a zero tolerance for CBS in fresh citrus fruit maintains its position that the climate in the EU is suitable for CBS establishment while the position taken by scientists in most non-European countries is that the EU climate is unsuitable or marginally suitable. Consequently, many disease modelling studies have been conducted to assess the suitability of the European climate to CBS but these have been ambiguous, partly because they were performed with different modelling approaches and often did not include locations with known disease prevalence and absence to enable adequate model calibration. Subsequently, an independent multi-model comparison, that included calibration with both low and high risk locations as well as blind model validation to prevent institutional bias was conducted (3.4.2). Four mechanistic models were used in the study including Magarey generic, ZedX generic, Bregaglio, and CRI-PhytRisk. All models performed well, on average being able to predict sites where CBS was *absent* with 86% accuracy in the validation data set. When the model-ensemble was used to predict the CBS suitability of 36 European locations, most models in the ensemble agreed in simulating an *absent* CBS suitability class in 28 out of 36 sites (77.8%).

Citrus black spot is widespread in citrus-growing regions but is absent within countries of the EU, where it is subject to phytosanitary legislation. In order to determine the occurrence and persistence of *Phyllosticta* spp. in Europe, surveys were conducted in citrus orchards, nurseries and gardens in the major citrus production areas of the EU (3.4.2). Once several *Phyllosticta* strains were isolated, phylogeny, morphology, genotypes, mating types and pathogenicity were studied. Four *Phyllosticta* spp. were found to occur in the EU but were not widespread and never associated with infections. Symptoms of CBS were also not observed during the surveys indicating that the fungi persisted but did not cause disease. Attempts to produce ascospores of *P. citricarpa* in culture were futile. The genomes of five *Phyllosticta* spp. were also sequenced. The availability of these genomic sequences provides an important resource in gaining a better understanding of the species' distribution, sexual morph, rapid detection and marker development.

The population structure, mode of reproduction and introductory pathways of *P. citricarpa* were determined in Project 977 (3.4.4). New polymorphic simple sequence repeat (SSR) markers developed in Project 977 were used, in combination with published markers, to genotype 383 isolates of *P. citricarpa* obtained from different citrus growing areas of the globe. Mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution, but the USA population harboured only a single mating type. High levels of connectivity among *P. citricarpa* populations in South Africa, Australia and Brazil found during the study were attributed to exchanges of plant material during the establishment of citrus industries in these countries. The population structure of *P. citricarpa* at the orchard spatial (distance) and temporal (seasonal) scales in South Africa was also investigated. Spatial analyses at the orchard scale indicated that subpopulations separated by shorter distances (within 200 m), were typically not significantly genetically differentiated, while those separated by longer distances were sometimes significantly differentiated. Temporal analyses showed that seasonal populations were not significantly genetically differentiated in a North West orchard, however, these were significantly differentiated in a Mpumalanga orchard.

Project RCE-6 (3.4.7) aimed at bridging the knowledge gap that exist with regards to maturation of fruiting structures and spore germination requirements of both the sexual and asexual morphs of the CBS pathogen. The effect of spore age, generation, temperature, germination medium and wetness interruption was investigated. High germination percentages were obtained with spores older than 1 day old and from the second and subsequent generations of spores oozing from pycnidia. Results also showed that 1.5% Valencia juice is not a requirement for germination, but considerable differences between germination of pycnidiospores on glass slides and leaves at different temperatures, were observed. Confocal laser microscopy images showed very similar trends for pycnidiospore germination and appressorium formation on lemon and lime leaves, which indicate that lime trees' tolerance to CBS is not linked to the germination process. Wetness interruption trials showed that pycnidiospores are able to germinate and survive a 3 h period of dryness, even as early as 4 h after inoculation. A qPCR protocol was developed but less than 1000 spores could not reliably

be quantified by the qPCR protocol. Subsequently, primers for a nested qPCR were developed to improve sensitivity of the qPCR. Attempts to produce ascospores in culture, using published crossing methods, failed.

In an effort to assist growers with better decision making in terms of timing of spray application and choice of fungicides, Project RCE-7 (3.4.8) integrated ascospore and pycnidiospore production, release and infection models together with accurate weather data into a web-based platform (CRI-PhytRisk). This platform is already in use and provides farmers with information about CBS risks, weather forecasts and suitable weather for spraying. The mobile phone version was launched in October 2017. Validation using actual measured weather data as input vs. weather data from YR.NO was conducted. The PhytRisk output based on predicted weather data appeared to be more liberal (i.e. more infection periods were predicted), mostly resulting from higher predicted rainfall figures. To further improve CRI-PhytRisk's accuracy, it was programmed to automatically upload measured weather data from a grower's weather station database (iLeaf was used as model platform), thereby giving CBS prediction based on weather conditions measured on-farm.

Methods described in the original proposal for Project RCE-8 (3.4.9), as well as amended methods, failed to generate data for 4 out of the 5 objectives set in the project. Consequently, the four objectives were discontinued and all focus was on the one objective in which data could be generated. Volumetric ascospore trapping was conducted, in geographically different citrus production areas in South Africa. Ascospore monitoring is complete in all areas. Ascospore release data generated is currently being analysed for use in validating and improving CBS models by Fourie et al. (2013).

Because of the need to get 100% control of diseases and pests of market access concern, including CBS and false codling moth, high volume citrus spray applications are often used in South Africa. Although these high volume sprays are effective in disease and pest control as well as improved deposition, they are costly and have negative impacts on the environment. Subsequently, research aimed at the use of reduced volume fungicide application in citrus orchards in South Africa was initiated (Projects 1132 (3.4.10) and 1089 (3.4.11)). Project 1132 evaluated the effect of reduced spray volumes on the control of CBS and false codling moth, while 1089 aimed at the development of a tree canopy characteristic calibration formula for reduced volume fungicide application in citrus orchards. The effect of different amounts of pruning on spray deposition quantity, uniformity and quality as well as on canopy density (canopy density as measured by the LIDAR) was evaluated in 1089. Evaluation of experiments for the control of CBS and insect pests in Project 1132 will be completed in June 2018 but reduced spray volumes were not effective in controlling insect pests such as red scale and mealy bug when compared to high volume sprays in the 2016/2017 season. Data on deposition parameters indicated that poorer deposition values were obtained in the 2017/2018 season when compared with the 2016/2017 season. The LIDAR successfully observed changes in tree canopy density after pruning in Project 1089. Pruning had no to little effect on spray deposition parameters when higher spray volumes were applied. However, results showed that light pruning had a marked effect on spray deposition when lower spray volumes were applied.

Due to the increasing pressure, from consumers, to reduce the number of detectable residues on citrus fruit, spray programmes aimed at reducing fungicide residues were developed and tested in project 750 (3.4.6). Different contact fungicides were combined at half their recommended rates and tested for efficacy against *Alternaria* brown spot, in an orchard under a hail net. Unfortunately, the disease pressure was extremely low and results were inconclusive.

Citrus black spot is effectively controlled by the use of strobilurins and two mancozeb applications before and after the strobilurin applications in October and January. The possibility that CBS may develop resistance to the strobilurins, justifies the continuous testing of novel control measures against CBS. Project 970 (3.4.5) evaluated new potential fungicides and alternative spray programmes for the control of CBS. All products tested showed promise in controlling the disease.

With an increasing tendency to erect shade nets over high value citrus cultivars, there are fears that the change in microclimate under nets could affect the development of diseases. Consequently, a study aimed to determine how the use of shade nets could influence the development and spread of CBS within citrus orchards was initiated (3.4.12). A higher risk of CBS infection was predicted in the orchard under the net when compared to the orchard outside the net, confirming that the microclimate in orchards is altered with the use of nets, subsequently affecting the development of CBS.

It has been demonstrated that fruit becomes resistant to CBS infection with maturity, *i.e.* ontogenic resistance development. However, this trait has not been demonstrated quantitatively and more conclusively. Project 1186 (3.4.13), therefore, aims to demonstrate ontogenic resistance development of citrus fruit to *P. citricarpa* infection. Evaluation of the experiments will occur in August 2018.

Program-opsomming

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 strem. Die fokus van die Vrug- en Blaar-program (met SSV) is om die epidemiologie en beheer-aspekte van hierdie siektes te bestudeer. Die epidemiologie van SSV word nie ten volle verstaan nie en streng regulasies is deur sekere van ons bestaande uitvoermarkte geïmplementeer op die gebruik van sekere produkte geregistreer vir beheer van ABV.

Sitrus swartvlek, veroorsaak deur *Phyllosticta citricarpa* McAlp van der Aa, is die onderwerp van 'n intense wetenskaplike en reglementêre debat. Die Europese Unie (EU) wat 'n zero-toleransie vir SSV in vars sitrusvrugte het, behou hul posisie dat die klimaat in die EU geskik is vir die vestiging van SSV, terwyl die posisie wat deur wetenskaplikes in die meeste nie-Europese lande ingeneem word, is dat die EU klimaat nie geskik of slegs marginaal geskik is. Gevolglik is baie siekte modelleerstudies uitgevoer ten einde die geskiktheid van die Europese klimaat vir SSV vas te stel, maar hierdie was twyfelagtig, gedeeltelik omdat hulle uitgevoer is met verskillende modelleerbenaderings, en het dikwels nie liggings ingesluit met bekende siektevoorkoms of -afwesigheid, ten einde voldoende modelkalibrasie moontlik te maak nie. Gevolglik is 'n onafhanklike multi-model vergelyking in Projek 1149 (3.4.2) uitgevoer, wat kalibrasie met beide lae en hoë risiko liggings ingesluit het, asook blinde modelbekragtiging ten einde institusionele bevooroordeeling te voorkom. Vier masjien-geboude modelle is in die studie ingesluit, insluitende Magarey generic, ZedX generic, Bregaglio en CRI-PhytRisk. Al die modelle het goed gedoen, en was oor die algemeen in staat om met 86% akkuraatheid areas te voorspel waar SSV *afwesig* was in die bekragtiging dataset. Wanneer die model-ensemble gebruik is om die SSV-geskiktheid van 36 Europese liggings te bepaal, het die meeste modelle in die ensemble saamgestem in die simulاسie van 'n *afwesig* SSV-geskiktheidsklas in 28 uit 36 liggings (77.8%).

Sitrus swartvlek is wydverspreid in sitrusproduksie-areas, maar is afwesig binne lande van die EU, waar dit aan fitosanitêre wetgewing onderhewig is. Ten einde die voorkoms en volharding van *Phyllosticta* spp. in Europa vas te stel, is opnames in sitrusboorde, kwekerye en tuine in die hoof sitrusproduksie-areas van die EU uitgevoer (3.4.3). Sodra verskeie *Phyllosticta* isolate geïsoleer is, is filogenie, morfologie, genotipes paringstipes en patogenisiteit bestudeer. Daar is gevind dat vier *Phyllosticta* spp. in die EU voorkom, maar was nie wydverspreid nie en is nooit met infeksies geassosieer nie. Simptome van SSV is ook nie tydens die opnames waargeneem nie wat aandui dat swamme volhard, maar nie siekte veroorsaak nie. Pogings om askospore van *P. citricarpa* in kultuur te produseer, was tevergeefs. Die basispaar-volgorde-bepaling ("sequencing") van die genome van die vyf *Phyllosticta* spp. is ook gedoen. Die beskikbaarheid van hierdie genoom basispaar-volgordes verskaf 'n belangrike hulpmiddel ten einde die spesies se verspreiding, geslagtelike vorm, vinnige waarneming en merker-ontwikkeling beter te verstaan.

Die populasie-struktuur, voortplantingswyse en introduksiebane van *P. citricarpa* is in Projek 977 (3.4.4) bepaal. Nuwe polimorfiese "simple sequence repeat" (SSR) merkers wat in Projek 977 ontwikkel is, is gebruik, in kombinasie met gepubliseerde merkers, ten einde die genotipe van 383 isolate van *P. citricarpa*, verkry vanaf verskillende sitrusproduksie-areas van oor die wêreld, te bepaal. Paringstipe analyses het getoon dat beide paringstipes teenwoordig was in die populasies vanaf Suid-Afrika, China, Australië en Brasilië, teen 'n ongeveer 1:1 verspreiding, maar die V.S.A. populasie het slegs 'n enkele paringstipe gehuisves. Hoë vlakke van samehang wat tydens die studie tussen *P. citricarpa* populasies in Suid-Afrika, Australië en Brasilië gevind is, kan toegeskryf word aan uitruil van plantmateriaal gedurende die vestiging van sitrus-industrieë in hierdie lande. Die populasie-struktuur van *P. citricarpa* op die boord ruimtelike (afstand) en tydelike (seisoenale) skale in Suid-Afrika, is ook ondersoek. Ruimtelike analyses op die boord skaal het aangedui dat sub-populasies wat deur korter afstande van mekaar geskei is (binne 200 m), tipies nie betekenisvol geneties gedifferensieer was nie, terwyl die wat deur langer afstande van mekaar geskei is, soms betekenisvol gedifferensieer was. Tydelike analyses het getoon dat seisoenale populasies nie betekenisvol geneties gedifferensieer in 'n Noordwes boord was nie, maar hierdie was egter betekenisvol gedifferensieer in 'n Mpumalanga boord.

Projek RCE-6 (3.4.7) het ten doel gehad om die gaping in kennis wat bestaan met betrekking tot ryppword van vrugstrukture, en spoor-ontkiemingsvereistes van beide geslagtelike en ongeslagtelike vorms van die SSV-patogeen, te oorbrug. Die effek van spoor-ouderdom, generasie, temperatuur, ontkiemingsmedium en natheid-onderbreking, is ondersoek. Hoë ontkiemingspersentasies is verkry met spore ouer as 1 dag oud en van die tweede en daaropvolgende generasies van spore wat uit piknidia uitstroom. Resultate het ook getoon dat 1.5% Valencia sap nie 'n vereiste vir ontkieming is nie, maar aansienlike verskille tussen ontkieming van piknidiospore op glasplaatjies en blare by verskillende temperature, is waargeneem. Konfokale lasermikroskopie beelde het baie soortgelyke neigings vir piknidiospore-ontkieming en appressoriumvorming op suurleroen- en lemmetjieblare getoon, wat aandui dat lemmetjiebome se bestandheid teen SSV nie aan die ontkiemingsproses gekoppel is nie. Natheid-onderbrekingsproewe het aangedui dat piknidiospore in staat is om te ontkiem en 'n 3 h droogteperiode te oorleef, selfs so vroeg as 4 h ná inokulasie. 'n qPKR protokol is ontwikkel maar minder as 1000 spore kon nie betroubaar deur die qPKR protokol gekwantifiseer word nie. Gevolglik is inleiers vir 'n "nested" qPKR ontwikkel om sensitiwiteit van die qPKR te verbeter. Pogings om askospore in kultuur te produseer deur van gepubliseerde kruisingsmetodes gebruik te maak, het misluk.

In 'n poging om produsente met beter besluitneming by te staan in terme van tydsberekening van spuittoediening en keuse van fungisiedes, het Projek RCE-7 (3.4.8) askospore- en piknidiosporeproduksie, vrystelling en infeksie Modelle, tesame met akkurate weerdata in 'n web-gebaseerde platform (CRI-PhytRisk) geïntegreer. Hierdie platform is reeds in gebruik en verskaf aan boere inligting oor SSV-risiko's, weervoorspellings en geskikte weer vir spuit. Die mobiele foon weergawe is in Oktober 2017 in gebruik geneem. Bekragtiging deur gebruik te maak van werklik gemete weerdata as invoer vs. weerdata vanaf YR.NO, is uitgevoer. Die PhytRisk-lewering, gebaseer op voorspelde weerdata, blyk meer liberaal te wees (meer infeksieperiodes is voorspel) wat meesal die resultaat van hoër voorspelde reënvalsyfers was. Ten einde CRI-PhytRisk se akkuraatheid verder te verbeter, is dit geprogrammeer om gemete weerdata vanaf 'n produsent se weerstasie databasis outomaties op te laai (iLeaf is as modelplatform gebruik), waardeur SSV-voorspelling verskaf word gebaseer op weerstoestand wat op die plaas gemeet word.

Metodes wat in die oorspronklike voorlegging vir Projek RCE-8 (3.4.9) beskryf is, asook aangepaste metodes, het misluk om data vir 4 uit die 5 doelwitte wat in die projek gestel is, te lewer. Gevolglik is die vier doelwitte beëindig en alle fokus is geplaas op die een doelwit waarin data gegenereer kon word. Volumetriese askospore lokval-stelling is in geografies verskillende sitrusproduksie-areas in Suid-Afrika uitgevoer. Askosporemonitering is in alle areas voltooi. Askosporevrystellingsdata wat gegenereer is, word tans geanaliseer vir gebruik in bekragtiging en verbetering van SSV-Modelle deur Fourie et al. (2013).

Weens die behoefte aan 100% beheer van siektes en plae wat van belang vir marktoegang is, insluitende SSV en valskodlingmot, word hoë volume sitrus spuittoedienings dikwels in Suid-Afrika gebruik. Hoewel hierdie hoë volume spuite effektief in siekte- en plaagbeheer, asook in verbeterde neerlegging is, is hulle duur en het hulle 'n negatiewe impak op die omgewing. Navorsing met die doel om verminderde volume fungisiedtoediening in sitrusboorde in Suid-Afrika te gebruik, is gevolglik geïnisieer (Projekte 1132 (3.4.10) en 1089 (3.4.11)). Projek 1132 het die effek van verminderde spuitvolumes op die beheer van SSV en valskodlingmot geëvalueer, terwyl 1089 ten doel gehad het om 'n boomlowerkenmerk kalibrasieformule te ontwikkel vir verminderde volume fungisiedtoediening in sitrusboorde. Die effek van verskillende hoeveelhede snoei op spuitneerlegginghoeveelheid, -uniformiteit en -kwaliteit, asook op lowerdigtheid (lowerdigtheid soos gemeet deur LIDAR) is in 1089 geëvalueer. Evaluasie van proewe vir die beheer van SSV en insekplae in Projek 1132 sal in Junie 2018 voltooi wees, maar verminderde spuitvolumes was nie effektief in die beheer van insekplae soos rooi dopluis en witluis, in vergelyking met hoë volume spuite in die 2016/2017 seisoen, nie. Data rakende neerleggingsparameters het aangedui dat swakker neerleggingswaardes in die 2017/2018 seisoen verkry is in vergelyking met die 2016/2017 seisoen. Die LIDAR het suksesvol veranderinge in boomlowerdigtheid ná snoei in Projek 1089 waargeneem. Snoei het geen tot min effek op spuitneerleggingsparameters gehad wanneer hoër spuitvolumes toegedien is nie. Resultate het egter getoon dat ligte snoei 'n merkbare effek op spuitneerlegging gehad het wanneer laer spuitvolumes toegedien is.

Weens die toenemende druk vanaf verbruikers om die hoeveelheid waarneembare residue op sitrusvrugte te verminder, is spuitprogramme wat op verminderde fungisiedresidue gemik is, ontwikkel en in projek 750 (3.4.6) getoets. Verskillende kontakfungisiedes is teen die helfte van hul aanbevole dosis gekombineer, en vir

effektiwiteit teen *Alternaria* bruinvlek, in 'n boord onder 'n haelnet, getoets. Die siektedruk was ongelukkig baie laag en resultate was dus onbeslis.

Sitrus swartvlek word effektief beheer deur die gebruik van strobilurine en twee mankoseb toedienings vóór en ná die strobilurien toedienings in Oktober en Januarie. Die moontlikheid dat SSV weerstand teen die strobilurine kan ontwikkel, regverdig die voortdurende toets van nuwe beheermaatreëls teen SSV. Projek 970 (3.4.5) het nuwe potensiële fungisiedes en alternatiewe spuitprogramme vir die beheer van SSV geëvalueer. Alle produkte wat getoets is, het belofte getoon in die beheer van die siekte.

Met die toenemende neiging om skadunette oor hoë waarde sitruskultivars te trek, is daar kommer dat die verandering in mikroklimaat onder nette die ontwikkeling van siektes kan affekteer. 'n Studie is gevolglik geïnisieer met die doel om te bepaal hoe die gebruik van skadunette die ontwikkeling en verspreiding van SVS binne sitrusboorde kan beïnvloed (3.4.12). 'n Hoër risiko vir SSV-infeksie is in die boord onder die net voorspel wanneer vergelyk word met die boord buite die net, wat bevestig dat die mikroklimaat in boorde deur die gebruik van nette verander word, en gevolglik word die ontwikkeling van SSV beïnvloed.

Daar is gedemonstreer dat vrugte met rypwording weerstandbiedend word teen SSV-infeksie, m.a.w. ontogeniese weerstandsontwikkeling. Hierdie kenmerk is egter nog nie kwantitatief en meer afdoende bewys nie. Projek 1186 (3.4.13), het dus ten doel om ontogeniese weerstandsontwikkeling van sitrusvrugte teen *P. citricarpa*-infeksie te demonstreer. Evaluasie van die eksperimente gaan in Augustus 2018 geskied.

3.4.2 PROGRESS REPORT: An independent model comparison for evaluating predictions of climate suitability of citrus black spot in Europe

Project 1149 (2016/7 - 2018/19) by JM Russo (ZedX), RD Magarey (North Carolina State University); Paul Fourie, Mareli Kellerman (CRI)

Summary

Citrus black spot (CBS), caused by the fungus *Phyllosticta citricarpa*, is the subject of an intense scientific and regulatory debate. The European Union (EU) has a zero tolerance for CBS in fresh citrus fruit and the issue has generated considerable scientific debate in the literature. The EU scientific position is that the climate is suitable and that fruit represents a possible pathway of introduction. The position taken by scientists in most non-European countries is that the climate is unsuitable or marginally suitable and that fruit is not a pathway for introduction. Recent evidence indicates that *P. citricarpa* is present in Europe, suggesting that the pathogen could survive in EU citrus areas, although disease may be limited by an unfavourable climate. Disease modelling studies to assess the suitability of the European climate to CBS have been ambiguous, partly because they were performed with different modelling approaches and often did not include locations with known disease prevalence and absence to enable adequate model calibration. We conducted an independent multi-model comparison that included calibration with both low and high risk locations as well as blind model validation to prevent institutional bias. Four mechanistic models were used in the study including Magarey generic, ZedX generic, Bregaglio, and CRI-PhytRisk. All models performed well, on average being able to predict sites where CBS was *absent* with 86% accuracy in the validation data set. The model-ensemble was then used to predict the CBS suitability of 36 European locations. Most models in the ensemble agreed in simulating an *absent* CBS suitability class in 28 out of 36 sites (77.8%), while simulation for the remaining 8 sites varied between *absent*, *low* and/or *moderate*. Weather data comparison between simulated grid-data used as model input and measured weather station data at orchard level indicated significant differences in temperature and RH, which explains some of the anomalous findings.

Opsomming

Sitrus swartvlek (SSV), veroorsaak deur die swam, *Phyllosticta citricarpa*, is die onderwerp van 'n intense wetenskaplike en reglementêre debat. Die Europese Unie (EU) het 'n zero-toleransie vir SSV in vars sitrusvrugte, en die geskilpunt het aansienlike wetenskaplike debat in die literatuur ontketen. Die EU se wetenskaplike posisie is dat die klimaat geskik is en dat vrugte 'n moontlike baan vir introduksie is. Die posisie wat deur wetenskaplikes in meeste nie-Europese lande ingeneem word, is dat die klimaat nie geskik of slegs marginaal geskik is en dat vrugte nie 'n baan vir introduksie is nie. Onlangse bewyse dui daarop dat *P.*

citricarpa in Europa teenwoordig is, wat voorstel dat die patogene in EU sitrus-areas kan oorleef, hoewel siekte deur 'n ongunstige klimaat beperk kan word. Siektemodelleerstudies om die geskiktheid van die Europese klimaat vir SSV te bepaal, was twyfelagtig, gedeeltelik omdat hulle uitgevoer is met verskillende modelleerbenaderings, en het dikwels nie liggings ingesluit met bekende siektevoorkoms of -afwesigheid ten einde voldoende modelkalibrasie moontlik te maak nie. Ons het 'n onafhanklike multi-model vergelyking uitgevoer, wat kalibrasie met beide lae en hoë risiko liggings ingesluit het, asook blinde modelbekragtiging ten einde institusionele bevoordeling te voorkom. Vier masjien-geboude modelle is in die studie ingesluit, insluitende Magarey generic, ZedX generic, Bregaglio en CRI-PhytRisk. Al die modelle het goed gedoen, en was oor die algemeen in staat om persele te voorspel waar SSV *afwesig* was met 86% akkuraatheid, in die bekragtiging datastel. Wanneer die model-ensemble gebruik is om die SSV geskiktheid van 36 Europese liggings te bepaal, het die meeste modelle in die ensemble saamgestem in die simulاسie van 'n *afwesig* SSV geskiktheidsklas in 28 uit 36 liggings (77.8%), terwyl simulاسie vir die oorblywende 8 persele tussen *afwesig*, *laag* en/of *matig* gevarieer het. Weerdata vergelyking tussen gesimuleerde rooster-data wat as model invoer gebruik is, en gemete weerstasiedata op boordvlak, het betekenisvolle verskille in temperatuur en RH aangedui, wat van die afwykende bevindinge verduidelik.

3.4.3 FINAL REPORT: Distribution and genetics of Citrus pathogens

RCE-9 (10/12/2014 – 31/03/2018) by P. Crous, (Westerdik Fungal Biodiversity Institute - Netherlands)

Summary

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

Opsomming

The genus *Phyllosticta* kom wêreldwyd voor, en verteenwoordig etlike plantpatogene, endofitiese en saprofitiese spesies. *Phyllosticta citricarpa* is die organisme wat Swartvlek (CBS) veroorsaak. Alhoewel die siekte wyd verspreid voorkom in lande waar citrus geplant word, is dit afwesig in Europa, waar streng fytosanitêre maatreels toegepas word. *Phyllosticta citricarpa* word maklik verwar met *P. capitalensis*, 'n nie-patogeniese endofiet wat algemeen in blare en vrugte voorkom (ook op ander gewasse). Drie addisionele *Phyllosticta* spesies word geassosieer met siekte simptome van *Citrus* spp. in Asië: *P. citriasiana*, *P. citrichinaensis* en *P. citrimaxima*, terwyl *P. citribraziliensis* voorkom as endofiet op citrus in Brazil. Europese citrus is oorspronklik gevestig uit materiaal afkomstig uit Asië ongeveer in die 4de eeu na Christus. Een doel van die projek was om die moontlike voorkoms (en oorlewing) van *Phyllosticta* spesies op citrus plant materiaal in Europa te ondersoek. Om dit vas te stel is verskeie opnames in verskillende lande, kwekerye en tuine in Europa uitgevoer. Alle *Phyllosticta* isolate wat geïsoleer is, is daarna onderwerp aan ondersoek (morfologie, filogenie, genotype en paringstipe bepaling, patogenisiteit en genoom data). In totaal is 64 isolate versamel, waarvan 52 gebruik is vir 'n multi-lokus DNA dataset van ITS, *actA*, *tef1*, *gapdh*, LSU en *rpb2*. Gebaseer op die data was vier *Phyllosticta* soorte geïdentifiseer op citrus in Europese lande. Die *Phyllosticta* soorte was

egter heel beperk in hul verspreiding, en was nooit geassosieer met siekte simptome in die veld nie. Geen siekte simptome is waargeneem nie, selfs nie gedurende een opvolg survey uitgevoer in 2017 in dieselfde gebiede nie. Die isolate was geisoleer uit dooie blare as endofiete, maar was nie geassosieer met siekte nie.

Introduction

The genus *Phyllosticta* was introduced by Persoon (1818), with *P. convallariae* (nom. cons.) (= *P. cruenta*) designated as the type species (Donk 1968). Species of *Phyllosticta* are known as plant pathogens of several hosts and responsible for various disease symptoms including leaf and fruit spots. Several *Phyllosticta* species have been associated with *Citrus* spp. worldwide (Baayen *et al.* 2002, Glienke-Blanco *et al.* 2002, Everett & Rees-George 2006, Baldassari *et al.* 2008, Wulandari *et al.* 2009, Glienke *et al.* 2011, Brentu *et al.* 2012, Wikee *et al.* 2013a). Citrus black spot (CBS) is a foliar and fruit disease of *Citrus* spp. caused by *P. citricarpa* (sexual morph *Guignardia citricarpa*) (Kotzé 1981, Baldassari *et al.* 2008). The pathogen affects fruits and leaves of several citrus hosts causing various symptoms (Kiely 1948a, 1949, Kotzé 1981, 2000, Snowdon 1990) with lemons and 'Valencia' oranges being more susceptible (Kotzé 2000). Hard spot is the most common symptom characterised by sunken, pale brown necrotic lesions with a dark reddish brown raised border; lesions often containing the pycnidia (asexual sporocarps). Several other kinds of lesions are known: virulent spot, a sunken necrotic lesion without defined borders mostly on mature fruit, false melanose consisting of small black pustules usually in a tear stain pattern, and freckle, cracked or speckled spot. Leaf symptoms are seldom seen except for lemons. They appear as round, small, sunken necrotic spots with a yellow halo (Schubert *et al.* 2010). The infected leaves, when fallen on the orchard floor, represent a substrate for the development and maturation of perithecia from which the primary inoculum, ascospores, are released for new infections (McOnie 1967). *Phyllosticta citricarpa* has never been found on plant species outside of the *Rutaceae*, and can be isolated from asymptomatic citrus tissues (Baldassari *et al.* 2008). Whilst infections affect the fruit rind only, severe losses to the fresh fruit industry might be incurred under disease-conducive weather conditions in the absence of disease control measures (Kiely 1948, Kotzé 1981, Dewdney *et al.* 2012). Moreover, costs for chemical control may be unsustainable for farmers (Kotzé 1981).

Significant progress in species differentiation was achieved with multi-locus phylogenetic analyses performed on a large number of *Phyllosticta* species (Wulandari *et al.* 2009, Glienke *et al.* 2011, Wang *et al.* 2012). Using three partial DNA regions, Wulandari *et al.* (2009) revealed three *Phyllosticta* clades associated with citrus in Thailand, namely *P. capitalensis*, *P. citricarpa* and *P. citriasiana*. Wang *et al.* (2012) described one new species associated with citrus in China, namely *P. citrichinaensis*, and also distinguished two subclades within *P. citricarpa*. Sequencing four partial regions of DNA, Glienke *et al.* (2011) distinguished a new species, *Phyllosticta citribraziliensis*, associated with *Citrus* sp. in Brazil. *Phyllosticta citriasiana* is a pathogen causing tan spot on pomelo (*Citrus maxima*) in Asia while *P. citrichinaensis* is a weak pathogen on various citrus species in Asia, and *P. citribraziliensis* is a non-pathogenic endophyte on citrus in Brazil (Glienke *et al.* 2011). A recent study added a sixth *Phyllosticta* species associated with citrus, namely *P. citrimaxima*, which was isolated from tan spots on fruit of *C. maxima* in Thailand (Wikee *et al.* 2013a).

Phyllosticta citricarpa has been recorded in Australia since the late 19th century, causing citrus black spot disease, particularly in coastal regions of New South Wales and Queensland (Benson 1895, Kotzé 1981, Miles *et al.* 2013). *Phyllosticta citricarpa* has also been recorded in several countries of all continents except Europe (Baker *et al.* 2014). Recent studies performed in Florida, USA (Zhang *et al.* 2015, Wang *et al.* 2016), supported the heterothallism of *P. citricarpa*, finding only MAT1-2-1 isolates present in Florida (based on 113 isolates) while 26 strains from Australia displayed an equal ratio of the two mating types. Amorim *et al.* (2017) recently showed that in Brazil, the two idiomorphs occur in a 1:1 ratio. Furthermore, Tran *et al.* (2017) reported for the first time the successful mating *in vitro* of *P. citricarpa*, confirming that this species is heterothallic and requires isolates of different MAT idiomorphs to be in direct physical contact. A broad survey was undertaken in citrus growing regions within EU countries to investigate the presence of *Phyllosticta* spp. associated with citrus plants. In 2015 to 2017 several surveys were conducted in the major citrus production areas of the EU to determine the occurrence of *Phyllosticta* spp. associated with citrus.

Objectives

A. *Phyllosticta* survey from citrus in southern EU member states.

Based on climate data models set up by CRI, a survey was conducted in *Citrus* growing regions in southern EU member states that are considered favourable for CBS. The fungi involved were isolated, cultured, identified using robust molecular techniques, and compared to the known species.

B. *In vitro* production of *P. citricarpa* ascospores. Developing such a technique will make it possible to produce ascospores from predetermined parents, which could subsequently be used in inoculation experiments needed to understand the infection biology of the pathogen.

C. Derive whole genome sequences of all *Phyllosticta* spp. associated with CBS. Whole genome sequences of all *Phyllosticta* spp. associated with *Citrus*, namely *P. citricarpa* (high coverage of a representative isolate and low coverage for isolates respectively from SA, Australia, Asia, Latin America and the USA), low coverage of *P. citriasiana* (China), high coverage of *P. citribraziliensis* (Brazil), high coverage of *P. capitalensis* (Brazil), high coverage of *P. citrichinensis* (China) and high coverage of *P. citrimaxima* (Thailand) were generated. These data will enable us to 1) obtain the mating types of these species, 2) obtain microsatellite markers of all species, which will facilitate research into the global spread and variation within these species. These data will also allow for the development of robust DNA barcodes for quick detection. A further aim was to do whole genome sequencing of a global set of isolates (40) of *P. citricarpa* to determine the intraspecific variation of the pathogen in different countries.

Materials and methods

Surveys were carried out during 2015 and 2016 in selected main citrus-producing regions of Europe. More surveys were performed during 2017 also to revisit the sites where *Phyllosticta* species were found, to confirm their presence and to observe possible presence of symptoms on fruits or leaves. Evaluations were conducted on a total of 95 orchards and five nurseries. Samples were collected from Andalusia, Mallorca, Valencia (Spain), Apulia, Calabria, Sicily (Italy), Algarve (Portugal), Crete, Mesolongi, Nafplio (Greece), Gozo and La Valletta (Malta) areas. Investigated citrus species included Australasian lime (*Citrus australasica*), citranges (*Citrus sinensis* × *Poncirus trifoliata*), citrons (*C. medica*, *C. medica* var. *sarcodactylis*), kumquat (*C. japonica*), limequats (*Citrus xfloridana*), calamondin (×*Citrofortunella microcarpa*), mandarins (*C. reticulata*), tangelo (*C. xtangelo*), oranges (*C. xaurantium*, *C. xbergamia*, *C. xsinensis*), pummelo (*C. maxima*), grapefruit (*C. paradisi*), limes (*C. xaurantifolia*, *C. xhystrix*, *C. xlatifolia*) and lemons (*C. xlimon*). The isolates collected in this study are maintained in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS culture collection), Utrecht, The Netherlands, and in the working collection of Pedro Crous (CPC), housed at the Westerdijk Institute.

Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions. Partial regions of six loci were amplified. The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. The primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998) were used to amplify part of the translation elongation factor 1- α gene (*tef1*). The primers ACT-512F and ACT-783R (Carbone & Kohn 1999) were used to amplify part of the actin gene (*actA*). The 28S large subunit nrDNA (LSU) was amplified using primers LR0R (Moncalvo *et al.* 1995) and LR5 (Vilgalys & Hester 1990). The RNA polymerase II second largest subunit (*rpb2*) was amplified with RPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR (Liu *et al.* 1999). Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was amplified using primers Gpd1-LM and Gpd2-LM (Myllys *et al.* 2002). For *P. citricarpa* isolates the alternative primers Gpd1 (Guerber *et al.* 2003) and GPDHR2 (Gliénke *et al.* 2011) were used to amplify *gapdh*. The PCR amplification mixtures and cycling conditions for ITS, *actA*, *tef1*, LSU and *gapdh* were followed as described by Gliénke *et al.* (2011). Due to the lack of available *rpb2* gene sequences of *Phyllosticta* isolates, we generated these sequences for all the strains used for this study (except for *P. citrimaxima* CPC 20276 = CBS 136059, culture not available anymore). Novel sequences generated in this study were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of different gene

regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed by using the MAFFT v. 7 online server (<http://mafft.cbrc.jp/alignment/server/index.html>) (Kato & Standley 2013), and then manually adjusted in MEGA v. 6.06 (Tamura *et al.* 2013). Additional reference sequences were selected based on recent studies on *Phyllosticta* species (Glienke *et al.* 2011, Wang *et al.* 2012, Wikee *et al.* 2013a). Phylogenetic analyses were based on both Bayesian Inference (BI) and Maximum Parsimony (MP) analyses.

A subset of *Phyllosticta* isolates collected in this study was morphologically characterised. After 14 d of incubation in the dark at 27 °C, the morphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at ×1 000 magnification were determined for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) optics.

The mating types of *P. citricarpa* strains were determined based on PCR amplification of a diagnostic region from each mating type idiomorph by using four primers, MAT111F3 (5'- GCAATGTGGCAGCGCAATCC-3') and MAT111R3 (5'- TCTGGACCATCGGACTCATC-3') for MAT1-1-1, and MAT121F6 (5'- GATCGTGGCAGGAGGCTTTG-3') and MAT121R6 (5'- AACGACCAGCGATCGGTAAG-3') for MAT1-2-1 (Amorim *et al.* 2017).

Two isolates of each of the four *Phyllosticta* species isolated from specimens collected in Europe, were inoculated into mature, untreated fruits of sweet orange (*Citrus sinensis* Osbeck), cultivar 'Valencia' (from Spain), following the method described by Perryman *et al.* (2014) to obtain indicative results about pathogenicity.

A subset of isolates has been selected for testing a new technique recently developed from other scientists, named "sandwich technique" (Tran *et al.* 2017 - Sexual Reproduction in the Citrus Black Spot Pathogen, *Phyllosticta citricarpa* – Phytopathology <http://dx.doi.org/10.1094/PHYTO-11-16-0419-R>). The experiments did not lead to the ascospore production.

The genomes of five *Phyllosticta* spp. as well as the transcriptome profiles have been sequenced. The genome of two strains were sequenced for *P. citricarpa*, and one strain for *P. capitalensis*, *P. citrichinaensis*, *P. citribraziliensis*, *P. paracitricarpa*. Species were selected based on their association with citrus hosts. For isolating DNA, QIAGEN Genomic 100/G tips (QIAGEN Benelux B.V., Venlo, The Netherlands) were used and for isolating RNA, the QIAGEN RNeasy Midi kit was used.

In order to provide a foundation for future studies, we analysed the mating type loci of these six *Phyllosticta* genomes and one of *P. citriasiana*, with the aim to identify which mating type(s) are present in these species and to characterize the mating type genes. In addition, we designed new *Phyllosticta*-specific mating type primers and developed a PCR method for rapid identification of the MAT1-1-1 and MAT1-2-1 genes in *P. capitalensis*, *P. citriasiana*, *P. citribraziliensis*, *P. citricarpa*, *P. citrichinaensis* and *P. paracitricarpa* strains.

Results and discussion

A total of 64 monosporic isolates resembling those of the genus *Phyllosticta* were collected. The *Phyllosticta* isolates were recovered from five species of *Citrus* at 10 different sites. The combined species phylogeny of *Phyllosticta* consisted of 100 sequences, including the outgroup sequences of *Neofusicoccum mediterraneum* (culture CBS 121718). Morphological observations, supported by phylogenetic inference, were used to distinguish two known species (*P. capitalensis* and *P. citricarpa*) and two novel species (*P. paracapitalensis* and *P. paracitricarpa*).

The *Phyllosticta* mating type primer sets were successful in amplifying the respective portions of the MAT1-1-1 or the MAT1-2-1 idiomorphs of the 21 *P. citricarpa* isolates tested. The primer pair MAT111F3–MAT111R3 amplified a fragment of approximately 600 bp in 8 isolates, and the primer pair MAT121F6–MAT121R6 amplified approximately 700-bp-fragments in the remaining 13 isolates.

After 25 d, some inoculation points showed atypical lesions. The lesions developed only on fruits inoculated with *P. citricarpa* and *P. paracitricarpa* isolates. No lesions were observed on fruits inoculated with *P. capitalensis*, *P. paracapitalensis* and on the negative control. The lesions caused by *P. citricarpa* and *P. paracitricarpa* were similar.

After the genomes study, based on a comparison of the genes known to be in *P. citricarpa* (Wang *et al.*, 2016), we selected the 40SS9, MAT1-1-1, MAT1-2-5, MAT1-2-1, OML1 and APN2 genes and then we used translated nucleotide blast (Altschul *et al.*, 1997) to identify the scaffolds in each assembly that contain fragments of the mating type locus genes. We identified that one European and one Australian strain of *P. citricarpa*, and the *P. paracitricarpa* strain have heterothallic idiomorphs containing the MAT1-2-1 gene. In contrast, *P. citriasiana* and *P. citribraziliensis* strains have heterothallic idiomorphs containing the MAT1-1-1 gene. *Phyllosticta citrichinaensis* is homothallic, containing the full MAT1-1 and MAT1-2 loci on different scaffolds. Finally, *P. capitalensis* seems to contain a hybrid of both MAT1-1 and MAT1-2 loci, with the MAT1-2-1 and MAT1-1-1 collinearly present between the 40SS9 and OML1 genes. The scaffolds with the MAT locus fragments were visualized with SimpleSynteny (Veltri *et al.*, 2016). Moreover, the *Phyllosticta*-specific mating type primers sets were successful in amplifying a portion of the MAT1-1-1 and MAT1-2-1 genes in several tested isolates. The primer pair MAT111deg-F2 (GCTCTCAACTCTTTCATGGC) and MAT111deg-R3 (TGGYKCGYYGCATCACGC) amplified a fragment of approximately 1,010 bp, whilst the primer pair MAT121deg-F1 (AACAYRTRARGCYCCGG) and MAT121deg-R1 (YAABCCTGGRTTYTCCATCG) amplified a 300-bp-fragment.

This study contributed significantly towards our understanding of the genotypic variation in *P. capitalensis* and *P. citricarpa*, splitting both groups into different taxa, and clearly showing that a multi-locus approach works well for distinguishing these species.

Conclusions

No evidence of CBS disease in European citrus trees was observed in this study. The *P. citricarpa* isolates were found in leaf litter of very old *C. limon* and *C. sinensis* trees (20 to 60 years old) that were situated in gardens, and not found in any of the younger commercial orchards surveyed. Considering that mature citrus fruit are resistant to *P. citricarpa* infection under field conditions (Kiely 1948b, Schutte *et al.* 2003, 2012, Miles *et al.* 2004, Baldassari 2006) and since the harsh artificial inoculation technique used in the pathogenicity assay did not resemble natural field infection (Kotzé 1963, McOnie 1967, Noronha 2002) these findings should be regarded as preliminary. The findings from our study indicate that *P. citricarpa* was able to persist but did not induce CBS disease or spread. These findings are, therefore, not contradictory to the findings of the climate modelling studies as they relate to CBS disease. Based on the whole genome analysis of several strains in this study, we identified the heterothallic behaviour in *P. citriasiana*, *P. citribraziliensis* and *P. paracitricarpa*, and the homothallism of *P. capitalensis* and *P. citrichinaensis*. Moreover, we partially sequenced the MAT1-1-1 and MAT1-2-1 genes present in additional isolates of all the tested species, by using new sets of specific primers designed in this study.

Future research

Future studies on the global distribution of mating types of *P. citricarpa* at the lesion, fruit, and tree level, and movement of spermatia on the plant at the infection stage, are necessary to test their primary role in ascospore production, and in order to explain the occurrence of sexual reproduction of the fungus on leaf litter under orchard conditions. Such data would provide a better understanding of all the spore types in the disease cycle of CBS, aiding the development of a good and fast method for pathogenicity testing, in order to screen various chemicals and/or biological agents. Moreover, larger populations of *P. citriasiana*, *P. citrichinaensis* and *P. paracitricarpa* are required to make it possible to obtain more details about the distribution of mating types for other pathogenic *Phyllosticta* spp.

The available genomic sequences for six *Phyllosticta* spp. provide an important resource to gain a better understanding regarding the distribution, sexual morph, rapid detection and marker development of these

important species. Further studies must be conducted on a wider global selection of *P. citricarpa* strains also to improve the knowledge of its host association and distribution. A broader sampling is required to collect additional populations from Europe, Asia and the Oceania countries for more detailed population studies. Population genetic inferences were strengthened by the development of several polymorphic SSR markers, but a complete picture of the introduction and global movement pathways has not yet emerged. Therefore, more informative markers are needed to examine the population structure and migration pathways of *P. citricarpa* and other *Phyllosticta* spp. Moreover, the development of new informative markers is crucial to accurately characterise clones and to track global movement and introductions of *P. citricarpa* along with plant material from Asia, where *Citrus* originally established, to other continents.

Technology transfer

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3.4.4 **FINAL REPORT: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely**
Project 977 (2010/11 – 2018) by E. Carstens (CRI)

Summary

Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa*, is a fungal disease that influences citrus industries worldwide. All commercial *Citrus* spp. are susceptible to the disease. The pathogen was first described from Australia and subsequently from summer rainfall citrus production regions in China, Africa, South America and recently, the United States. Limited information is available on the pathogen's population structure, mode of reproduction, and introduction pathways on a global scale and on a regional scale in South Africa. This is also true for the effect of distance, season and *Citrus* spp. on population structure at the orchard scale in South Africa. The population structure of *P. citricarpa* was investigated at a global scale in populations from South Africa, the United States, Australia, China, and Brazil. Fifteen polymorphic SSR markers were used for genotyping populations. The Chinese and Australian populations had the highest genetic diversities, whereas populations from Brazil, the United States, and South Africa exhibited characteristics of founder populations. Based on population differentiation and clustering analyses, the Chinese populations were distinct from the other populations. With the exception of the clonal United States populations that only contained one mating type, the other populations contained both mating types in a ratio that did not deviate significantly from 1:1. The effects of distance and season on the population structure of *P. citricarpa* were investigated over two seasons in two lemon orchards; one in Mpumalanga and the other in North West. Spatial analyses indicated that subpopulations separated by a short distance (within 200 m) were typically not significantly genetically differentiated, but that those separated by longer distances were sometimes significantly differentiated. Temporal analyses in the North West orchard showed that seasonal populations were not significantly genetically differentiated. In contrast, seasonal populations from the Mpumalanga orchard were significantly differentiated, most likely due to higher rainfall and disease pressure. Studies on a provincial scale in South Africa showed that ten *P. citricarpa* populations, representing the five CBS provinces were not significantly genetically differentiated. Based on gene and genotypic diversities and private allele richness, KwaZulu-Natal or Limpopo are likely the provinces where the pathogen was first introduced. The Eastern Cape was confirmed as being the province where the latest introduction occurred in South Africa. Despite lemon trees having overlapping fruit crops, potentially providing increased opportunities for clonal reproduction, *Citrus* spp. (lemons vs. oranges) did not have an effect on population structure.

Opsomming

Sitrus Swartvlek (SSV) is 'n swamsiekte wat deur *Phyllosticta citricarpa* veroorsaak word, en wat sitrusbedrywe wêreldwyd beïnvloed. Alle kommersiële *Citrus* spp. is vatbaar vir die siekte. Die patogeen is vir die eerste maal

in Australië beskryf en daarna vanuit sitrus produserende streke in somerreënval gebiede in Sjina, Afrika en Suid-Amerika en mees onlangs die Verenigde State. Beperkte inligting oor die patogeen se populasie-struktuur, wyse van voortplanting en introduksie roetes is op 'n globale vlak beskikbaar, sowel as op 'n provinsiale vlak in Suid-Afrika. Op 'n boordvlak, is inligting ook beperk oor die effek wat afstand, seisoen en *Citrus* spp. op die populasie-struktuur het. Die populasie-struktuur van *P. citricarpa* is op 'n globale vlak in populasies van Suid-Afrika, die Verenigde State, Australië, Sjina en Brasilië ondersoek. Vyftien polimorfiese mikrosatelliet merkers is gebruik om die populasies te genotipeer. Die Sjinese en Australiese populasies het die hoogste genetiese diversiteit getoon, terwyl populasies van Brasilië, die Verenigde State en Suid-Afrika eienskappe van stigterspopulasies toon. Gebaseer op populasie-differensiasie en groeiperings-analises verskil die Sjinese populasies van die ander populasies. Met die uitsondering van die klonale populasies van die Verenigde State, met net een paringstipe, het die ander populasies beide paringstipes gehad in 'n verhouding wat nie beduidend van 1: 1 afwyk nie. Die effek van afstand en seisoen op die populasie-struktuur van *P. citricarpa* is oor twee seisoene in twee suurlemoenboorde ondersoek; een boord in Mpumalanga en die ander in Noordwes. Ruimtelike analises het getoon dat subpopulasies wat deur 'n kort afstand (binne 200 m) geskei word, tipies nie betekenisvol geneties gedifferensieer was nie, maar dat die wat deur langer afstande geskei is, soms betekenisvol gedifferensieer was. Temporale analises in die Noordwes boord het getoon dat seisoenale populasies nie betekenisvol geneties gedifferensieer was nie. In teenstelling hiermee, was seisoenale populasies van die Mpumalanga-boord betekenisvol gedifferensieer, waarskynlik weens hoër reënval en siektedruk. Studies op 'n provinsiale vlak in Suid-Afrika het getoon dat tien *P. citricarpa*-populasies wat die vyf SSV provinsies verteenwoordig, nie betekenisvol geneties gedifferensieer was nie. Gebaseer op geen- en genotipiese diversiteit en die aantal privaat allele, is KwaZulu-Natal of Limpopo waarskynlik die provinsies waar die patogeen eerste gevestig het. Daar is bewys dat die Oos-Kaap die provinsie is waar die laaste introduksie in Suid-Afrika plaasgevind het. Ten spyte daarvan dat suurlemoenbome wat oorvleuende oeste het, moontlik verhoogde geleenthede vir klonale voortplanting bied, het *Citrus* spp. (suurlemoene vs. lemoene) nie 'n effek op die populasie-struktuur gehad nie.

Introduction

The fungal pathogen, *Phyllosticta citricarpa* McAlpine) Aa, is the causative organism of the disease known as Citrus Black Spot (CBS). This fungal disease influences global citrus production and trade. CBS has an almost worldwide distribution, but is currently not known to occur in Europe, Chile, Japan and New Zealand. In the countries where CBS does occur, it often does not exist in all the production areas (Miles *et al.*, 2008; Carstens *et al.*, 2012; Wang *et al.*, 2012). Some of South Africa's most important trading partners such as the European Union, Japan, United States of America (USA), India, Iran and Reunion have identified CBS as being of quarantine importance. *Phyllosticta citricarpa* was reported for the first time from Australia in 1895 (Benson, 1895). The pathogen and disease was first reported in South Africa (SA) in 1929 from the KwaZulu-Natal province (Doidge, 1929). Although the epidemiology of the CBS disease in SA and other countries has been unravelled and well documented (Kotzé, 1963, 1981, 2000; Spósito *et al.*, 2007, 2008, 2011; Truter, 2010), limited information is available on the pathogen's global population structure, mode of reproduction, global introduction pathways and the effect of distance, season and *Citrus* spp. on the population structure in South Africa. Currently, it is believed that this pathogen originated with its host from South East Asia from where several migrations across the globe occurred. This hypothesis, however, requires investigation using a population genetics approach. The fungus can infect fruit, leaves and twigs. Two types of spores can be produced namely waterborne conidia (asexual) and windborne (sexual) ascospores.

Objectives

1. To develop additional informative genetic markers (SSR markers) to determine how many genetically differentiated populations of *P. citricarpa* exist in the world in order to infer introduction pathways and possibly the centre of origin of the pathogen and to investigate the mode of reproduction of *P. citricarpa* on a global scale.
2. To determine the relative contribution of sexual and asexual spores to disease development in two citrus orchards located in different climatic production regions in South Africa (spatial and temporal analysis of CBS populations).

3. To determine the population structure of *P. citricarpa* in different citrus production areas in South Africa and to investigate the effect of *Citrus* spp. (lemons vs. oranges) on the population structure.

Materials and methods

To develop additional informative genetic markers (SSR markers) to determine how many genetically differentiated populations of *P. citricarpa* exist in the world in order to infer introduction pathways and possibly the centre of origin of the pathogen and to investigate the mode of reproduction of *P. citricarpa* on a global scale.

Samples were collected from different geographical areas on five different continents, namely China, Brazil, Australia, South Africa and the United States of America. International collaborators assisted with sampling. A total number of 383 isolates were obtained.

Since there were no polymorphic markers available for use in population genetic studies in *P. citricarpa*, new markers were developed. Firstly, sequence data from known gene regions were investigated for polymorphisms. Very low levels of sequence polymorphisms were obtained when four known gene regions (Chitin synthase I, Calmodulin, second largest subunit of RNA polymerase II and β -tubulin) were sequenced in a subset of *P. citricarpa* isolates. As these markers could not be used for population genetic analyses, 8 SSR markers were developed using next generation sequencing techniques. A total of 383 isolates were genotyped with 15 SSR markers (the eight markers developed in this study as well as seven SSR markers published in 2016 (Wang *et al.*, 2016). Data was analysed using Genemapper (Applied Biosystems), GeneAlex v 6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.*, 2014; R Core Team, 2013) to determine the gene and genotype diversity amongst and within populations. Mating type primers, developed by Wang *et al.* (2016) in the USA, were used to determine the mating types of 196 isolates that represented a clone-corrected dataset.

To determine the relative contribution of sexual and asexual spores to disease development in two citrus orchards located in different climatic production regions in South Africa (spatial and temporal analysis of CBS populations).

The study was conducted in two lemon orchards in South Africa (the one orchard in Mpumalanga (subtropical) and the other in North West (semi-arid)), over two seasons (2012 and 2013). A total of 599 isolates were genotyped with the 15 SSR markers. Data was analysed using Genemapper (Applied Biosystems), GeneAlex v 6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) to determine the gene and genotype diversity amongst and within populations. Mating type primers, developed by Wang *et al.* (2016) in the USA, were used to determine the mating types of 203 isolates that represented a clone-corrected dataset.

To determine the population structure of *P. citricarpa* in different citrus production areas in South Africa and to investigate the effect of *Citrus* spp. (lemons vs. oranges) on the population structure.

In order to determine the population structure of the pathogen in South Africa and to determine the effect of *Citrus* spp. on the population structure, *P. citricarpa* isolates of ten populations from five different production areas were analysed. A total of 274 isolates were genotyped with 15 SSR markers. Data was analysed using Genemapper (Applied Biosystems), GeneAlex v 6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) to determine the gene and genotype diversity amongst and within populations. Mating type primers, developed by Wang *et al.* (2016) in the USA, were used to determine the mating types of 133 isolates that represented a clone-corrected dataset.

Results and discussion

To develop additional informative genetic markers (SSR markers) to determine how many genetically differentiated populations of *P. citricarpa* exist in the world in order to infer introduction pathways and

possibly the centre of origin of the pathogen and to investigate the mode of reproduction of *P. citricarpa* on a global scale

A total of 383 *P. citricarpa* isolates (12 populations from five countries) were genotyped with seven published polymorphic simple sequence repeat (SSR) markers and eight newly developed SSR markers (15 SSR markers in total). Data was analysed using Genemapper (Applied Biosystems). GeneAlex v6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) were used to determine the gene and genotype diversity amongst and within populations. To determine the relationships among populations, minimum spanning networks were constructed based on Bruvo's distance using the R package Poppr. One hundred and forty nine (149) multilocus genotypes (MLGs) were identified. The populations from China and Australia contained the highest gene and genotypic diversity. Principal component analyses (PCoA) also differentiated the Chinese populations from all other populations. Pairwise *PhiPT* values indicated that the genetic differentiation varied significantly amongst some, but not all countries and supported the PCoA results. The low genetic diversity among the five South African *P. citricarpa* populations and in the Brazilian and USA populations is an indication of founder populations. The high levels of connectivity among the *P. citricarpa* populations in South Africa, Australia and Brazil is most likely due to exchanges of plant material dating back to the establishment of the citrus industries in these countries. In all the populations from Australia, Brazil, China and South Africa, the mating-type frequencies did not deviate significantly from a 1:1 ratio based on Chi-square analyses (Fisher and Yates, 1963). In contrast, all the isolates obtained from the USA, contained only a single mating type.

To determine the relative contribution of sexual and asexual spores to disease development in two citrus orchards located in different climatic production regions in South Africa (spatial and temporal analysis of CBS populations)

Two commercial lemon orchards, situated in the Mpumalanga and North West provinces, with contrasting climates and a history of CBS, were included in the study. The orchard in the North West province was a 15-year-old Eureka lemon orchard located near Brits, with a BSh Köppen-Geiger climate classification. BSh climates are described as semi-arid; low relative humidity, warm summers and mild winters. The Mpumalanga orchard was a 9-year-old Eureka lemon orchard, located near Mbombela (Nelspruit), with a Cwa Köppen-Geiger classification. Cwa climates are described as humid, subtropical; the summers are hot and the winters are dry. The two orchards were separated by approximately 400 km.

A total of 599 *P. citricarpa* isolates (373 isolates were from the North West orchard (200 in 2012 and 173 in 2013) and 226 isolates were from the Mpumalanga orchard (130 in 2012 and 96 in 2013) were obtained and genotyped with the 15 polymorphic simple sequence repeat (SSR) markers. Mating type specific primer pairs were used to determine the mating type of 203 isolates that represented a clone-corrected dataset.

The isolates were collected according to a structured orchard sampling strategy over two consecutive production seasons (2012 and 2013). The number of sampling sites and the layout varied between the orchards. In the North West orchard, four sampling sites were selected. The distances of sample sites varied based on their relative location to each other. Sample sites were separated at distances ranging from 50 m (sampling sites 1 vs. 2; 2 vs. 3; 3 vs. 4), to 100 m (sampling sites 1 vs. 3; 2 vs. 4), or 200 m (sampling sites 1 vs. 4) (Fig. 1A). The number of rows of trees between the samples sites varied with the highest number (34 rows) being between sample sites 1 and 4. In the Mpumalanga orchard, three sampling sites were selected. Sample sites were separated by 200 m (sampling sites 1 vs. 2), 300 m (sampling sites 1 vs. 3), or 400 m (sampling site 2 vs. 3) (Fig. 1B). The number of rows of trees between the sampling sites varied with the highest number (48 rows) being between sample sites 2 and 3, and the lowest being 35 rows between sampling sites 1 and 2. A sampling site consisted of a total of five trees located in three different rows; three of the sampled trees were in the middle row, and the other two trees were in the adjacent rows (cross figure).

Data was analysed using Genemapper (Applied Biosystems). GeneAlex v 6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) were used to determine the gene and genotype diversity amongst and within populations. An analysis of molecular variance (AMOVA), a pairwise *PhiPT* analyses and a discriminant analysis of the principal components (DAPC) was performed to determine the genetic differentiation of populations and subpopulations within and among orchards.

Sixty (60) MLGs were identified in the North West orchard in 2012 and 46 MLGs in 2013. In the Mpumalanga orchard, 55 MLGs were identified in 2012 and 36 MLGs in 2013. Spatial analyses at the orchard scale indicated that subpopulations that were separated by a short distance (within 200 m) were typically not significantly genetically differentiated, but those that were separated by larger distances (200 m to 400 m) were sometimes significantly differentiated. Temporal analyses in the North West orchard showed that seasonal populations were not significantly genetically differentiated (Fig. 2). In contrast, seasonal populations from the Mpumalanga orchard were significantly differentiated (Fig. 3). In both orchards, linkage disequilibrium analyses indicated that populations were sexual. Sexual reproduction was supported by the clone corrected mating type ratios not deviating significantly from a 1:1 ratio. Asexual clonal reproduction was evident from low genotype evenness values for seasonal- and some subpopulations. On a regional scale, the Mpumalanga seasonal populations were significantly genetically differentiated from the North West populations.

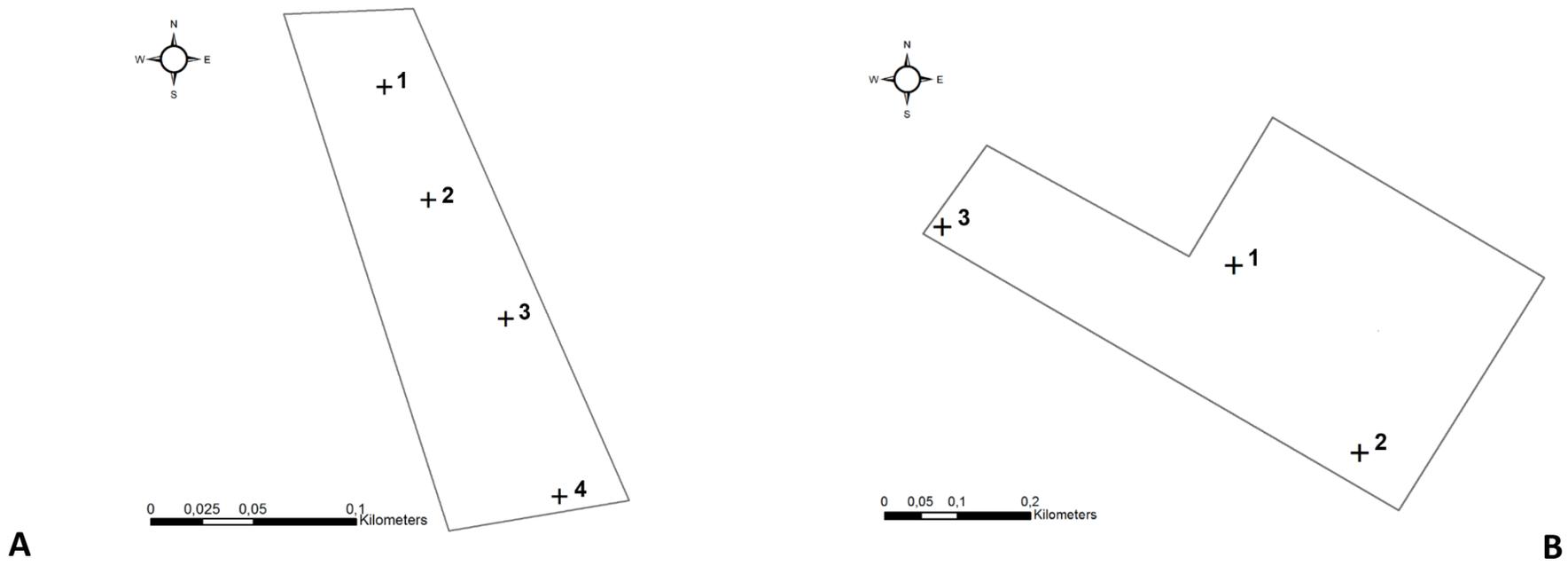


Figure 1. Sampling strategy used for studying the population structure of *Phyllosticta citricarpa* at the spatial- and temporal scales in two lemon orchards situated in the (A) North West and (B) Mpumalanga provinces in South Africa. In each orchard, the selected sampling sites are indicated by “+” along with the *P. citricarpa* subpopulation numbers. At each of the sampling sites, five trees located in a cross over two rows were selected.

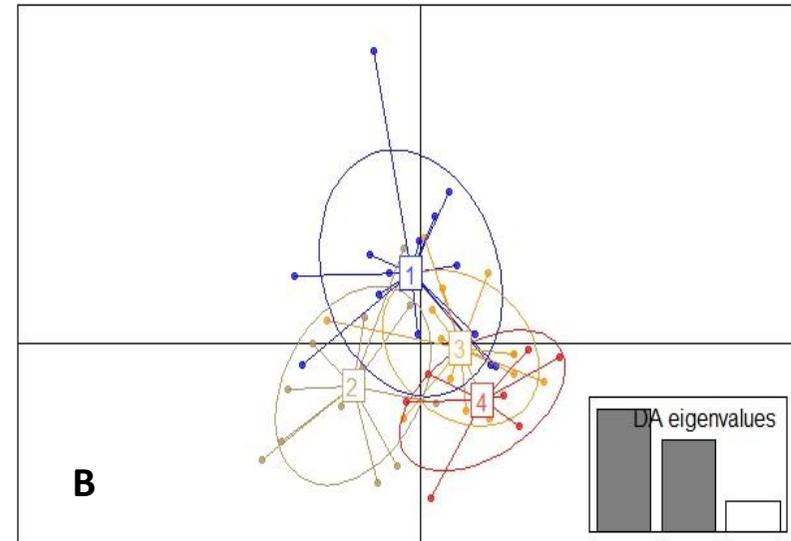
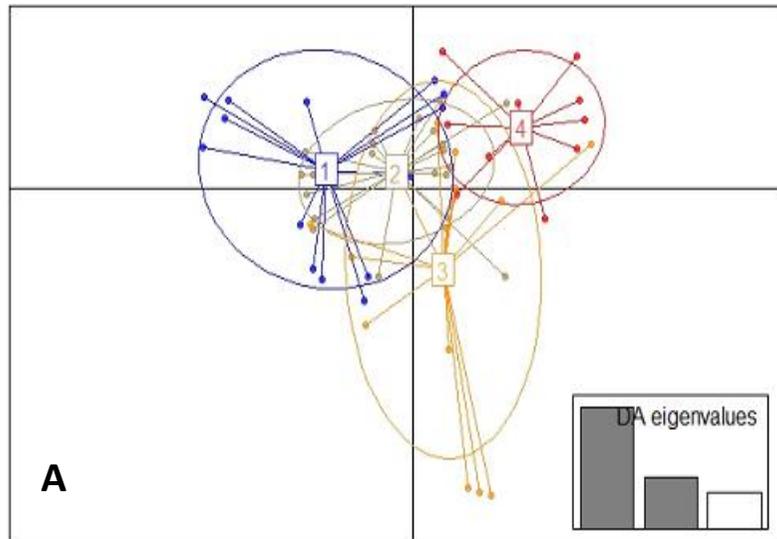


Figure 2. A discriminant analysis of principal components (DAPC) of *Phyllosticta citricarpa* subpopulations (clone corrected) sampled from a lemon orchard in the North West province in the (A) 2012 and (B) 2013 seasons. Subpopulations are indicated by numbers and different colours. The number of axes retained for the principal component analysis was 16 and 2 for the discriminant analysis. The eigenvalues represented 80% of the variation in both seasons.

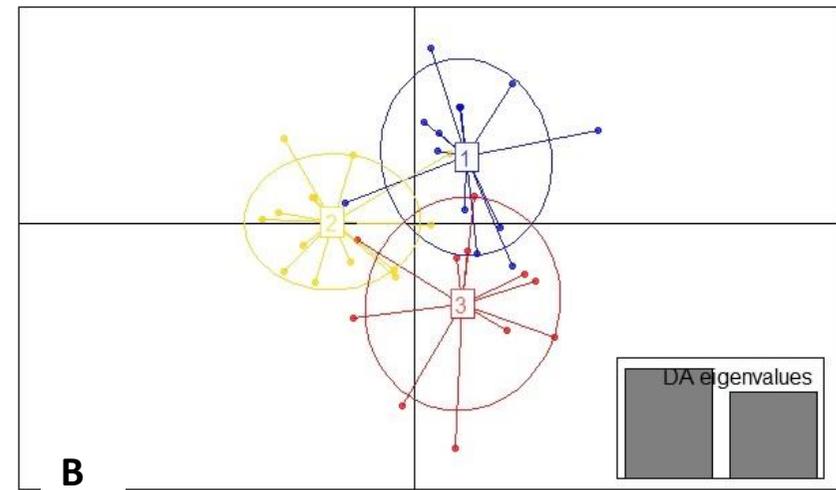
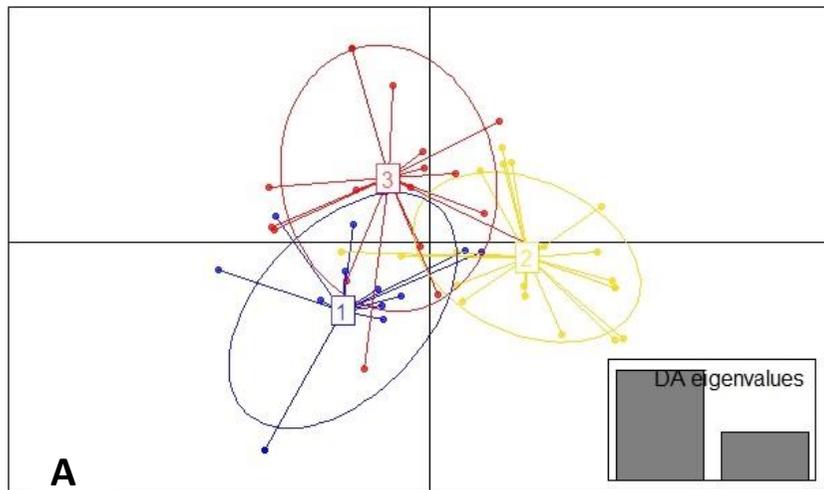


Figure 3. A discriminant analysis of principal components (DAPC) of *Phyllosticta citricarpa* subpopulations (clone corrected) sampled from a lemon orchard in the Mpumalanga province in the (A) 2012 and (B) 2013 seasons. Subpopulations are indicated by numbers and different colours. The number of axes retained for the principal component analysis was 24 and 2 for the discriminant analysis. The eigenvalues represented 80% of the variation in both seasons.

To determine the population structure of *P. citricarpa* in different citrus production areas in South Africa and to investigate the effect of *Citrus* spp. (lemons vs. oranges) on the population structure

A total of ten *P. citricarpa* populations were investigated to determine if the population structure of the pathogen differed in the five provinces where CBS occurs in South Africa (Fig. 4). Four populations were from the Eastern Cape, two from Mpumalanga, two from Limpopo, and one from each of the North West and KwaZulu-Natal provinces. Six of the populations were from two previous studies (Carstens *et al.*, 2017; Chapter 3 of the PhD dissertation). The other four populations were newly isolated in 2016. To study the effect of *Citrus* spp. (orange and lemon) on the population structure of *P. citricarpa*, nine populations (20 to 30 isolates per population) were investigated which were also used to compare populations from the different provinces. One lemon- and one orange population were included for Limpopo and Mpumalanga and one lemon population from the North West. In the Eastern Cape, two orange and two lemon populations were included.

A total of 274 *P. citricarpa* isolates were obtained and genotyped with the 15 SSR markers. Mating type specific primer pairs were used to determine the mating type of 133 isolates that represented a clone-corrected dataset. Data was analysed using Genemapper (Applied Biosystems). GeneAlex v6.5 (Peakall and Smouse, 2012) and R package Poppr (Kamvar *et al.* 2014; R Core Team, 2013) were used to determine the gene and genotype diversity amongst and within populations. An analysis of molecular variance (AMOVA), a pairwise *PhiPT* analyses and a discriminant analysis of the principal components (DAPC) was performed to determine the genetic differentiation of populations and subpopulations within and among orchards. A total of 89 MLGs were identified. The number of MLGs varied between the provinces. One of the populations from Limpopo had the highest number of MLGs. All four populations from the Eastern Cape had the lowest numbers of MLGs (Fig. 5).

AMOVA analysis showed that most genetic variation (88%) was distributed within populations and only 2% among citrus provinces. Populations from the five provinces were not significantly genetically differentiated. The Eastern Cape was confirmed as being the province into which the last introduction of *P. citricarpa* occurred as was evident from low gene and genotypic diversities of all populations within this province. The KwaZulu-Natal (only one population sampled) and Limpopo provinces had higher gene and genotypic diversities than the North West and Mpumalanga provinces. The Limpopo province had the highest private allele richness, followed by KwaZulu-Natal. Therefore, KwaZulu-Natal or Limpopo are the regions where the pathogen was likely first introduced. There might have been at least two separate introductions of the pathogen into South Africa, based on principal coordinate analyses, pairwise *PhiPT* analyses and the sharing of multilocus genotypes (MLGs) between populations (Fig. 6 and Fig. 7). All ten populations reproduced sexually based on linkage disequilibrium analyses. Asexual reproduction was evident from low genotype evenness values for some populations, which furthermore indicates clonal reproduction. Despite lemon trees having overlapping fruit crops, which potentially provide increased opportunities for clonal reproduction, *Citrus* spp. (lemon vs. oranges) did not have an effect on population structure as not all lemon populations were significantly genetically differentiated from all orange populations.

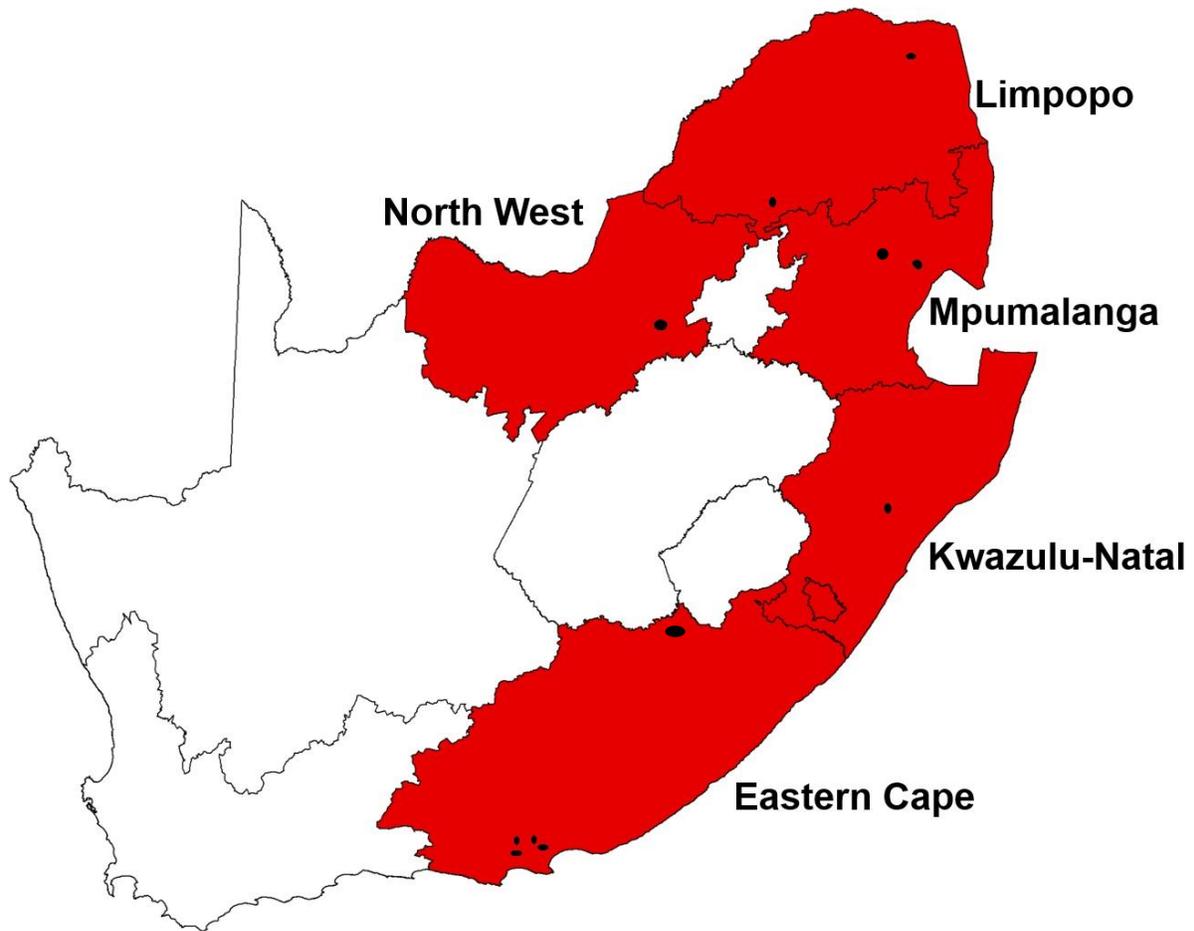


Figure 4. Five provinces (North West, Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape) in South Africa where *Phyllosticta citricarpa* populations were sampled from *Citrus* spp. orchards. The populations were used in studies to determine if *P. citricarpa* populations differed in the provinces and if *Citrus* spp. had an effect on population structure.

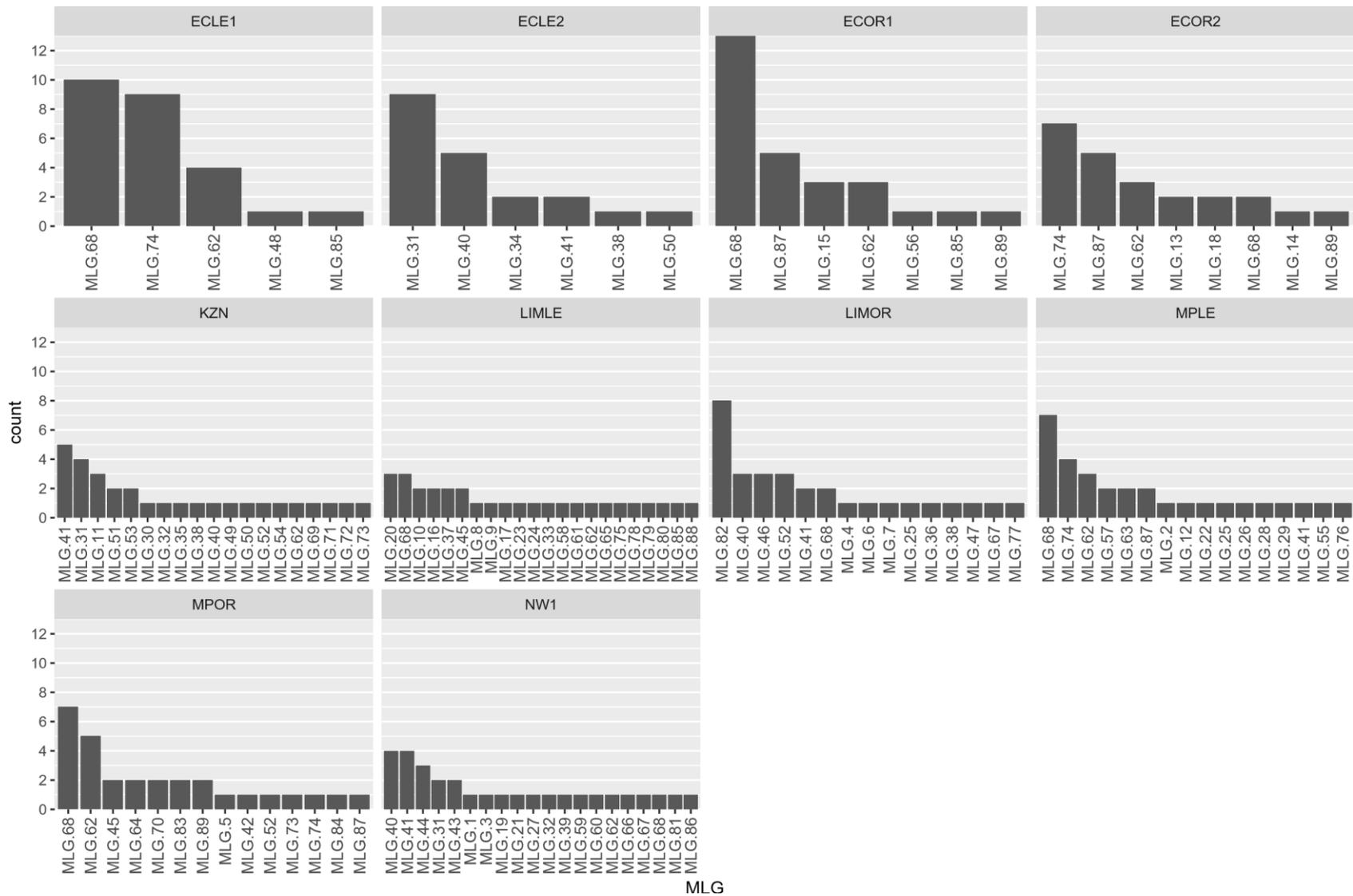


Figure 5. Occurrence of *Phyllosticta citricarpa* multilocus genotypes (MLGs) in ten South African citrus orchards.

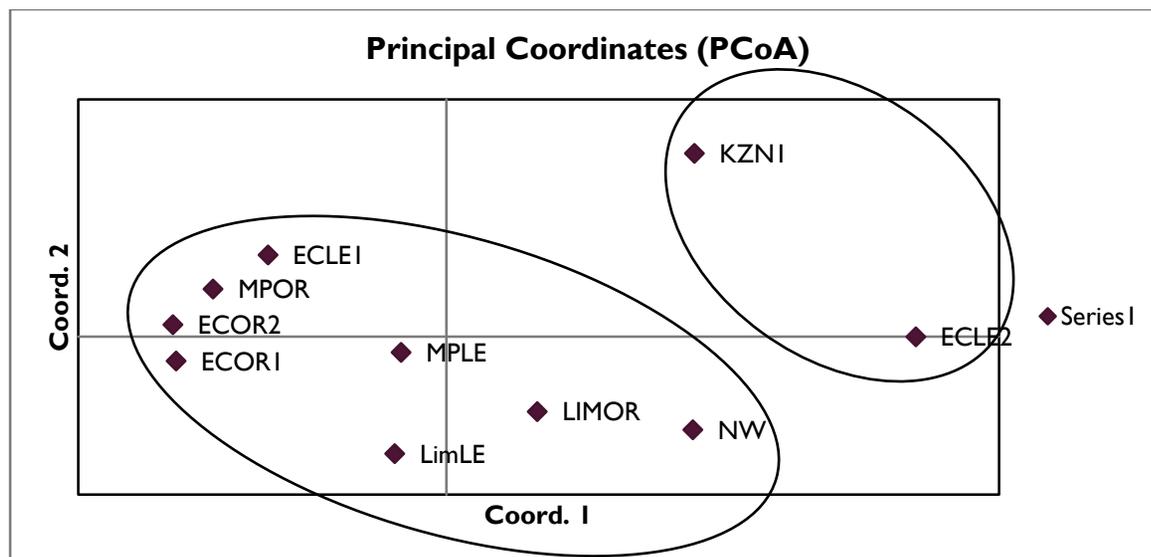


Figure 6. Principal coordinate analysis (PCoA) for ten *Phyllosticta citricarpa* populations collected in five provinces in South Africa, including the North West (NW), Mpumalanga (MPLE, MPOR), Eastern Cape (ECOR1, ECOR2, ECLE2 and ECLE1) and KwaZulu-Natal (KZN). Populations sampled from lemon orchards included ECLE1, ECLE2, LIMLE, MPLE and NW. The KZN population was obtained from a grapefruit orchard. The remaining populations were obtained from orange orchards. The axes explained 82.19% variation for Coordinate 1 and 8.66% for Coordinate 2.

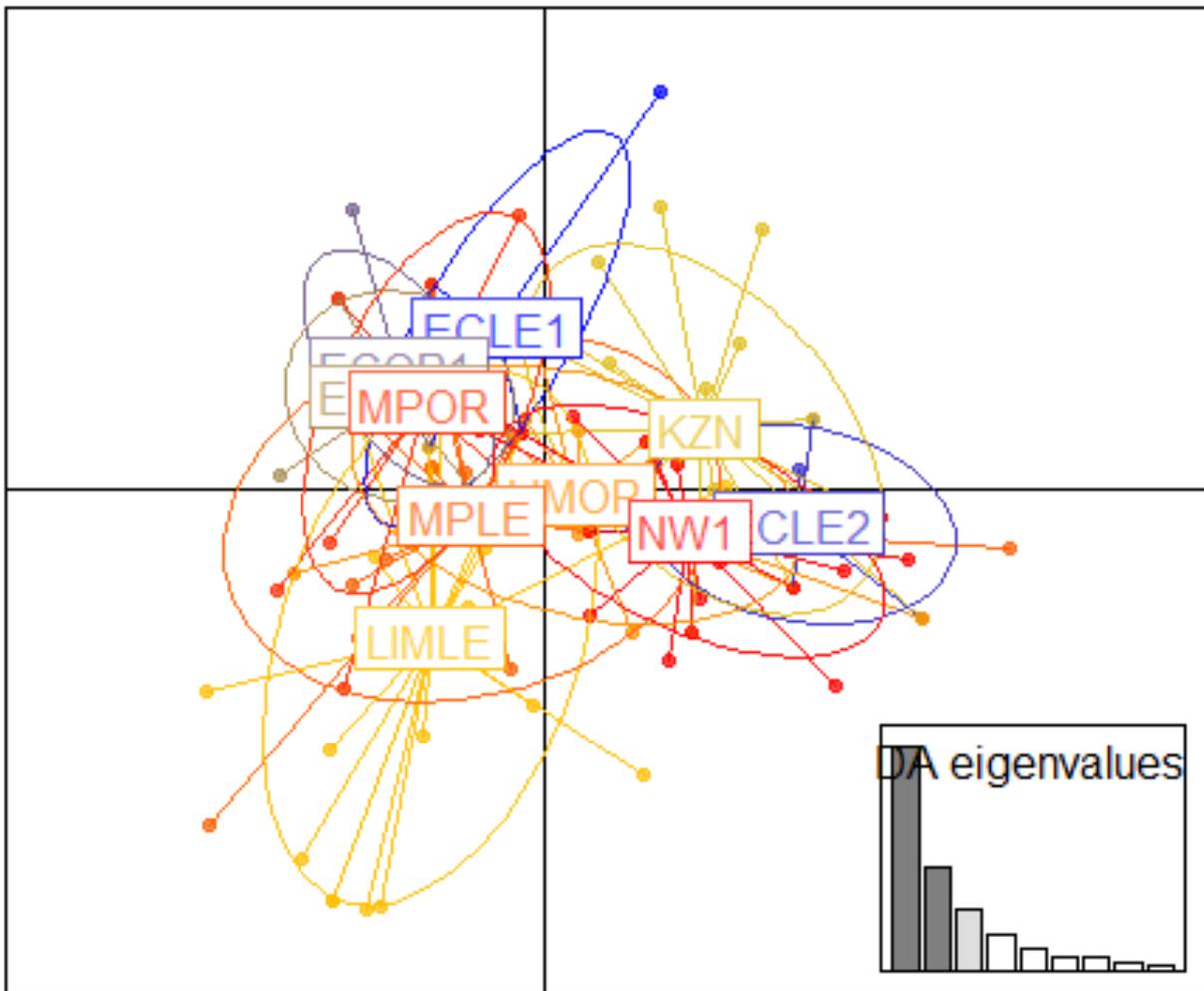


Figure 7. A discriminant analysis of principal components (DAPC) of *Phyllosticta citricarpa* populations (clone corrected) sampled from five provinces in South Africa, including the North West (NW), Mpumalanga (MPLE, MPOR), Eastern Cape (ECOR1, ECOR2, ECLE2 and ECLE1) and KwaZulu-Natal (KZN). Populations sampled from lemon orchards included ECLE1, ECLE2, LIMLE, MPLE and NW. The KZN population was obtained from a grapefruit orchard. The remaining populations were obtained from orange orchards. Populations are indicated by different colours. The number of axes retained for the principal component analysis was 17 and 3 for the discriminant analysis. The eigenvalues chosen represented more than 80% of the total variation.

Conclusions

Citrus black spot is a fungal disease, caused by *P. citricarpa*, which influences global citrus production and trade. The epidemiology of *P. citricarpa* has been studied in many countries where the disease is present, but limited information is available on the pathogen's population genetic structure. Markers available to conduct population genetic studies have identified very low or no polymorphisms in *P. citricarpa* populations (Wang *et al.*, 2016). The reproductive system of *P. citricarpa* was only recently resolved, where the mating type locus containing the MAT 1-1-1 or MAT 1-2-1 genes was identified (Wang *et al.*, 2016, Amorim *et al.*, 2017) and the mating of opposite mating type isolates was achieved under artificial culture conditions (Tran *et al.*, 2017). The aims of this project were to develop more informative markers to determine the distribution of genetic variation in global and South African *P. citricarpa* populations, to investigate the reproductive mode of the pathogen on an international and national scale and to determine the effect of distance (orchard scale), season (temporal - orchard and regional scale) and *Citrus* spp. on the population structure of *P. citricarpa*.

The findings of objective 1 were based on the results and analyses of genotyping data of *P. citricarpa* populations from South Africa, USA, Australia, China and Brazil, using seven published (Wang *et al.*, 2016) and eight newly developed polymorphic simple sequence repeats (SSR). The study showed that populations differed in their connectivity and differentiation from each other. Limited connectivity was found between the Chinese populations and the populations from the other countries. There was, however, high levels of connectivity between South Africa, Australia and Brazil, as well as between South Africa, Australia and the USA. These findings are most likely due to exchanges of plant material and the associated *P. citricarpa* genotypes dating back to the establishment of the citrus industries in these countries. The Chinese and Australian populations had the highest level of genetic diversities, which correlates with the origin of the *Citrus* host and the first description of CBS in Australia (Benson, 1895). This finding is consistent with a co-evolutionary relationship between the pathogen on its wild hosts. The populations from Brazil, USA and South Africa exhibited characteristics of founder populations, which correlates with the known history of CBS in these countries. Therefore, the source of the South African population could be from the Far East or Australia. Australia or South Africa were identified as a likely source of the Brazilian population. Australia or South Africa can also be the source of the USA population.

Migration has thus, played an important role in determining the population structure of *P. citricarpa* in several countries. Both mating types were found in the populations from South Africa, Australia, China and Brazil. The USA populations, however, contained one mating type only. Linkage disequilibrium analyses indicated the occurrence of sexual reproduction and that asexual reproduction may be important in the pathogen's life cycle. The reproductive structure of the pathogen and presence or absence of both mating types will thus be very important in determining the population's structure and epidemiology of the disease in different countries.

To further investigate the population structure of *P. citricarpa* in South Africa at the orchard spatial (distance) and temporal (seasonal) scales, as well as the reproductive system, a detailed study in two orchards was conducted (objective 2). Populations were sampled according to a distance based structure over two seasons (2012 and 2013), from two lemon orchards differing in climate (Mpumalanga province - sub-tropical and North West province - semi-arid). The populations were genotyped using 15 SSR markers. Spatial analyses at the orchard scale indicated that subpopulations separated by shorter distances (within 200 m), were typically not significantly genetically differentiated, while those separated by longer distances were sometimes significantly differentiated. Temporal analyses of the North West orchard showed that seasonal populations were not significantly genetically differentiated. In contrast, seasonal populations from the Mpumalanga orchard were significantly differentiated, most likely due to higher rainfall and disease pressure in the Mpumalanga orchard. Mating type ratios in both orchards did not deviate significantly from a 1:1 ratio. Linkage disequilibrium analyses indicated that *P. citricarpa* reproduces sexually and asexually.

The effect of *Citrus* spp. on population structure in South Africa, and whether *P. citricarpa* populations differ among the five provinces where CBS occurs, were investigated along with the reproductive system in objective 3. Ten populations from five provinces were genotyped. To study the effect of *Citrus* spp., nine of these populations (obtained from oranges and lemons) were analysed. Analyses of the provincial population

structure indicated that the KwaZulu-Natal and Limpopo populations had the highest genetic diversities, while the Eastern Cape had the lowest. This correlates with the historical records of the time period that the disease has been established in the different provinces. Results indicated that there was most likely two separate introductions of the pathogen into South Africa. Populations from the different provinces were not significantly genetically differentiated. *Citrus* spp., specifically oranges and lemons, did not have an effect on *P. citricarpa* population structure. Linkage disequilibrium analyses and mating type ratios further supported the findings of objective 2 and 3 in that *P. citricarpa* reproduces sexually and asexually.

This was the first study using a population genetics approach to better understand the biology and epidemiology of CBS at a global, regional and orchard scale. The study provides insight into CBS introduction pathways. Although China is considered to be the centre of origin of the host and the pathogen, no connectivity could be identified between the western and eastern countries. Both mating types were present in all the countries, except for the USA. The study has shed new light on the population structure of *P. citricarpa* globally and in South Africa. Important evolutionary forces that affect the population structure of *P. citricarpa* at the global scale include the reproductive system of the pathogen and migration.

Technology transfer

Peer reviewed article:

Carstens, E., Linde, C.C., Slabbert, R., Miles, A.K., Donovan, N.J., Hongye, L., Dewdney, M.M., Glienke, C., Schutte, G.C., Fourie, P.H. and McLeod, A. 2017. A Global Perspective on the Population Structure and Reproductive System of *Phyllosticta citricarpa*, a pathogen of citrus. *Phytopathology* 107: 758-768.

Oral presentations:

Friday Forum – Department Plant Pathology, University of Stellenbosch: Spatial and temporal analysis of *Phyllosticta citricarpa* populations in two South African lemon orchards.

PhD Defence – Department Plant Pathology, University of Stellenbosch: Population structure, sex and spatial distribution of *Phyllosticta citricarpa*, the citrus black spot pathogen.

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

Project 970 (Ongoing) by C. Kotze (CRI)

Summary

Trials were conducted at two different sites during 2016-17, one on Valencia oranges at Crocodile Valley Co. and the other on Eureka Lemons at Fountains Lemon Project. At both sites, the disease pressure was not ideal, which was surprising as it was a season very conducive to infection. However, all products tested at Crocodile Valley Co. yielded more than 99.4% clean fruit compared to the 97.2% clean fruit of the untreated control, while the experimental treatments evaluated at Fountains Lemon Project yielded more than 94.5% clean fruit compared to the untreated control's 82.4%.

Opsomming

Gedurende die 2016-17 seisoen is daar van twee onderskeie proefpersele gebruik gemaak, een op Valencia lemoene te Crocodile Valley Co. en die ander op Eureka suurlemoene te Fountains Lemon Project. By beide van die proefpersele was die siektedruk nie ideaal gewees nie, wat baie verbasend was omdat kondisies voordelig was vir infeksie. Daar kon agter bepaal word dat alle behandelings wat getoets was by die Crocodile Valley Co. perseel meer as 99.4% skoon uitvoerbare vrugte gelewer het in ter vergelyking met die 97.2% van onbehandelde kontrole, terwyl die behandelings by die Fountains Lemon Project gelei het tot meer as 94.5% skoon uitvoerbare vrugte teenoor die 82.4% van die onbehandelde kontrole.

Introduction

Citrus black spot (CBS), caused by *Phyllosticta citricarpa* (McAlpine) van der Aa, affects all commercial citrus cultivars only in the summer rainfall regions of the world. Control of the disease is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in the southern hemisphere (Kotze 2000). The most important inoculum source of CBS is airborne ascospores. Pseudothecia

of the fungus develop on dead infected leaves on the orchard floor within 40-180 days after leaf drop, depending on the temperature and frequency of wetting (Kiely 1948; Kotze 1963, 1981, 2000). Once mature, ascospores are discharged during rain spells. Ascospores are dependent on converging currents and favourable environmental conditions to reach a suitable host substrate, since the maximum vertical distance of ascospore ejection from a pseudothecium is 10-12 mm and the horizontal disease dispersion occurs at distances below 24.7m (Kiely 1948; Kotze 1963). When protective fungicides such as copper and dithiocarbamates are used to control CBS, spray applications have to be carefully timed to coincide with the critical infection period (McOnie 1964). Spore trapping with an Interlock volumetric spore trap® and sampler is used to determine the first onset of ascospore release in South Africa (Kotze 2000).

A four-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes (Brodrick 1970; Schutte *et al*, 1997). However, alternating copper fungicides with mancozeb in a four-spray programme solved this problem (Timmer *et al*, 1998). Protective fungicides became less popular after the release of post-infection benzimidazole fungicides such as benomyl. (Kellerman and Kotze, 1977) In 1971, the introduction of a single benomyl application in a tank mixture with mancozeb and mineral spray oil came as a breakthrough as it replaced copper and dithiocarbamates that must be applied in a four-spray protective schedule. Since the detection of *G. citricarpa* resistance to benomyl in South Africa in 1981, emphasis has shifted back to the use of contact fungicides for disease control (Kellerman and Kotze, 1977; Kotze 1981). Field evaluations using strobilurins for the control of CBS in 1993 also came as a breakthrough. Two applications of kresoxim-methyl and azoxystrobin at respective rates of 0.10 and 0.075 g a.i./liter in tank mixtures with mancozeb (1.2 g a.i./liter) and mineral oil (0.5% [vol/vol]/liter of water) were initially recommended (Schutte *et al*. 1996; 2003). The possibility that CBS may develop resistance to the strobilurins justifies the incorporation of two additional mancozeb applications before and after the strobilurin applications in October and January. Testing of novel control measures against CBS, is therefore regarded as a priority even if it includes tank mixtures with current registered fungicides (Nel 2003).

A newly developed product from River Bioscience, called RB1™ (Dipotassium phosphate) has just been registered for the control of citrus black spot. The product is registered in a four spray-programme which alternates RB1™ either with a copper or a dithiocarbamate formulation at 28 day intervals. Ultimately RB1™ will be used as an alternative to mancozeb in a standard strobilurin CBS spray programme or as a four-spray programme on its own.

Furthermore, in a quest to lower mancozeb residues on fruit, the three different copper formulations (copper hydroxide, cuprous oxide and copper oxychloride) could be applied individually in tank mixtures with mancozeb at half of each active ingredients' registered rates. A new copper hydroxide formulation, Product A, has been submitted for evaluations for the control of CBS this season. Product A has a smaller particle size than the other registered copper hydroxide formulations, which equates to a better coverage.

Objective

To evaluate any potential fungicides and alternative spray programmes for the control of CBS.

Materials and methods

Two trial sites were used during the 2016-17 season, a 29-year-old "Valencia" orange (*Citrus sinensis* (L.) Osbeck) orchard grafted onto Volckameriana rootstock (*Citrus x limon* (L.) Osbeck) at Crocodile Valley Co. in Nelspruit, Mpumalanga and a 10-year-old Eureka lemon (*Citrus limon* (L.) Osbeck) orchard grafted on Rough lemon (*Citrus jambhiri* Lush) rootstock at Fountains Lemon Project in White River, Mpumalanga. Applications occurred at predetermined intervals starting in October 2016 and ending in February 2017. At the first trial site, RB1™ was evaluated as a standalone four-spray programme at 100 and 200ml/100L and in two experimental CBS spray programmes consisting of only two applications during the season (Table 1). Rates of copper oxychloride (Demildex; 100g/100L), cuprous oxide (Nordox; 45g/100L) and copper hydroxide (Kocide; 75g/100L) were, respectively, halved in tank mixtures with a half rate of mancozeb (100g/100L). At the second trial site, Product A was tested at two different rates of 175g/100L and 350g/100L, respectively, in a tank

mixture with a generic azoxystrobin and mineral oil (Table 2). RB1™ was evaluated as well at this site, once again in a standalone four-spray programme at two different rates. The trial was laid out such that each treatment consisted of five randomised singletree plots; these trees were selected based on uniformity in canopy density and tree size. Treatments were applied using a trailer-mounted, high-volume, high-pressure (2,500 - 3,000 kPa) sprayer with two hand-held spray guns. All trees were sprayed to the point of runoff ($\pm 10000\text{l/ha}$) to ensure thorough coverage. At fruit maturity in July or August, CBS severity was to be rated on 100 fruit per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data was to be analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Table 2. Application dates and rates of fungicides applied for the control of Citrus black spot at Crocodile Valley Co. in Nelspruit, Mpumalanga during the period 12 October 2016 to 16 January 2017

No	12 October 2016	7 November 2016	16 November 2016	5 December 2016	12 December 2016	3 January 2017	16 January 2017
1	Untreated Control	-	-	-	-	-	-
2	Dithane (200g)	X		X		X	
3	Demildex (200g)		X		X		X
4	Dithane/Demildex (100g/100g)	X		X		X	
5	Dithane/Demildex (100g/100g)		X		X		X
6	Dithane/Kocide (100g/75g)	X		X		X	
7	Dithane/Kocide (100g/75g)		X		X		X
8	Dithane/Nordox (45g/100g)	X		X		X	
9	Dithane/Nordox (45g/100g)		X		X		X
10	RB1 (100ml)	X		X		X	
11	RB1 (200ml)	X		X		X	
12	Benlate/Azoxy/Oil (25g/20ml/250ml)		X				X
13	RB1/Azoxy/Oil (100ml/20ml/250ml)		X				X
14	Benlate/RB1/Oil (25g/100ml/250ml)		X				X

Table 2. Application dates and rates of fungicides applied for the control of Citrus black spot at Fountains Lemon Project in White River, Mpumalanga during the period 18 October 2016 to 14 February 2017

No	18 October 2016	15 November 2016	24 November 2016	13 December 2016	5 January 2017	9 January 2017	14 February 2017
1	Untreated Control	-	-	-	-	-	-
2	Dithane (200g)	X		X		X	
3	RB1 (100ml)	X		X		X	
4	RB1 (200ml)	X		X		X	
5	Dithane (200g)	Dithane/Ortiva/Oil (150g/20ml/250ml)			Dithane/Ortiva/Oil (150g/20ml/250ml)	X	Dithane (200g)
6	Product A* (175g)		Product A/Azoxy/Oil (175g/20ml/250ml)		Product A/Azoxy/Oil (175g/20ml/250ml)		Product A (175g)

7	Product A (350g)		Product A/Azoxy/Oil (350g/20ml/250ml)		Product A/Azoxy/Oil (350g/20ml/250ml)		Product A (350g)
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*Spray programme only commenced on 25 October 2016 due to the late arrival of the product

Results and discussion

Trial conducted at Crocodile Valley Co.

With very low natural infection levels [97.2% symptomless fruit in control (Table 3)], all the experimental programmes and treatments had significantly more clean fruit than the untreated control. Other than the untreated control, only one treatment did not have 100% clean fruit and that was the 100 mL/100 L rate of RB1. It was nonetheless still statistically cleaner than the untreated control. The low level of natural infection in the orchard was quite surprising as it has a history of very high CBS infection. The results suggest that at some critical infection stage, the orchard might have been sprayed by the grower. However, if this was not the case, all the tested treatments and tank mixtures were then efficient in controlling CBS under low disease pressure conditions.

Trial conducted at Fountains Lemon Project

All the treatments evaluated had statistically more symptomless fruit than the untreated control except for the industry standard, Dithane, which yielded 94.5% clean fruit; a figure not significantly different from the 82.4% obtained with the untreated control (Table 4). The amount of control achieved by Dithane was, however, not significantly different from that achieved by the other experimental treatments. The programme containing Product A and the generic azoxystrobin yielded 97% and 95.7% clean fruit at the respective rates of 175 g/100 L and 350 g/100 L. This suggests that Product A is more effective at a lower rate. Control achieved by Product A, regardless of rate applied, was not statistically different from that achieved by the industry standard mancozeb, strobilurin and mineral oil spray programme which yielded 98.3% clean fruit. RB1, at the tested rates of 100 mL/100 L and 200 mL/100 L, had more clean fruit (98.4% and 98.0%, respectively) than the Dithane treatment, but not statistically so. Although the disease pressure was not ideal, all treatments performed decently under moderate disease conditions.

Table 3. Evaluation of spray programmes for the control of Citrus black spot conducted at Crocodile Valley Co., Nelspruit, Mpumalanga during the 2016-17 season

Treatment		Dosage (g/ml per 100L water tank mixture)	Percentage of fruit in each class		
			Lesions/fruit ^x		
			0	1-3	≥4
1	Untreated Control	-	97.2b	1.8a	1.0a
2	Dithane ^y	200g	100.0a	0.0b	0.0b
3	Demildex	200g	100.0a	0.0b	0.0b
4	Demildex/Dithane (25 day interval)	100g/100g	100.0a	0.0b	0.0b
5	Demildex/Dithane (35 day interval)	100g/100g	100.0a	0.0b	0.0b
6	Kocide/Dithane (25 day interval)	75g/100g	100.0a	0.0b	0.0b
7	Kocide/Dithane (35 day interval)	75g/100g	100.0a	0.0b	0.0b
8	Nordox/Dithane (25 day interval)	45g/100g	100.0a	0.0b	0.0b
9	Nordox/Dithane (35 day interval)	45g/100g	100.0a	0.0b	0.0b
10	RB1	100ml	99.6a	0.4b	0.0b

11	RB1	200ml	100.0a	0.0b	0.0b
12	Benomyl/Ortiva/Oil	25g/20ml/250ml	100.0a	0.0b	0.0b
13	RB1/Ortiva/Oil	100ml/20ml/250ml	100.0a	0.0b	0.0b
14	Benomyl/RB1/Oil	25g/100ml/250ml	100.0a	0.0b	0.0b

*Means followed by the same letter in the same column do not differ significantly

^ySpray dates were: 12 October 2016; 7 November 2016; 16 November 2016; 5 December 2016; 12 December 2016; 3 January 2017; 16 January 2017

Table 4. Evaluation of spray programmes for the control of Citrus black spot conducted at Fountains Lemon Project, Nelspruit, Mpumalanga during the 2016-17 season

Treatment	Dosage (g/ml per 100L water tank mixture)	Percentage of fruit in each class			
		Lesions/fruit ^x			
		0	1-3	≥4	
1	Untreated Control	-	82,4b	2,8a	14,8a
2	Dithane ^y	200g	94,5ab	0,0b	5,5ab
3	RB1	100ml	98,4a	0,0b	1,6b
4	RB1	200ml	98,0a	0,0b	2,0b
5	Dithane/Dithane+Ortiva+Oil/ Dithane+Ortiva+Oil/Dithane	200g/150g+20ml+250ml/ 150g+20ml+250ml/200g	98,3a	0,0b	1,7b
6	Product A/Product A+Strobe+Oil/ Product A+Strobe+Oil/Product A	175g/175g+20ml+250ml/ 175g+20ml+250ml/175g	97,0a	0,0b	3,0b
7	Product A/Product A+Strobe+Oil/ Product A+Strobe+Oil/Product A	350g/350g+20ml+250ml/ 350g+20ml+250ml/350g	95,7a	0,0b	4,3ab

^xMeans followed by the same letter in the same column do not differ significantly

^ySpray dates were: 18 October 2016; 25 October 2016; 15 November 2015; 24 November 2016; 13 December 2016; 5 January 2017; 14 February 2017

Conclusions

Although the disease pressure was not ideal at both trial sites, all the experimental treatments and tank mixtures were successful in controlling citrus black spot. RB1 as a treatment on its own performed well under the circumstances, but should be evaluated as a replacement of mancozeb in the industry standard strobilurin spray programme. Furthermore, the two-application programme consisting of RB1, mineral oil and a strobilurin should be investigated in future season's trials as there is a market for this in the areas of low pest prevalence. Further research should be conducted on the copper/mancozeb tank mixtures as the initial results under low infection levels suggest some merit in this type of programme. The effect that the use of half rates could have on the fruit MRL's should also be investigated. Finally, the use of the experimental Product A in conjunction with any strobilurin should also be investigated further as the product has a lower metallic copper content than its competitors. This will in turn have a smaller impact on the environment over time, while still being as efficient.

Future Research

Research for the coming season will consist of further evaluation of the promising spray programmes as well as treatments identified during the current season. Furthermore, more focus should be on the development of cost effective programmes. This can be achieved by reducing the number of sprays during the season without sacrificing efficiency. There is still a lot of market pressure on several of our most effective actives, such as benzimidazoles and dithiocarbamates. Alternatives should be identified and developed.

Technology transfer

Talks at study groups and workshops; while the results will be presented at the biennial CRI Symposium in August 2018.

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3.4.6 **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 C. Kotze (CRI)

Summary

With a growing tendency in the South African citrus industry to erect netting over high value citrus cultivars, there were fears that the change in microclimate could contribute to a change in disease severity in traditionally low risk areas. Therefore, a suitable Nova trial site under net was selected at the JB Group situated between Ohrigstad and Hoedspruit. Of late there has been growing pressure from certain European retailers to reduce the number of detectable residues on citrus, the focus this season was on developing programmes that can possibly reduce these by combining different contact actives at half rates and without sacrificing efficacy. Unfortunately, the disease pressure was extremely low due to very dry conditions in the area and effectivity data could not be analysed sufficiently.

Opsomming

Weens die groeiende tendens in die oprig van net strukture oor hoë waarde sitrus varieteite, was daar vrese dat hierdie die mikroklimaat tot so 'n mate kon verander dat dit 'n effek op siektedruk kon hê in tradisionele lae druk areas. Daar is toe besluit op 'n 10 jaar oue 'Nova' mandaryn boord van die JB Groep, wat geleë is tussen Ohrigstad en Hoedspruit. Daar is ook groeiende druk van veral die Europese kettingwinkels om die

hoeveelheid opspoorbare residue op sitrus te verlaag, om hierdie rede is daar gedurende die seisoen gekyk na die verlaging hiervan deur twee tipes aktiewe produkte teen halwe dosisse van elk te meng. So kon ons die effektiwiteit bepaal sowel as nuwe effekte van die mengels. Ongelukkig, weens die droogte wat in die area geheers het, was die infeksie druk vreeslik laag en kon die effektiwiteitsdata nie noemenswaardig ontleed word nie.

Introduction

Alternaria brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa (Dalikilic *et al*, 2005; Peever *et al*, 2005). Susceptibility to ABS is a dominant trait that is transferred from 'Dancy' mandarin to its progeny. Dancy mandarin hybrids and some cultivars of unknown origin, such as 'Murcott', 'Emperor' and 'Ponkan', are affected by the disease (Dalikilic *et al*, 2005). The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the 'Nova', 'Minneola' and 'Mor'.

ABS attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. ABS sporulates 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 and twig dieback (Pegg 1966; Peever *et al*, 2004; Peever *et al*, 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, fruit are only susceptible from petal fall until they reach about 5 cm in diameter. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit, which are unacceptable to the consumer.

Cultural measures, such as wider tree spacing and pruning to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards (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.

With a growing emphasis on the reduction in detectable residues on fruit destined for the export market, it has become increasingly important to focus on remedies with very low or no MRL's, especially when two different chemistries are used during post-harvest treatments alone. Unfortunately, very little has happened with regards to new chemistry and the new chemistry that has been effective against Citrus black spot is not as effective against ABS.

Another consideration with regards to the control of ABS, should be the rate at which sensitive cultivars are being covered by netting structures. Not only will this affect the microclimate and disease pressure, but disease control as well through the weakened effect weathering would have on residue breakdown. This could contribute to longer spray intervals or on the flipside negatively affect MRL levels.

Objectives

To evaluate different spray programmes on a susceptible 'Nova' mandarin orchard that is covered by net. Spray programmes would consist of mancozeb and copper in tank mixtures at half of their registered rates. The evaluation of RB1 will also continue as previous results were inconclusive.

Materials and methods

A susceptible 10 year old 'Nova' mandarin orchard under net was selected at the JL Group, located between Ohrigstad and Hoedspruit. The efficacy of RB1™ was evaluated on its own (Table 1) and in a programme

consisting of alternated sprays with a mancozeb and copper oxychloride tank mixture at half their registered rates. The mancozeb/copper tank mixture was evaluated as a standalone programme at a 25 and 35 day spray interval. The trial was randomized as a single tree block design, each treatment consisting of 5 single tree plots per replicate. 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. Unsprayed trees served as the untreated control.

At fruit maturity in May 2017, 100 fruit per data tree were evaluated according to an infection scale where: 0 = fruit with no brown spot lesions, 1 = fruit with one to five lesions and 2 = fruit with six or more lesions. Data accumulated were analysed using the statistical package XLSTAT and the mean percentages compared using the Fischer's student t-test of least significant difference (LSD). Fruit were evaluated for any sign of phytotoxicity by randomly harvesting 15 fruit from each treatment and visually inspecting them.

Results and discussion

Unfortunately, a drought was experienced in the Ohrigstad area during the trial period and this contributed to very low disease pressure at the trial site. Only 6 fruit from the untreated control were observed with ABS lesions, which led to a 1.2% infection rate. The data was statistically analysed and can be seen in Table 2

RB1 applied at rates of 100 and 200ml/100L water

At both rates (100 and 200ml/100L) RB1™ yielded statistically less fruit with ABS than the untreated control, although only 1.2% of evaluated fruit from the untreated control had 1-4 lesions. This can be attributed to the fact that all of the other treatments yielded no fruit with ABS lesions. There was no fruit observed with 5 or more lesions. All of the fruit observed had no visible signs of phytotoxicity (Fig. 1A and 1B).

RB1 alternated with a mancozeb and copper oxychloride tank mixture

As previously stated, the disease pressure was very low in the trial orchard. There was, however, no lesions found on any of the fruit evaluated from the experimental programmes. Fruit evaluated for phytotoxicity did reveal a delaying of fruit colour (Fig. 1C).

Mancozeb and copper oxychloride tank mixture at 25 and 35 day intervals

Both spray intervals did not result in ABS lesions. This was the same in all the other treatments, including the standard recommended programme (No 6; Table 2). However, as with the previously mentioned experimental programme there was a delay in fruit colouring. This could be due to the copper oxychloride present in both programmes. The occurrence was observed from both spray intervals, though not as prominent on the fruit sprayed at 35 day intervals (Fig. 2D and 2E).

Table 1. Application dates and rates of fungicides applied for the control of Alternaria brown spot at JB Group, Ohrighstad, South Africa for the period 26 September 2016 to 3 April 2017.

No	26 Sep 2016	24 Oct 2016	31 Oct 2016	21 Nov 2016	5 Dec 2016	14 Dec 2016	3 Jan 2017	10 Jan 2017	16 Jan 2017	6 Feb 2017	14 Feb 2017	27 Feb 2017	06 Mar 2017	14 Mar 2017	03 Apr 2017
1	Untreated Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	MZ (200g)	MZ (200g)		MZ (200g)		MZ (200g)		MZ (200g)		MZ (200g)			MZ (200g)		MZ (200g)
3	CuOCl (200g)		CuOCl (200g)		CuOCl (200g)				CuOCl (200g)			CuOCl (200g)			CuOCl (200g)
4	RB1 (100ml)	RB1 (100ml)		RB1 (100ml)		RB1 (100ml)		RB1 (100ml)		RB1 (100ml)			RB1 (100ml)		RB1 (100ml)
5	RB1 (200ml)	RB1 (200ml)		RB1 (200ml)		RB1 (200ml)		RB1 (200ml)		RB1 (200ml)			RB1 (200ml)		RB1 (200ml)
6	MZ (200g)	MZ (200g)		Azoxy (20ml) + MZ (200g) + Oil (250ml)			Azoxy (20ml) + MZ (200g) + Oil (250ml)				MZ (200g)			MZ (200g)	
7	MZ (100g) + CuOCl(100g)	MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)			MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)
8	MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCl(100g)		MZ (100g) + CuOCL(100g)				MZ (100g) + CuOCl(100g)			MZ (100g) + CuOCl(100g)			MZ (100g) + CuOCl(100g)
9	MZ (100g) + CuOCl(100g)	RB1 (100ml)		MZ (100g) + CuOCl(100g)		RB1 (100ml)		MZ (100g) + CuOCl(100g)		RB1 (100ml)			MZ (100g) + CuOCl(100g)		RB1 (100ml)
10	MZ (100g) + CuOCl(100g)	Fighter (570ml)		MZ (100g) + CuOCl(100g)		Fighter (570ml)		MZ (100g) + CuOCl(100g)		Fighter (570ml)			MZ (100g) + CuOCl(100g)		Fighter (570ml)

Table 2. Evaluation of RB1 and tank mixtures of mancozeb and copper oxychloride in standard spray programmes applied from 26 September 2016 to 3 April 2017 for the control *Alternaria* brown spot at JB Group, Ohrighstad, South Africa.

Treatment		Dosage (g/ml per 100L water tank mixture)	Percentage of fruit in each class		
			Lesions/fruit ^x		
			0	1-5	≥6
1	Untreated control		98.8a	1.2b	0.0a
2	Mancozeb	200g	100.0b	0.0a	0.0a
3	Copper oxychloride	200g	100.0b	0.0a	0.0a
4	RB1™	100ml	100.0b	0.0a	0.0a
5	RB1™	200ml	100.0b	0.0a	0.0a
6	Mz/Mz/Mz + Ortiva + Oil/ Mz + Ortiva + Oil/Mz/Mz	200g/200g/150g + 20ml + 250ml/ 150g + 20ml + 250ml/200g/200g	100.0b	0.0a	0.0a
7	Mz + CuOCl (25 day)	100g + 100g	100.0b	0.0a	0.0a
8	Mz + CuOCl (35 day)	100g + 100g	100.0b	0.0a	0.0a
9	Mz + CuOCl/RB1™	100g + 100g/100ml	100.0b	0.0a	0.0a
10	Mz + CuOCl/Fighter	100g + 100g/570ml	100.0b	0.0a	0.0a

^xMeans followed by the same letter in the same column do not differ significantly at P <....

^ySpray dates were: 26 September 2016; 24 October 2016; 31 October 2016; 21 November 2016; 5 December 2016; 14 December 2016; 3 January 2017; 10 January 2017; 16 January 2017; 6 February 2017; 14 January 2017; 27 February 2016; 6 March 2017; 14 March 2017 and 3 April 2017.



Fig. 1A-C. Fruit collected from the trial orchard at JB Group, Ohrigstad one week before harvest. A) RB1™ at a rate of 100ml/100L; B) RB1™ applied at a rate of 200ml/100L and C) RB1™ applied at 100ml/100L of water in an alternating spray programme with a tank mixture of mancozeb and copper oxychloride.



Fig. 2D and E. Fruit collected from the trial orchard at JB Group, Ohrigstad one week before harvest. D) A tank mixture of mancozeb and copper oxychloride at a rate of 100g/100L each, applied every 25 days. E) A tank mixture of mancozeb and copper oxychloride at a rate of 100g/100L each, applied every 35 days.

Conclusion to date

Although there was a very low disease pressure in the trial orchard, all of the treatments and experimental programmes still yielded clean fruit without any ABS lesions. Which were in all cases statistically less than the untreated control. It should, however, be mentioned that even though copper oxychloride was applied at half of its registered rate it still had an effect on the colouring of fruit, including in the programmes where it was alternated bi-monthly with another fungicide.

Technology transfer

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

Future objectives and work plan

Research in future will focus on the inclusion of new chemistry into already existing spray programmes, with the ultimate goal of decreasing the amount of applications during the season. As mancozeb remains a problem in most market, alternatives will have to be identified.

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

Project RCE-6 (Apr 2015 – Dec 2018) by P.H. Fourie, M. Kellerman and Laurika Swart (CRI)

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa, because of its quarantine status in major export countries. Epidemiology of CBS is not fully understood, since there is currently no method to distinguish between its ascospores and those of the non-pathogenic *P. capitalensis* and although generic infection models are available, actual infection has not been measured yet. Knowledge gaps also still exist with regards to fruiting body maturation and spore germination requirements, for both asexual and sexual proliferation cycles. Germination studies were done with pycnidiospores, where the effect of spore age, generation, temperature, germination medium and wetness interruption was investigated. Washing spores 3x in distilled water had a significant beneficial effect on germination. For *in vivo* trials, 1.5% Valencia juice was not a requirement for germination, as was the case for *in vitro* trials on microscope slides. Differences were observed between germination on glass slides and leaves, and whether leaves were sterilised or not. Various trials were conducted to study the temperature effects on germination and infection; however, all attempts at measuring infection were unsuccessful. Wetness interruption trials showed that pycnidiospores are able to germinate and survive a 3 h period of dryness, even as early as 4 h after inoculation. Less than 1000 spores could not reliably be quantified by the qPCR protocol, but >1000 spores.mL⁻¹ could be quantified. The qPCR protocol was improved by developing primers to conduct a nested qPCR, which proved to be more sensitive. Various crossing attempts were conducted according to the published methods, but to date ascospores could not be produced in culture.

Opsomming

Sitruswartvlek (SSV) is die belangrikste swamsiekte op sitrus in Suid-Afrika, vanweë die kwarantynstatus wat dit geniet in uitvoerlande. Die epidemiologie van SSV word nie ten volle verstaan nie, omdat daar nie tans 'n metode is om tussen askospore van die patoogeen en nie-patogeniese *P. capitalensis* te onderskei nie, en ook omdat daar slegs generiese infeksie Modelle bestaan, en werklike infeksie nie gemeet is nie. Daar is steeds

gapings in die kennis oor vrugstruktuur rypwording en spoorontkieming se vereistes, vir beide die seksuele en nie-seksuele lewensiklus. Spoorontkiemingstudies is gedoen met piknidiospore, en die effek van spoorouderdom, -generasie, temperatuur, ontkiemingsmedium, en onderbreking van natheidsperiode is ondersoek. Deur spore 3x te was in gedistilleerde water het ontkieming betekenisvol verbeter. Vir *in vivo* proewe, is gevind dat 1.5% Valenciasap nie nodig is vir ontkieming, soos die geval was vir *in vitro* proewe op mikroskoopglasplaatjies nie. 'n Groot verskil is gesien in ontkieming tussen spore op glasplaatjies en op blare by verskillende temperature, asook wanneer blare gesteriliseer was of nie. Natheidsperiode onderbrekingsproewe het gewys dat piknidiospore 'n 3h-droë periode kan oorleef, selfs so vroeg as 4 h na inokulasie. Minder as 1000 spore kon nie betroubaar gekwantifiseer word deur qPCR nie, maar >1000 spore.mL⁻¹ kon. Die qPCR protokol is verbeter deur nuwe 'inleiers te ontwikkel om 'n meer sensitiewe geneste qPCR te kan doen. Verskeie kruisingseksperimente is volgende die gepubliseerde tegniek gedoen, maar askospore kon tot dusver nie in kultuur gekweek word nie.

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

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

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa. Using proper spore dispersal and maturation models together with accurate weather data, integrated into easy to use web-based software, better decision making in terms of timing of spray application and choice of fungicides used can be made. CBS risk can also be determined more accurately from season to season. CRI-PhytRisk (www.cri-phytrisk.co.za) was developed and launched for computer (August 2016) and mobile (October 2017) platforms. PhytRisk warnings are emailed to registered users with links to the website predictions. PhytRisk has 470 users, and the site was viewed 3629 times from 1 Feb 2017 to 22 May 2018. Validation led to some changes in the algorithm for better accuracy, and it is planned to upload measured weather data as input to further improve accuracy. The PhytRisk CBS disease prediction models have also been evaluated with three other international CBS models in a CRI funded project 1149.

Opsomming

Sitrus swartvlek is die belangrikste swamsiekte in sitrus in Suid-Afrika. Deur behoorlike spoor vrystelling en rypwording modelle te gebruik saam met akkurate weerdata, geïntegreer in verbruikersvriendelike web-gebaseerde sagteware, kan dit die produsent help om beter besluite op plaasvlak te neem in terme van tydsberekening van spuit toediening en keuse van swamdoder. CBS risiko kan ook van seisoen tot seisoen bepaal word. CRI-PhytRisk (www.cri-phytrisk.co.za) is ontwikkel vir rekenars (Augustus 2016) en slimfone (Oktober 2017). PhytRisk epos waarskuwings aan geregistreerde gebruikers met skakels na die webtuiste voorspellings. PhytRisk het 470 gebruikers wie die webtuiste 3629 keer besoek het van 1 Februarie 2017 tot 22 Mei 2018. Die PhytRisk infeksie-modelle is ook saam drie ander internasionale CBS modelle in CRI projek 1149 geëvalueer.

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

Project RCE-8 (2014/12– 2019/03) by Susan du Raan (QMS Laboratories)

Summary

Ascospore release data for citrus black spot (CBS) for geographically different citrus production areas in South Africa was generated. Data were generated for areas including Letsitele, Hoedspruit, Noordgrens, Tshipise, Origstad, Burgersfort, Nelspruit and Eastern Cape (Kirkwood/Addo). The data are currently being used to validate and improve current models for CBS. No data were, however, generated for further objectives of the study (objectives 2-5), due to different reasons including failure of proposed methods to yield positive results.

Opsomming

Askospoor vrystellingsdata vir sitrus swartvlek (SSV) vir geografies verskillende sitrusproduksie-areas in Suid-Afrika, is gegeneer. Data is gegeneer vir areas, insluitende Letsitele, Hoedspruit, Noordgrens, Tshipise, Origstad, Burgersfort, Nelspruit en Oos-Kaap (Kirkwood/Addo). Die data word tans gebruik om huidige modelle vir SSV te bekragtig en te verbeter. Geen data is egter gegeneer vir verdere doelwitte van die studie nie (doelwitte 2-5), weens verskillende redes, insluitende tekortkoming van voorgestelde metodes om positiewe resultate te lewer.

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

Project 1132 (2014/15 – 2018/9) by J. van Niekerk (CRI), C. Kotze (CRI), T. van Wyk (USPP) and P. Fourie (CRI)

Summary

Spray application forms the backbone of pre-harvest pest and disease management strategies in South African citrus production. Due to zero tolerance status of Citrus black spot (caused by *Phyllosticta citricarpa*) for export to the European Union, growers tend to use high volume fungicide application ranging from 6000 to 12000 L/ha. However, high spray volumes are costly in terms of off-target losses and environmental pollution, amount and cost of water, fuel and plant protection product (PPP), the strain on equipment, and it is more labour intensive. The potential of reduced volume applications for the control of citrus black spot have been investigated in various previous studies. However, the potential must be proven through seasonal bio-efficacy trials. Following on the 2016/2017 season trials, in the 2017/2018 season, three trials were done in three different growing regions with spray volumes ranging from 750 L/ha to 8800 L/ha, which included a repeat of the Groblersdal trial of 2016/2017. With results obtained so far, the data indicated that poorer deposition values were obtained in comparison with the 2016/2017 results. In the Patensie and Groblersdal trials the FPC% of the leaves and fruit were all below the FPC75 benchmark of 4.14. Deposition uniformity (CV%) from both these trial sites performed poorly on the leaves, being significantly better on the fruit for high and low volumes. The deposition quality (ICD%) on the leaves and fruit were better, with different results achieved by the various spray volumes. The Citrusdal trials showed promising results in terms of all deposition parameters for the fruit and leaves. Deposition quantity with values above the FPC75 benchmark on the leaves as well as fruit were achieved. In terms of deposition uniformity and quality, the results indicated better performance on the outer canopy positions for all the treatments. The efficacy of the applications will be confirmed as soon as the final biological evaluation is completed for relevant pests and diseases in June 2018. However, from the 2016/2017 results it was clear that lower volume applications led to poorer control of insect pests such as red scale and mealybug.

Opsomming

Spuittoediening vorm die ruggraat van voor-oes plaag- en siektebestuurstrategieë in Suid-Afrikaanse sitrusproduksie. Weens zero-toleransie status van Sitrus swartvlek (veroorzaak deur *Phyllosticta citricarpa*) vir uitvoer na die Europese Unie, neig produsente daartoe om hoë volume fungisiedtoedienings te gebruik wat van 6000 tot 12000 L/ha wissel. Hoë spuitvolumes is egter duur in terme van nie-geteikende verliese en omgewingsbesoedeling, hoeveelheid en koste van water, brandstof en plantbeskermingsmiddel, die stremming op toerusting, en die feit dat dit meer arbeidsintensief is. Die potensiaal van verminderde volume toediening vir die beheer van sitrus swartvlek is in verskeie vorige studies ondersoek. Die potensiaal moet egter deur seisoenale bio-effektiwiteit proewe bewys word. Volgende op die 2016/2017 seisoen proewe, is in die 2017/2018 seisoen, drie proewe in drie verskillende produksie-areas met spuitvolumes wat van 750 L/ha tot 8800 L/ha gewissel het, uitgevoer, wat 'n herhaling van die Groblersdal proef van 2016/2017 ingesluit het. Met resultate so ver verkry, dui data daarop dat swakker neerleggingswaardes in vergelyking met die 2016/2017 resultate verkry is. In die Patensie en Groblersdal proewe, was die FPC% van die blare en vrugte almal onder die FPC75 hoogtemerk van 4.14. Neerleggingsuniformiteit (CV%) van beide hierdie proefpersele

het swak op die blare gedoen, en betekenisvol beter op die vrugte vir hoë en lae volumes. Die neerleggingskwaliteit (ICD%) op die blare en vrugte was beter, met verskillende resultate verkry deur die verskeie spuitvolumes. Die Citrusdal proewe het belowende resultate getoon vir die vrugte en blare in terme van al die neerleggingsparameters. Neerleggingskwantiteit met waardes bó die FPC75 hoogtemerk, is op die blare, asook die vrugte bereik. In terme van neerleggingsuniformiteit en -kwaliteit, het die resultate aangetoon dat beter vertoning op die buitenste lowerposisies vir alle behandelings verkry is. Die effektiwiteit van die toedienings sal bevestig word sodra die finale biologiese evaluasie vir relevante plaë en siektes in Junie 2018 voltooi word. Resultate van 2016/2017 toon egter dat dit duidelik is dat laer volume toedienings tot swakker beheer van insekplaë soos rooi dopluis en witluis gelei het.

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

Project 1089 (2014/04 – 2019/04) by J. van Niekerk (CRI), T. van Wyk (USPP) and P.H. Fourie (CRI)

Summary

In the 2017/2018 season progress was made in that it was shown that the LIDAR are able to clearly differentiate between citrus trees with variation in canopy densities. This was achieved by scanning trees with varying canopy densities in a diseased Nules Clementine orchard. Following on this, two trials were done to determine the effect of different amounts of pruning on canopy density, the canopy density as measured by the LIDAR and the effect on spray deposition quantity, uniformity and quality. Results indicated that the LIDAR successfully observed the changes in tree canopy density after pruning. It was furthermore seen that at higher spray volumes, pruning had no, to little effect on spray deposition parameters. However, when applying lower spray volumes, it was shown that light pruning had a marked effect on spray deposition, improving it markedly in comparison to the results seen for unpruned or heavily pruned trees. It was shown that if lower spray volumes are employed, tree canopy manipulation through pruning must be done to get proper spray deposition.

Opsomming

In die 2017/2018 seisoen is vordering gemaak deurdat aangetoon is dat die LIDAR in staat is om duidelik tussen sitrusbome, met variasie in lowerdigthede, te onderskei. Dit is bereik deur bome met variërende lowerdigthede in 'n siek 'Nules' clementine boord, te skandeer. Volgende hierop is twee proewe uitgevoer ten einde die effek van verskillende hoeveelhede snoei op lowerdigtheid, die lowerdigtheid soos gemeet deur die LIDAR, en die effek op spuitneerleggingshoeveelheid, -uniformiteit en -kwaliteit, te bepaal. Resultate het aangedui dat die LIDAR suksesvol die veranderinge in boomlowerdigtheid ná snoei waargeneem het. Daar is verder waargeneem dat teen hoër spuitvolumes, snoei geen tot min effek op spuitneerleggingsparameters gehad het nie. Wanneer laer spuitvolumes toegedien is, is aangedui dat ligte snoei 'n merkbare effek op spuitneerlegging gehad het. Spuitneerlegging is merkbaar verbeter in vergelyking met die resultate gesien vir ongesnoeide of hewig gesnoeide bome. Daar is aangetoon dat indien laer spuitvolumes gebruik word, boomlower manipulasie deur snoei gedoen moet word ten einde voldoende spuitneerlegging te verkry.

3.4.12 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, Charl Kotze, Jan van Niekerk 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*, which cause citrus black spot (CBS). Thus, the use of shade nets

can directly or indirectly affect the development of these diseases in citrus orchards. This study aimed to determine how the use of shade nets could influence the development and spread of CBS within citrus orchards. Weather data obtained from two orchards (one under the net and uncovered) were analysed in CRI-PhytRisk to compare the risks of CBS infection in covered vs. uncovered orchards. The number of days with 3-hour periods in which pycnidiospore and ascospore infections were predicted, using the CRI-PhytRisk platform, were compared between the shaded and open orchard. A higher risk of CBS infection was predicted in the orchard under the net when compared to the orchard outside the net, confirming that the microclimate in orchards is altered with the use of nets, subsequently affecting the development of CBS.

Opsomming

Die gebruik van skadu- of haelnette neem toe in Suid-Afrikaanse boorde. Hierdie nette word hoofsaaklik gebruik om die verlies aan opbrengs weens nadelige klimaatstoestande, insluitende temperatuur-uiterses, haelstorms en hoë winde, te verminder. Hulle beskerm ook gewasse teen insekte en voëls. Die gebruik van haelnette kan egter tot modifikasie van die boord mikroklimaat lei, veral humiditeit en temperatuur, wat baie belangrik is in die groei en ontwikkeling van patogene soos *Phyllosticta citricarpa*, wat sitrus swartvlek (CBS) veroorsaak. Die gebruik van skadunette kan dus direk of indirek die ontwikkeling van hierdie siektes in sitrusboorde beïnvloed. Hierdie studie het ten doel gehad om te bepaal hoe die gebruik van skadunette die ontwikkeling en verspreiding van CBS binne sitrusboorde kan beïnvloed. Weerdata vanaf twee boorde verkry (een onder die net en een onbedek) is in CRI-PhytRisk geanaliseer ten einde die risiko's van CBS-infeksie in bedekte teenoor oop boorde te vergelyk. Die aantal dae met 3-uur periodes waarin piknidiospor- en askospor-infeksies voorspel is, deur gebruik te maak van die CRI-PhytRisk platform, is tussen die bedekte en oop boord vergelyk. 'n Hoër risiko vir CBS-infeksie is in die boord onder die net voorspel, in vergelyking met die boord buite die net, wat bewys lewer dat die mikroklimaat in boorde deur die gebruik van nette verander word, wat gevolglik die ontwikkeling van CBS beïnvloed.

3.4.13 **PROGRESS REPORT: Susceptibility period of sweet orange fruit to *Phyllosticta citricarpa* in commercial orchards**

Project 1186 (2017/04- 2020/03) by Providence Moyo, Charl Kotze, Jan van Niekerk and Paul H. Fourie (CRI); Geraldo J. Silva Junior, Franklin Behlau & Fabricio Lanza (Fundecitrus, Brazil)

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 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 program. The trials will be evaluated at fruit maturity (August), where the incidence and severity of CBS symptoms will be assessed.

Opsomming

Sitrus swartvlek (CBS) is een van die belangrikste swamsiektes van sitrus wêreldwyd. Die siekte word deur 'n lang latente periode gekenmerk, waartydens simptome nie noodwendig voorkom totdat die vrugte ryp is nie, en die erns van die siekte hang van 'n aantal faktore af, waaronder die ouderdom van die vrugte tydens infeksie. Daar is al gedemonstreer dat vrugte soos hul ouer word, weerstandbiedend word teen CBS-infeksie, m.a.w. ontogeniese weerstandsontwikkeling. Onlangse navorsing het egter aangetoon dat vrugte vir langer periodes vatbaar vir infeksie is as wat voorheen aangeneem is, gevolglik word 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 geïnkuleer, of aan *P. citricarpa* natuurlike infeksie by verskillende tye blootgestel deur ’n trapsgewyse (“staggered”) spuitprogram. Die proewe sal tydens vrugrypheid (Augustus) geëvalueer word, waar die voorkoms en erns van CBS-simptome geëvalueer sal word.

3.5 PROGRAMME: POSTHARVEST DISEASES

Programme coordinator: Wilma du Plooy (CRI)

3.5.1 Programme summary

The *in vitro* and *in vivo* use of essential oils (EOs) as alternatives to synthetic fungicides for the simultaneous control of sour rot (*Galactomyces citrii-aurantii*) and green mould (*Penicillium digitatum*) on citrus were investigated (3.5.2). A chemometric approach was tested in an attempt to identify EOs with the ability to control these pathogens *in vitro*. Several researchers have prepared EOs encapsulated in silver nanoparticle (SNP), and coated with chitosan for postharvest control. The antifungal activities of these structures were compared to those of the EOs alone. More than a hundred EOs were screened *in vitro* against both pathogens at 2000 $\mu\text{L/L}$ using the toxic medium assay. Chemometric models, linking the antifungal activities and the chemical profiles were constructed. Further screening of the active oils was done at 1000 $\mu\text{L/L}$. Various other *in vitro* assays were also established for a more quantitative approach towards measuring the antifungal activities of the EOs. The encapsulation modifications were considered in this study to determine the effect on the efficacy and rate of release of the oils to allow for more consistent control. The application of EOs as a fumigant was also investigated. Accurate analysis is crucial for the successful development of natural fumigants. A mobile gas chromatograph (GC) was used to determine that the concentrations of the main components of the headspace of the EOs were considerably different from those in the liquid form, which implies that the antifungal activity of the liquid and the headspace may be different. The chemometric approach could not be successfully implemented for the identification of active EO constituents. However, screening at 1000 $\mu\text{L/L}$ allowed the identification of several highly active oils. The silver nanoparticle-encapsulated EOs did not lower the concentration at which the oils were effective, but the addition of silver ions yielded a marked improvement in the activity of the oils. The chitosan-coated EOs proved to be more active than the chitosan or EOs alone. Extensive packhouse trials using Kumquat fruit in Levubu indicated that fruit treated with spearmint applied as a dip-tank solution improved the quality of the product upon arrival in Europe compared to the controls. Essential oils with excellent multitarget *in vitro* efficacy against two important citrus postharvest pathogens were identified.

Two ring tests were undertaken and several postharvest products investigated (3.4.4). Several products were evaluated, with varying results. The most successful were once again an azoxystrobin formulation against *Penicillium digitatum* (PD), while the bioflavonoid and fruit acid-based sanitisers did not have any useful effect on PD. Further investigations into products combining hydrogen peroxide and acetic acid were conducted as alternative sanitizers in the fungicide bath. The PAA products are compatible with the postharvest products currently used, but the corrosivity of most is a concern. In addition, phytochemical conditions were observed on fruit treated at higher concentrations. Very inconsistent results are being achieved with ozone in actual packhouses, despite the active yielding very good results in the laboratory. Further investigations were also conducted on the powder formulation of PAA. A number of products based on bioflavonoids were investigated, although none of these had any useful sanitation action.

Drench application of postharvest chemicals were studied in two separate projects (3.5.3 and 3.5.6). As part of a resistance management strategy, pyrimethanil (PYR) and thiabendazole (TBZ) is regarded as the main components of the prepackhouse drench application. The compatibility between sanitisers such as chlorine and PAA, PYR and TBZ was observed in project 1126, with addition of both sanitisers contributing to sour rot control. A powder PAA was effective at a short exposure time (1 – 3 min) at the high pH used in this study (> 10), and it can only be incorporated with commercial drenching when pH is not regulated. Differential PYR residue loading was seen between the top and bottom bin levels during commercial packhouse trials with the upper level loading higher residue levels compared to the bottom level. The physical parameters of pH,

temperature, exposure time, and their effect on chemicals used in the dip tank were optimised, and an industry recommendation were completed. In project 1141 drench application of propiconazole (PPZ) was studied to determine the time frame (6, 14, 18, 24 h) in which the fungicide has to be applied from harvest until fruit arrives on the packline. Propiconazole as a postharvest fungicide recently became available to the South African citrus industry. Baseline sensitivity against PPZ has been tested for South African isolates of *G. citri-aurantii*, and *Penicillium digitatum*. Additionally, the exposure time (1, 2, 3 min) required to obtain adequate coverage of the fungicide applied to the fruit was determined for three cultivars ('Nules' mandarins, 'Eureka' lemon, navel orange). Results from inoculations of lemons with a *G. citri-aurantii* isolate indicated that propiconazole can effectively control sour rot infection when treatment is applied at 600 mg/L with 1 min exposure time (flow rate approximately 500 L/min), if applied within 14 h of inoculation. In comparison, 'Clementine' mandarin treatments had to be applied within 6 hours after inoculation to reach full effectivity

Plants produce an extensive diversity of compounds known as phytochemicals, all of which are functional in the plant in some or other way. The role of phytochemicals in either eliciting or inhibiting the ability of *Phyllosticta citricarpa* (citrus black spot, CBS) to infect citrus fruit is being investigated (3.5.5). Cultivars with varying susceptibility to CBS are being investigated, looking at the apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycosides) fractions in the rind phytochemistry. 'Eureka' lemons, 'Bitter Seville' orange, and 'Late' Valencia were sampled from fruit set and throughout the season. 'Nagami' kumquat and 'Tahiti' lime have been added as a CBS free control. Rind from the different growth stages were collected and frozen at -80°C for processing. Samples were freeze dried and extracted. Polar and apolar fractions are currently being analysed using chromatographic techniques. Chemometric analysis of the results are presented as principal component analysis (PCA) score plots. Variability of cultivar/type susceptibility needs more expansion, however, the resources available at the moment are the limiting factors.

Recent fungal contamination on wooden pallet bases used for export highlighted a problem of which the source is apparently unknown (3.5.7). This degradation of pallet bases when the consignment reaches its market has raised questions on the quality of the wood used in the manufacturing of these bases. Further concerns were the contribution of packhouse storage methods to the fungal contamination of the bases, and the possible role of environmental factors (for instance moisture, UV degradation, and insect infestation). However, it is fungal decay that is currently the hazard with the highest priority. Heavily contaminated pallets are a point source of fungal spore dissemination that poses a deterioration risk to the boxes stacked on these pallets, increases the spore load in cold rooms, and presents a phytosanitary concern. This study looks at the microbiome on contaminated pallet bases, manufacturing aspects of the bases, the role of storage at packhouses, options for wood treatment, as well as possible contribution from the shipping containers to the degradation of wooden pallet bases.

Program-opsomming

Die *in vitro* en *in vivo* gebruik van essensiële olies (EOs) as alternatiewe vir sintetiese fungisiedes vir die gelyktydige beheer van suurvrot (*Galactomyces citrii-aurantii*) en groenskimmel (*Penicillium digitatum*) op sitrus, was ondersoek (3.5.2). 'n Chemometriese benadering vir die identifikasie van die EOs wat hierdie patogene *in vitro* kan beheer, was getoets. Verskeie navorsers het EOs wat ge-enskapsuleer was met silwer nanopartikels (SNP), of chitosan, voorberei vir na-oesbeheer. Hierdie gemodifiseerde partikels is vergelyk met skoon EOs. Meer as eenhonderd EOs was onderworpe aan 'n *in vitro* sifting met beide patogene. Die olies was getoets teen 2000 µL/L, met gebruik van die toksiese medium toets. Chemometriese modelle wat die antifungus aktiwiteite en die chemiese profiele in verband bring met mekaar, was gekonstrueer. Verdere sifting van die aktiewe olies was gedoen teen 1000 µL/L. Verskeie ander *in vitro* siftings is aangepak om 'n meer kwantitatiewe benadering tot die meting van EO antifungus aktiwiteit te behaal. Die enkapsuleringsveranderinge aan die olies was bereken om die tempo en effektiwiteit van oliëvrystelling te beheer vir meer gelykmatige beheer. Die aanwending van EOs as 'n natuurlike berokingsmiddel was ook oorweeg. Akkurate analiese is belangrik vir suksesvolle ontwikkeling van sulke berokingsmiddels. 'n Mobiele gaschromatograaf (GC) het uitgewys dat die konsentrasies van die hoofkomponente in die bo-lug van die EOs aansienlik verskil het dit wat in die olies in vloeistofvorm voorkom, wat impliseer dat die antifungus aktiwiteit van die olies self en die bo-lug ook mag verskil. Die chemometriese benadering vir die identifikasie van aktiewe

EO komponente kon nie suksesvol geïmplimenter word nie. Die sifting teen 1000 µL/L het egter die suksesvolle identifkasië van verskeie aktiewe olië moontlik gemaak. Die SNP ge-enskapsuleerde EO's het nie die konsentrasie waar die olië aktief is, verlaag nie, maar die byvoeging van die silwer ione het 'n noemenswaardige verbetering in die olie aktiwiteit tot gevolg gehad. Die chitosan-ge-enskapsuleerde olië was minder effektief as chitosan op sigself, of as die olië op sigself. Uitgebreide toetse met koemkwartse in Levubu het aangedui dat vrugte behandel met kruisement in die dipbad se kwaliteit met aankoms in Europa beter was as die van die kontroles. Essensieële olie met uitstekende veel-teiken *in vitro* effektiwiteit teen die twee patogene is geïdentifiseer.

Twee ringtoetse is onderneem en verskeie produkte was getoets. Verskeie produkte was ge-evalueer, met baie variërende resultate (3.4.4). Die mees suksesvolle produk was weereens 'n azoxytrobieë formulasië teen *Penicillium digitatum* (PD), terwyl die bioflavonoïde vrugtesuur-gebaseerde produkte nie enige bruikbare waarde tot na-oesbeheer kon toevoeg nie. Daar was weereens klem gelê op sanitasië produkte met PAA en osoon as aktiewe molekules. PAA lyk of dit goed verenigbaar is met na-oesprodukte, maar die korrosiwiteit van die meeste is 'n bekommernis. Verder is daar fitochemiese reaksies waargeneem by hoër konsentrasies van PAA. Baie wisselvallige resultate word met osoon behaal in pakhuisse, ten spyte daarvan dit baie goed werk onder laboratoriumtoestande. Die poeier formulasië van PAA het verdere ondersoek gehad rondom die oplosbaarheid van die produk. 'n Aantal produkte wat gebaseer is op bioflavonoïde was ondersoek. Geeneen van hierdie produkte het noemenswaardige sukses behaal as saniteermiddels nie.

Stortvloedaanwending van na-oes chemikalieë was in twee afsonderlike projekte bestudeer (3.5.3 en 3.5.6). Pirimetaniël (PYR) en tiabendasoël (TBZ) word beskou as die hoofkomponente in die weerstansbestuurstrategie van die voorpakhuisstort toediening. Die verenigbaarheid van hierdie produkte met saniteerders soos chloor en PAA met PYR, en TBZ was opgevolg in projek 1126, waar beide saniteermiddels bydra tot suurvrotbeheer in die vloedtoediensoplossing. So ook is die funksiedkonsentrasie en residuvlakke wat nodig is vir effektiewe groenskimmel beheer, vergelyk. 'n Poeie perasynsuur formulasië was effektief binne 'n kort blootstellingstyd (1 – 3 min) teen hoë pH in hierdie studie (> 10), wat aandui dat dit slegs ingesluit kan word in kommersiële stortbaddens waar die pH nie gereguleer word nie. Die PYR residu lading in die plukkratte het gewissel van bo na onder, met die boonste kratte wat beter residu laai in vergelyking met die onderstes. Optimisering van die fisiese parameters (pH, temperatuur en blootstellingstyd) en hul effek op die chemikalieë vir die dompeltank is voltooi, en industrie aanbevelings was voltooi. In projek 1141 is die stortvloedaanwending van propikonasool (PPZ) bestudeer om die tyd (6, 14, 18, 24 h) waarbinne die swamdoder moet aangewend word, te bepaal: vanaf pluk tot die vrugte op die paklyn verwerk word. Propikonasool was redelik onlangs beskikbaar gemaak as 'n na-oes-swamdoder in die Suid-Afrikaanse sitrusbedryf. Basislyn sensitiwiteit vir PPZ is getoets vir Suid-Afrikaanse isolate van *G. citri-aurantii* en *Penicillium digitatum*. Hiervoor was 'Eureka' suurlemoene, nawel lemoene en 'Nules' mandaryne gebruik. Verder was die blootstellingstyd (1 min, 2 min, 3 min) wat nodig is om goeie bedekking van die swamdoder op die vrugte te kry, getoets. Toetse op suurlemoene met 'n *G. citri-aurantii* isolaat, het aangedui dat propikonasool suurvrot infeksie effektief kan beheer, mits die behandeling aangewend word teen 600 mg/L vir 1 min binne 14 uur van inenting (vloei tempo ongeveer 500 L/min). Toetse met 'Clementine' mandaryne het aangedui dat die behandeling binne 6 ure aangewend moet word.

Plante produseer 'n baie uitgebreide diversiteit van verbindings bekend as fitochemikalieë, wat almal op een of ander wyse funksioneel is in die plant. Die rol wat fitochemie speel om die infeksie deur *Phyllosticta citricarpa* (sitrus swartvlek, SSV) òf aan te moedig, òf te inhibeer, word ondersoek (3.5.5). Kultivars met verskillende vatbaarhede vir SSV word ondersoek deur bestudering van die apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloïede, glikosiede) in die vrugskil. 'Eureka' suurlemoene, 'Bitter Seville' lemoene, en 'Late' Valencia is gemonster vanaf die aanvang van die nuwe seisoen. 'Nagami' koemkwat en 'Tahiti' lemme'tjies is bygevoeg as SSV-vrye kontroles. Skille van verskillende groeistadiums is versamel en by -80°C gevries vir prosesering. Monsters word gevriesdroog en geëkstreer. Polêre en apolêre fraksies word huidiglik geanaliseer met chromatografiese tegnieke. Chemometrie analises van die resultate word grafies aangebied as hoofkomponent analiese (HKA) waardes. Veranderlike vatbaarheid van sitrus tipe en/of kultivar moet uitgebrei word, maar huidiglike vermoë en kapasiteit is die beperkende faktore.

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

3.5.2 FINAL REPORT: Application of nanotechnology to decrease the volatility of effective essential oils in different applications against citrus postharvest fungi

Project 66/2014 – PHI 66 (2015/2016 - 2017): by Sinclair Bopaima (TUT), Katlego Phala (TUT), Sandra Combrinck (TUT), Thierry Regnier (TUT), Wilma Augustyn (TUT), Wilma Du Plooy (CRI)

Summary

The *in vitro* and *in vivo* use of essential oils (EOs) as alternatives to synthetic fungicides for the simultaneous control of sour rot (*Galactomyces citrii-aurantii*) and green mould (*Penicillium digitatum*) on citrus were investigated. A chemometric approach was tested in an attempt to identify EOs with the ability to control these pathogens *in vitro*. Several researchers have prepared EOs encapsulated in silver nanoparticle (SNP), and coated with chitosan for postharvest control. The antifungal activities of these structures were compared to those of the EOs alone. More than a hundred EOs were screened *in vitro* against both pathogens at 2000 $\mu\text{L/L}$ using the toxic medium assay. Chemometric models, linking the antifungal activities and the chemical profiles were constructed. Further screening of the active oils was done at 1000 $\mu\text{L/L}$. Various other *in vitro* assays were also established for a more quantitative approach towards measuring the antifungal activities of the EOs. The encapsulation modifications were considered in this study to determine the effect on the efficacy and rate of release of the oils to allow for more consistent control. The application of EOs as a fumigant was also investigated. Accurate analysis is crucial for the successful development of natural fumigants. A mobile gas chromatograph (GC) with the ability to accurately monitor the concentrations of active ingredient in air containing an antifungal fumigant was designed and constructed to allow the concentrations of volatile EO components to be determined in headspace by GC. The system was validated for accuracy. *In vivo* trials using EOs and encapsulated EOs were conducted on kumquat in Levubu. It was clear that the concentrations of the main components of the headspace of the EOs were considerably different from those in the liquid form, which implies that the antifungal activity of the liquid and the headspace may be different.

The chemometric approach could not be successfully implemented for the identification of active EO constituents. However, screening at 1000 $\mu\text{L/L}$ allowed the identification of several highly active oils. The silver nanoparticle-encapsulated EOs did not lower the concentration at which the oils were effective, but the addition of silver ions yielded a marked improvement in the activity of the oils. The chitosan-coated EOs proved to be more active than the chitosan or EOs alone. Extensive packhouse trials using Kumquat in Levubu indicated that fruit treated with spearmint applied as a dip-tank solution improved the quality of the product upon arrival in Europe compared to the controls. It was clear that EOs applied in wax did not inhibit pathogen development due to physiological changes, since the rind has a relatively large surface area. Essential oils with excellent multitarget *in vitro* efficacy against two important citrus postharvest pathogens were identified.

Opsomming

Die *in vitro* en *in vivo* gebruik van essensiële olies (EOs) as alternatiewe vir sintetiese fungisiedes vir die gelyktydige beheer van suurvrot (*Galactomyces citrii-aurantii*) en groenskimmel (*Penicillium digitatum*) op sitrus, was ondersoek. 'n Chemometriese benadering vir die identifikasie van die EOs wat hierdie patogene *in vitro* kan beheer, was getoets. Verskeie navorsers het EOs wat ge-enskapsuleer was met silwer nanopartikels (SNP), of chitosan, voorberei vir na-oesbeheer. Hierdie gemodifiseerde partikels is vergelyk met skoon EOs.

Meer as eenhonderd EOs was onderworpe aan 'n *in vitro* sifting met beide patogene. Die olies was getoets teen 2000 $\mu\text{L/L}$, met gebruik van die toksiese medium toets. Chemometriese modelle wat die antifungus aktiwiteite en die chemiese profiele in verband bring met mekaar, was gekonstrueer. Verdere sifting van die aktiewe olies was gedoen teen 1000 $\mu\text{L/L}$. Verskeie ander *in vitro* siftings is aangepak om 'n meer kwantitatiewe benadering tot die meting van EO antifungus aktiwiteit te behaal. Die enkapsuleringsveranderinge aan die olies was bereken om die tempo en effektiwiteit van olievystelling te beheer vir meer gelykmatige beheer. Die aanwending van EOs as 'n natuurlike berokingsmiddel was ook oorweeg. Akkurate analiese is belangrik vir suksesvolle ontwikkeling van sulke berokingsmiddels. 'n Mobiele gaschromatograaf (GC) wat die konsentrasies van die aktiewe bestanddele van antifungusmiddels in die lug kan monitor, was ontwerp en gebou. Hierdie apparaat maak die bepaling van die bo-lug samestelling met GC moontlik. Die stelsel was bekragtig vir akkuraatheid. *In vivo* behandelings met EOs en ge-enkapsuleerde EOs was uitgevoer op koemkwarte in Levubu. Dit was duidelik dat die konsentrasies van die hoofkomponente in die bo-lug van die EOs aansienlik verskil het van dit wat in die olies in vloeistofvorm voorkom, wat impliseer dat die antifungus aktiwiteit van die olies self en die bo-lug ook mag verskil.

Die chemometriese benadering vir die identifikasie van aktiewe EO komponente kon nie suksesvol geïmplementeer word nie. Die sifting teen 1000 $\mu\text{L/L}$ het egter die suksesvolle identifikasie van verskeie aktiewe olies moontlik gemaak. Die SNP ge-enkapsuleerde EOs het nie die konsentrasie waar die olies aktief is, verlaag nie, maar die byvoeging van die silwer ione het 'n noemenswaardige verbetering in die olie aktiwiteit tot gevolg gehad. Die chitosan-ge-enkapsuleerde olies was minder effektief as chitosan op sigself, of as die olies op sigself. Uitgebreide toetse met koemkwarte in Levubu het aangedui dat vrugte behandel met kruisement in die dipbad se kwaliteit met aankoms in Europa beter was as die van die kontroles. Verder is dit duidelik dat EO in die waks nie die inhibisie van die patogeen moontlik maak deur fisiologiese veranderinge nie, aangesien die skiloppervlak relatief groot is. Essensieële olie met uitstekende veel-teiken *in vitro* effektiwiteit teen die twee patogene is geïdentifiseer.

Introduction

The use of synthetic fungicides for postharvest control of fungi on fruit is threatened by increased consumer awareness and restrictions imposed on fungicide application (Beresford, 2010). An increase in microorganism resistance towards fungicides, rendering them ineffective, has also contributed to an interest in complex natural products as alternatives to their synthetic counterparts that usually contain only a single active compound. Essential oils (EO) are known to exhibit potent antimicrobial, antioxidant and medicinal properties and their uses in food are recognised as safe practice (Tabassum & Vidyasagar, 2013).

Nanoencapsulation can extend the period of release of volatile antifungal agents (Scrinis and Lyons, 2007). Metal nanoparticles such as silver and zinc oxide have gained considerable attention because of their unique antibacterial and antifungal properties (Jasim, 2014). Nanosilver is the most studied and utilized nanoparticle for bio-systems. It is known to have a broad spectrum of antimicrobial activities (Prasad and Swamy, 2013). Vilas *et al.* (2014) prepared SNPs using EOs (*Myristica fragrans*) as a reducing and stabilizing agent. Several studies have been conducted recently with chitosan (CHI) as a model for edible coatings to improve the safety and shelf-life of foods (Cerqueira *et al.*, 2011). Chitosan (1,4-linked 2- amino-2-deoxy-b-D-glucan) is a linear polysaccharide derived from deacetylation of chitin (Vieira *et al.*, 2016). It has gained wide consideration due to its characteristics that include biocompatibility, biodegradability, antimicrobial activity, and its non-toxic profile. It has been reported to have excellent carrier properties for functional substances, acting as antimicrobial and antioxidant agents (Yang *et al.*, 2014). Furthermore, the incorporation of EOs in CHI-coatings reduces water permeability and can improve the antimicrobial efficacy because the EOs constituents are released onto the fruit surface over time (Sánchez-González *et al.*, 2011). A study on grapes revealed the efficacy of CHI alone, and as coating matrix for EOs, to inhibit fungal pathogens (dos Santos *et al.*, 2009).

Their volatile nature and bioactivity in the vapour phase, renders EOs suitable for use as fumigants for the protection of stored products. The use of EOs as fumigants has several advantages such as increased penetrability, for example into wounds on the rind. Low concentrations can be applied, which reduces costs, while the sensory properties of the fruit are not affected (Somda *et al.*, 2007, Faleiro, 2011). Slow release of

volatile active components is necessary to ensure long-term protection of fruit. Several researchers have investigated the use of nanoparticles to slow down the evaporation of volatile substances, including EO-encapsulating zeolites, nanoparticles and micelles (van Vuuren et al., 2010; Vilas et al. 2014). Differences in volatility of the oil components may cause the headspace to have a different activity towards fungal organisms than the oil in its liquid form. Knowledge of the accurate headspace composition is therefore necessary. The quantification of EOs is an important factor in in-house quality control measures. However, accurate quantification of EOs is not easily achieved, since some aspects of the quantification remain ambiguous (Bicchi et al., 2008). Furthermore, difficulties in method validation and sampling reproducibility make it difficult to quantify active compounds in the vapour phase EOs using the available headspace techniques for research and quality assurance purposes. These problems hamper the development of EOs as fumigant products for postharvest crop protection products.

In the world of natural product research, new strategies have been developed that allow the plant extract or isolate to be viewed in its entirety. Plants without a host of natural compound weapons would lose the battle against a hostile world of invaders of various types, while remaining immobile and vulnerable. Many researchers have tried to link the chemical profiles of plant extracts (and pure isolates) to the biological activities of the compounds, with varying degrees of success. Maree et al. (2014) followed a non-targeted metabolomic approach to investigate the antimicrobial activity and chemistry of various commercial EOs tested three Gram-positive and two Gram-negative bacterial organisms as well as two yeasts. The construction of chemometric models allowed them to identify components that were linked to good activity of the oils against specific pathogens. We decided to test whether this approach could be used to identify EOs with good activity against two postharvest fungal pathogens of citrus. More than 100 commercial oils were selected to broaden the compound base as much as possible.

The aim of this study was to investigate the potential of EOs as postharvest protection agents, when applied in various forms, to inhibit *Penicillium digitatum* (green mould) and *Galactomyces citri-auranti* (sour rot). The specific objectives were to:

- screen a large number of EOs to identify those with high multi-target efficacy;
- establish various *in vitro* assays and compare the results;
- encapsulate EOs with silver nanoparticles (SNPs) or chitosan or/and zein and test them as fumigants and in dips and coatings, and
- design and construct a (mobile) system for accurate headspace analysis of EOs so that differences between the activities of the headspace and liquid EO can be better understood.

Performance chart

Milestone	Target Date	Extension Date	Date completed
1. Use a chemometric approach to flush out EO components active against both pathogens.	January 2016		January 2016
2. Apply a reductionist approach to identify the most active multi-target EOs.	September 2016		September 2016
3. Synthesize EO-encapsulated SNPs according to published methods using individual EOs.	June 2015		June 2015
4. Characterise EO-encapsulated SNPs using scanning electron microscopy and ultraviolet/visible spectrophotometry.	September 2015	Some aspects completed Dec 2015.	Other nanoparticles prepared by Sept 2017.
5. Prepare a solution of CHI-coated EOs and evaluate the antifungal activities of the prepared SNPs and EO-coated CHI <i>in vitro</i> . Zein and a	Not an original target, but included because micelles were too		Completed Oct 2016.

combination of zein and CHI were also tested as a vehicle for encapsulating EOs.	expensive and reagents scarce.		
6. Test various methods of wounding fruit for inoculation. Also establish standardised <i>in vitro</i> procedures.	May 2016.		May 2016
7. Test pure EOs, as well as modified EOs <i>in vivo</i> .	Completed August 2016	Extended to Sept 2017 to test on fruit in new season	Completed September 2017
8. Design and construct a headspace sampling system for GC analysis of EO-fumigant components.	Not an original target of the project		Completed July 2016
9. Validate the analytical system.	Not an original target	Extension was given to Sept 2017	Completed July 2017
10. Journal publication(s) – final PHI newsletter article Peer-reviewed article (CHI-coated EOs) Peer-reviewed article (headspace measurement EO fumigants)	Dec 2016 Dec 2016	May 2017 July 2017	Dec 2016 Not completed results not at publication stage.

Materials and methods

Pathogens

Isolates of *Geotrichum citri-aurantii* (sour rot) and *Penicillium digitatum* (green mould) were obtained from Citrus Research International (CRI). Spore suspensions of 1×10^8 spores/mL for sour rot and 1×10^6 for green mould were prepared from an 8-day old culture inoculated on the surface of a Potato Dextrose Agar (PDA, Oxoid, Johannesburg, South Africa) plate prior to the addition of sterile distilled water amended with Tween 80 [1 drop per 100 mL (Regnier *et al.*, 2014)]. These spore concentrations were used throughout.

In vitro experimental procedures to determine the effects of EOs and encapsulated EOs against both pathogens

Screening of EOs for multi-target activity using the toxic medium method

Certified essential oils ($n = 106$) were obtained from Pranarom (South Africa). These were screened using the toxic medium assay by incorporating the oils at a final concentration of 2000 μ L/L into sterilized potato dextrose agar medium using Triton-X100 as surfactant. The control consisted of un-supplemented agar containing only the equivalent amount of Tween. Once the agar was set, a 5 μ L volume of a spore suspension of sour rot was placed in the centre of the agar and the plates were incubated for six days at 25 °C. The zones of growth were measured using digital calipers. The trial was repeated with green mould. Plates with no visible mycelial growth were considered to successfully inhibit both pathogens and were considered highly active. Guazatine and Imazalil (both from Makhteshim-Agan SA (Pty) Ltd) were supplemented into media and served as positive controls for each of the pathogens, respectively. Five replicates were prepared for each concentration of the oils against the pathogens and the data were expressed as percentage inhibition of mycelial growth relative to the control, according to the method described by Plaza *et al.* (2004). Plates with between 50 and 100% inhibition against both pathogens were classified as intermediate activity and those with less than 50% inhibition against one or both pathogens were considered inactive.

Gas chromatography-flame ionisation detection/mass spectrometry (GC-FID/MS) analysis

The oils were prepared as 20% (v/v) solutions in dichloromethane. A 1 µl volume of each solution was analysed using a gas chromatograph (Agilent 6890N GC system coupled simultaneously to a 5973 MS and a flame ionization detector), equipped with an autosampler. A split ratio of 200:1 and an inlet temperature of 250 °C were applied. Separation was achieved by using an HP-Innowax polyethylene glycol column (60 m × 250 µm i.d. × 0.25 µm film thickness) and the following oven temperature programme: 60 °C for the first 10 min, increased to 220 °C at a rate of 4 °C/min and held for 10 min, before rising to 240 °C at a rate of 1 °C/min. Helium was used as carrier gas at a constant flow of 1.2 mL/min. The ion source was operated in electron impact mode (ionization energy 70 eV), while maintaining the GC-MS interface temperature at 260 °C. The relative percentage peak areas of the individual compounds were obtained from the FID chromatograms. Relative retention indices (RRI) were determined for individual components using *n*-alkanes as reference compounds. The identification of the compounds was carried out by comparing mass spectra and retention indices using NIST®, Mass Finder® and Flavour® data libraries and the Başer Library of Essential Oil Constituents.

Multivariate analysis

Chemometric models were constructed using Simca (Umetrics, Sweden). Principal component analysis (PCA) models were constructed from the data, which was aligned (chromatographic peaks) according to the retention times and mass/charge ratios. Scaling of the data was done. Thereafter the EO samples were divided into three, and later two classes, based on the activity against the fungi, for the construction of orthogonal projection to latent structures-discriminant analysis (OPLS-DA) models. By using the loadings plots as a guide, the number of variables was reduced in the model.

Toxic medium testing of EO mixtures at different ratios and concentrations

Mixtures of lemongrass/spearmint, lemongrass/cinnamon and cinnamon/spearmint at a 1:1 ratio were tested at three concentrations (100, 250, 500, 750 and 1000 µL/L) to determine possible synergistic or additive effects between the oils. Thereafter, the lowest concentration of the mixture where complete inhibition was observed was tested at various ratios consisting of 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8 and 1:9. All test substances were pre-mixed with two drops of surfactant and tested using the toxic medium assay as described against both green mould and sour rot. Negative controls consisted of PDA plates containing surfactant, but without any supplementation, while Guazatine and Imazalil (250 and 500 µL/L) were supplemented into media and served as positive controls. Five replicates were prepared for each concentration of the oils against the pathogens and the data were expressed as percentage inhibition of mycelial growth relative to the control, according to the method described by Plaza *et al.* (2004). Average values and standard deviations for replicate measurements were determined using Microsoft Office Excel 2010 Version 14.0. No tests were done to determine whether differences were significant or not, since the sole criterion used to determine efficacy was total pathogen control.

Microtitre assay to determine the effect of oils against the pathogens

Essential oils were diluted by pre-mixing with 100 µL Tween 80 to yield 10 mL volumes of concentrations of 64 and 48 µL/L in falcon tubes containing Sabouraud broth (Sigma Aldrich (Pty) Ltd, Johannesburg, South Africa). The oils were tested individually and in combination at a 1:1 ratio. A fixed aliquot (100 µL) of the broth was transferred into each well of a microplate. Thereafter, serial dilutions were performed by dispensing 100 µL of the EO solution into the first well, mixing and then removing the same aliquot and transferring to the next tube to yield concentrations of 32, 24, 16, 12, 8.0, 6.0, 4.0, 3.0, 2.0, 1.5, 1.0 and 0.75 µL/L. The positive control (without antimicrobial agent) consisted of Sabouraud broth instead of EO. Subsequently, 50 µL of fungal spore suspension of sour rot and green mould were dispensed into all the wells. After covering with a lid and sealing with parafilm, the microtitre plate was incubated at 25 °C for 48 hours in a shaking incubator, which was allowed to shake for 40 rpm throughout the incubation period to avoid spores deposition on the surface of the well. Thereafter, 0.4 mg/mL of *p*-iodonitrotetrazolium violet solution (INT) was added to each well (40 µL), and left to incubate for a further 24 hours at 40 cfm. The lowest dilution with no colour change was considered as the

minimum inhibition concentration for that oil. The assays were undertaken in triplicate, and further repetitions were conducted where necessary to ensure the accuracy of the results.

The failure to obtain reproducible results encouraged us to observe the well contents using a microscope and to determine how the exposed pathogens had been affected by plating out the suspension (5 μL) onto set PDA medium in petri-dishes. For the microscope observation method, the effect of the oils on arthroconidium germination was observed. After 24 h of incubation, 10 μL of each suspension was transferred to a glass slide. The spores were fixed using Lactophenol blue and observed with a BH-2 light microscope (Olympus, Japan) at 40X magnification. It was found that higher concentrations of oils than those originally used were needed to prevent spore germination, so the concentrations were increased substantially to evaluate this aspect. For the inoculation of the suspension in each well, a 5 μL volume from each well was plated on the surface of PDA set in petri-dishes.

Evaluation of the ability of AgNO_3 and its combination with the oils to inhibit both pathogens

Silver nitrate was assessed to establish its effect against the pathogens, with and without oil, when added to medium. Different concentrations of silver nitrate namely 1.0; 0.50; 0.010 and 0.0010% (m/v) were prepared and used as solvent for the preparation of PDA by adding medium powder and autoclaved. The prepared medium was allowed to cool to 50 $^\circ\text{C}$ and was then transferred to petri-dishes. For the mixture of silver nitrate with EOs, the medium was still prepared using the solution of silver nitrate as solvent and the oils were supplemented when the medium cooled to 50 $^\circ\text{C}$. The agar amended silver nitrate was allowed to set in petri-dishes; where after an aliquot consisting of a drop of fungal mycelia was placed aseptically onto the centre of each. The EO concentration was selected corresponding to the concentration where the oil inhibited mycelial growth to some degree, but did not completely inhibit the pathogen. Five replicates were prepared for each concentration. Negative controls consisted of PDA plates without any supplementation while Guazatine and Imazalil served as positive controls. After 6 days incubation at 25 $^\circ\text{C}$, the measurement of the mycelial growth was recorded as described above.

Synthesis and characterization of essential-oil encapsulated silver nanoparticles

This experiment was conducted as described by Vilas et al. (2014), as follows; the oils were diluted in acetone at a ratio of 1:170. The EO was added to 30 mL of 2.14×10^{-4} M AgNO_3 (pH 7) solution at 100 $^\circ\text{C}$ to obtain the colloid. The experiment was repeated by transferring 2.0, 3.0, 4.0 and 5.0 mL of the diluted oil to another flask containing 30 mL of 2.14×10^{-4} M AgNO_3 (pH 7), respectively. Colour changes from colourless to yellow or golden yellow indicate the formation of Ag nanoparticles (NPs). Diluted oil was added with vigorous stirring to 30 mL boiling solution of AgNO_3 (2.14×10^{-4} M) at varying pH conditions of 7, 8, 9 and 10, respectively. Colour changes were observed. The optimal conditions were used for the further preparation of the nanoparticles. The SNPs were characterised by UV-vis spectrophotometry and scanning electron microscope to determine their stability. Samples were scanned from wavelength 200-600 nm using a UV-vis spectrophotometer (Helios, England). Prior to sample analysis, unheated silver solution with EOs was used as the blank. To confirm the formation of SNPs FT-IR was used to identify functional groups of interest. The infrared spectra of SNPs were recorded in the range 4000-400 cm^{-1} (wavenumbers) using a PerkinElmer spectrometer mounted with a Universal attenuated total reflectance (UATR) diamond crystal. The liquid sample was placed directly onto the surface of the crystal diamond, the liquid was allowed to evaporate and the spectral data was captured in the absorbance mode after performance of background correction using PerkinElmer Spectrum software. A total of four automated scans were accumulated for each sample with a spectral resolution of 4 cm^{-1} . The analyses were done in triplicate and the spectra obtained were visually inspected. The suspensions containing the synthesized SNPs were freeze-dried and the powdered sample was sent to the CSIR for analysis by scanning electron microscopy.

Preparation of oil-coated chitosan solution

Chitosan solution was obtained by dissolving the polymer (30 mg/mL) in 1% (v/v) glacial acetic acid for 24 h at 25 $^\circ\text{C}$ (120 rpm). Serial dilutions (1:1) were performed in Sabouraud broth (Himedia, India) to obtain solutions of different concentrations (15, 7.5, 3.75 and 1.88 mg/mL). Concentrations of 3000 and 2000 $\mu\text{L/L}$ of the EOs

were prepared in Sabouraud broth. The oils were pre-mixed with Tween 80 (two drops in Eppendorf tubes) before dissolving in Sabouraud. Successive dilutions (1:1) were performed in the same broth to obtain solutions of different concentrations (15, 10, 7.5, 5, 3.75, 2.5, 1.88, 1.25 and 0.06 $\mu\text{L}/10\text{ mL}$).

The oil-coated CHI solutions were obtained by preparing the CHI solution as above, but using the MIC and the sub-MIC concentration of the CHI. The solution was left to stir for 6 h at 25 °C. Thereafter, the sub-MIC concentrations (first two) were added to the CHI solution. Tween 80 (1%) was transferred into the solution. The solution was left to stir for an extra 18 h at the same temperature and 120 rpm (dos Santos *et al.*, 2012).

***In vitro* antifungal activities of the prepared silver nanoparticles**

Different volumes (100, 200, 300, 400 and 500 μL) of each sample of synthesized nanoparticles suspension (prepared using lemongrass and/or spearmint) were transferred into sterile Eppendorf tubes containing 100 μL of the pathogen (green mould or sour rot). The control was prepared using the same volume of the pathogen together with 100 μL of PDA. The content of the Eppendorf tubes were vortexed prior to the incubation at 25 ± 1 °C for 24 hours in a shaking incubator at 40 rpm. Thereafter, two actions were taken. The first consisted of inoculating the surface of PDA media containing petri-dishes with an aliquot of the suspension (5 μL). The plates were incubated at 25 ± 1 °C and evaluated after one and four days of incubation. The second consisted of the microscopic observation to assess spore germination. A volume of 10 μL of the suspension was transferred and mixed with a drop of Lactophenol cotton blue on the surface of the glass slide. A cover slide was placed on the fixed suspension and the samples were examined with a light microscope (Olympus, Japan) at 40x magnification. The microscopic examination consisted of examining the presence of spore germination only (no spore germination count and germinated length were done).

***In vitro* antifungal activity of essential oils coated in chitosan**

Antifungal assay:

The MICs for CHI and EOs were determined using the broth macrodilution technique. Successive serial dilution was performed from the prepared CHI solution and EOs as explained above in test tubes. The final volume in the tubes containing different concentrations of the CHI and EOs was 5 mL. Then, 1 mL of freshly prepared fungal suspensions of sour rot or green mould and 4 mL of Sabouraud broth were added to the tubes. The volume was subsequently adjusted to 10 mL. The solution was incubated at 25 °C for 7 days, where after the turbidity of the medium was measured. The lowest CHI and EOs concentration that exhibited no visible fungal growth was considered the minimum inhibitory concentration (MIC) (Sharma & Tripathi, 2008). In the control experiment, the fungal suspension was inoculated in Sabouraud broth without CHI or EOs.

Inhibition of fungal mycelial growth

The ability of the EO coated with CHI to inhibit mycelial growth (dry mass weight) was determined by means of the poisoned growth substrate technique (dilution in broth) by inoculating 10 mL of each fungal suspension in 40 mL of Sabouraud broth supplemented with 50 mL of different oil-coated concentrations and incubated at 25 °C. After 3, 5 and 7 days of exposure, the mycelial mass was weighed and recorded. The mycelial mass was obtained by filtering the suspension through a pre-weighed paper filter (Sartorius AG, Goettingen, Germany) using a periplasmic pump (Merck Millipore, Germiston, South Africa). The emptied flask containing the suspension was rinsed with a suitable volume of chloroform (Merck (Pty) Ltd, Modderstein, Gauteng, South Africa) which was then filtered to rinse the mycelia on the surface of the filter paper. Then, the filter papers were placed in an oven for 10 min and weighed. In the control experiment, the fungal suspension was inoculated in Sabouraud broth without CHI and EOs. The results were expressed as percentage inhibition of the fungal mycelial mass obtained after different time intervals.

***In vivo* experimental procedures of the effect of the solutions against both pathogens**

CRI semi-commercial trial

Trial 1: To determine the efficacy of essential oils applied to wound-inoculated 'Valencia' oranges

The fruits were washed; surface sterilized using ozone and allowed to dry. Four wounds were inflicted in the rind on opposite sides of each fruit using a Stainless steel rod with a 2 mm long circular tip for green mould infection, and for sour rot, the three sharp points on the back of a picture hanger was used to inflict a wound. The instruments were sterilised throughout by dipping in ethanol, while the inoculum was applied after dipping them into an inoculum suspension of sour rot or green mould. The wound-inoculated fruits were treated after six and twelve hours of incubation with the pathogens. A total number of 144 healthy fruits were used for each treatment, as well as for the controls. The EOs (lemongrass and spearmint EO, alone and as a mixture) were added after mixing with Tween 80 to the dip tank of the semi-commercial packline at CRI at 3000 µL/L in the absence of synthetic fungicide. The negative control consisted of infected fruits that were not treated with EOs; while fruits treated with guazatine (1000 µL/L) and imazalil (500 µL/L) served as positive controls for sour rot and green mould, respectively. The fruits were packed as 12 fruits per box. All the boxes containing fruits infected with green mould were wet by adding a fixed volume (50 mL) of water to the cardboard insert to ensure sufficient humidity for the growth of the pathogen. The treated fruits were incubated till decay by sporulation was observed from the negative control. The fruit results were compared to those obtained using the conventional fungicide.

All the treatments used for this experiment are summarised in Table 1 below.

Table 1. Treatments applied to kumquat in the laboratory for Trial 1. For wax applications, Citrashine was used.

Treatment	Code	Composition
1	W	Citrashine
2	LG/SP3W	Lemongrass/spearmint (3000 µL/L; ratio 60:40) in Citrashine
3	LG3W	Lemongrass/citrashine (3000 µL/L)
4	SP3/W	Spearmint/citrashine (3000 µL/L)
5	CIN3/W	Cinnamon/citrashine (3000 µL/L)
6	LG/SP (P)	CHI/spearmint
7	CIN (P)	CHI/lemongrass

W = wax; P = paper

After seven days of storage at 25 °C, the fruits were assessed for decay and the results were expressed as percentage decay per treatments. Each treatment was applied in triplicate.

Trial 2: To determine the efficacy of essential oils applied using the conventional wax coating solution

The second trial consisted of challenging sour rot with the solution containing oil coated with CHI. The surfaced sterilized fruits were wounded and inoculated with the pathogens and incubated for 18 hours prior to treatment. The treatment included lemongrass (5000 µL/L) and spearmint (5000 µL/L) amended wax (Citrashine), individually, as well as a mixture of these oils at a total concentration of 5000 µL/L. The treatments were applied by means of a paint brush wetted with a fixed volume of the test solution. The positive controls consisted of Guazatine and Imazalil-supplemented wax; while the negative control comprised fruit coated with wax alone. The fruit were examined when the negative control had grown sufficiently.

Sixty fruits were packed into individual boxes where after ¼ of the fruits in each box were wound-inoculated with freshly prepared spore suspensions of sour rot as before and the remaining ¾ was just wounded. The inoculation and wounding were performed 24 hours prior to the treatment, after which the fruits were stored at room temperature. After seven days of storage at 25°C, the fruits were assessed for decay and the results were expressed as percentage decay per treatments. Each treatment was applied in triplicate (Table 2).

Table 2. Treatments applied to kumquat in the laboratory for Trial 2 at TUT.

Treatment No:	Code	Composition
1	W	Citrashine (Control)
2	CHI	CHI (15 mg/mL)
3	CHI/LG 1	CHI (15 mg/mL)/lemongrass (500 µL/L)
4	CHI/LG 2	CHI (15 mg/mL)/lemongrass (1000 µL/L)
5	CHI/SP 1	CHI (15 mg/mL)/spearmint (500 µL/L)
6	CHI/SP 2	CHI (15 mg/mL)/spearmint (1000 µL/L)

CHI = chitosan

Laboratory *in vivo* pre-commercial pack-house trials on kumquat conducted at TUT

Freshly harvested, untreated export quality kumquats were obtained during the 2017 season from a pack-house near Levubu (Limpopo Province, South Africa). The fruits were packed into boxes and transported to the Tshwane University of Technology (TUT) laboratory facility (Department of Biotechnology and Food Technology), where they were stored in the cold room (5 °C).

Prior to the curative treatment, the fruits were surface-sterilized with 70% ethanol for 3 minutes and allowed to dry on the surface of the laboratory bench covered with paper towel at room temperature (20±1 °C). The fruits were packed to a 100-count in each box. Thereafter, one quarter (¼) of the fruits in each box were wound-inoculated with freshly prepared spore suspensions of sour rot using the points on the back of a picture frame holder, while the remaining ¾ of the fruit were just wounded. The inoculation and wounding were performed 24 hours prior to the treatment, after which the fruits were stored at room temperature.

The control consisted of a wax application (Citrashine) alone (W) as indicated in Table 1. The essential oil treatments consisted of mixtures or pure oils added to the wax at 3000 µL/L and applied using a paintbrush. Two treatments (6 and 7) consisted of paper impregnated with 3000 µL/L of oils as indicated. The paper was wetted with the solution and placed above the fruit in the box without touching. Cinnamon oil was added since a report had indicated that the oil has good antifungal properties towards decay pathogens.

Trial 3: Effect of dip tank temperature on the quality of the kumquats

Trial 3 was aimed at investigating whether the quality of the fruit was affected by the temperature of the dip tank. Fruit (each sample consisting of 20 fruit) were dipped in a bath maintained at 30, 35, 37, 40, 45 and 50 °C for 1 min and left to dry at room temperature on a paper towel. To determine whether the exposure to a specific temperature had caused the immune response of the fruit to be stimulated, the phenolic content of the peels were determined. Peels were removed immediately from ten of the twenty fruits per sample and stored in a freezer until required, while the remaining ten fruit were stored in the cold room (5 °C) for 34 days. The control consisted of undipped fruit. After 34 days storage, the peels of fruits from each sample were removed and stored at -80 °C. Thereafter, peels from all samples were freeze-dried (Telstar, LyoQuest-55, Spain) and then ground using a mortar and pestle. The ground peels were sieved (<500 µm), to guarantee uniformity of particle size. The powdered peels of each sample were transferred to falcon tubes.

Powdered peel (0.5 g) was placed in teflon extractor tubes (Mad Technology, South Africa) and suspended in 15 mL of methanol:acetone:water (7:7:1) for 30 min. Thereafter, the soluble phenolic compounds were extracted in triplicate by means of an industrial microwave oven (MarsX Express microwave extractor, Mad Technology, South Africa). The temperature of the mixture was ramped to 75 °C within 5 min and held for 15 min. The extraction of samples was repeated three times. After each extraction, the supernatant was transferred to 50 mL falcon tubes (Lasec, South Africa) and 15 mL of the solvent was transferred into the Teflon extractor tubes containing the peel residues. A total volume of 45 mL resulted from each sample. Thereafter, the extract was concentrated to about 10 mL using a Genevac evaporator (EZ-2 Personal Evaporator (United Scientific, South Africa).

Total phenolic contents were determined by a modified Folin Ciocalteu method reported by Augustyn *et al.* (2014) with minor modification. The assay was carried out in flat-bottom 96-well Elisa plates (Merck, Germany).

Distilled water (165 µL), Folin Ciocalteu (25 µL) reagent (Sigma, South Africa), plant extract (5 µL) and freshly prepared 20% (w/v) sodium carbonate (50 µL) solution were placed in each well, following this order. The plates were incubated at 40°C for 30 min. After incubation, the absorbance of the contents of each well was measured at 690 nm against the blank using a Spectramax 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA). A standard curve was determined using gallic acid, and the total phenolic content was expressed as mg gallic acid equivalents (GAE) per 0.5 g dry extract using a standard curve. All samples were analysed in triplicate.

Trial 4: Effect of dip tank temperature on the development of postharvest pathogens

The same experiment was performed to assess the effect of different temperatures (30, 35, 37, 40, 45 and 50 °C) on pathogen development. The fruit were placed in aluminium foil containers. Sixty fruit were dipped in a warm water bath set at the above-stated temperature for one minute. The fruits were allowed to dry at room temperature, and then stored at 25 °C for 14 days and in the cold room (5 °C) for 34 days. After cold storage, the fruits were stored at 25 °C for an additional four days. The fruits were examined for the presence of pathogens (rind softening, appearance of mould, shrivelling, blister-forming, browning) and weighed.

Commercial trials at Levubu

Three trials were conducted at the Levubu Pack-house between June and September 2017. The first trial evaluated the potential use of lemongrass, spearmint EOs and their mixture as well as SNPs applied alone or in impregnated paper. The second trial evaluated the possibility of using the essential oils (spearmint and cinnamon leaf) in high concentrations (up to 8000 µL/L), their encapsulated forms as well as their SNP-mediated synthesis solution. The third trial was designed to formally evaluate the best two oils at 3000 µL/L, either by wax dip or by paper impregnation with the aim of evaluating the efficacy of the treatments under export condition.

Trial 1: Potential use of lemongrass, spearmint EOs and their mixture as well as SNPs applied alone or in impregnated paper

Various treatments (30 boxes x 12 treatments) were used to test the efficacy of pathogen control on kumquat are summarised in Table 3 below.

Table 3. List of treatments applied in Trial 1 to kumquat in Levubu.

Treatment no:	Code	Composition
1	C	Control: water bath at 37 °C
2	LG2	Oil 1 concentration 1 (2000 µL/L)
3	LG3	Oil 1 concentration 2 (3000 µL/L)
4	SP2	Oil 2 concentration 1
5	SP3	Oil 2 concentration 2
6	MIX	Mixture oil 1 and 2 (concentration 2)
7	CN2	Oil 3 concentration 1
8	CN3	Oil 3 concentration 2
9	W	Wax
10	WMIX	Wax + Mixture
11	NLG	Lemongrass mediated synthesis of silver nanoparticle
12	P	Paper impregnated with Lemongrass oil

All boxes were left in the pack-house overnight before storage. Furthermore, 24 boxes of each treatment with 15 boxes of treatment No 11 were stored for 26 days at 5 °C in the pack-house facility. The remaining five boxes per treatment were transported to the Department of Biotechnology and Food Technology at Tshwane University of Technology (TUT) (Pretoria), where 2 boxes were stored in a cold room at 5 °C for 34 days, while

3 boxes were stored at 18 °C for 4 days. The initial weight of each box was recorded prior to storage. The results were recorded as percentage weight loss and decay per treatment.

Trial 2: Possibility of using EOs (spearmint and cinnamon leaf) in high concentrations (up to 8000 µL/L), with encapsulated forms as well as SNP-mediated synthesis solution

The treatments (15 boxes of 100 fruit x 14 treatments) were used for the second trial as summarised in Table 4.

Table 4. List of treatments used for Trial 2 in Levubu.

Treatment no:	Code	Composition
1	C	Control: water bath at 37 °C
2	SP8	Oil 2 concentration 3 (8000 µL/L)
3	CIN8	Oil 3 concentration 3 (8000 µL/L)
4	M	Mixture oil 2 and 3 (concentration 3)
5	W	Wax
6	WSP	Wax + Oil 2
7	WCIN	Wax + Oil 3
8	WM	Wax + Mixture
9	NSP	Spearmint mediated synthesis of silver nanoparticle
10	NCIN	Cinnamon mediated synthesis of silver nanoparticle
11	NM	SP/CIN mediated synthesis of silver nanoparticle
12	NMW	SP/CIN mediated synthesis of silver nanoparticle + Wax
13	ZnEO	Zein + EO
14	ZnCHEO	Zein + CHI + EO

With the exception of the last treatment, which was used as a dip for 2 minutes, all the other treatments were applied using the pack-line sprayer. All treated fruits went through the pre-wetted brush roller of the sprayer with the same treating solution and packed. Each treatment consisted of 15 boxes of 100 fruits each and the initial weight of each box was recorded. All boxes were left in the pack-house overnight before storage. Five boxes of each treatment were stored for 26 days at 5 °C in the pack-house facility, while 10 boxes of each treatment were taken back to TUT. Five boxes were stored in a cold room at 5 °C for 34 days while the remaining five were stored at 20 °C for 10 days.

Trial 3: Evaluating the efficacy of the treatments under export condition

The treatments used for the third trial are summarized in Table 5. This trial was aimed at confirming the efficacy of the selected treatment after export to Europe.

Table 5. List of treatments applied on the fruits during Trial 3 in Levubu.

Treatment no:	Code	Composition
1	C	Control: water bath at 37 °C
2	SP3	Oil 2 concentration 2 (3000 µL/L)
3	CIN3	Oil 3 concentration 2 (3000 µL/L)
4	WM	Wax + Mixture oil 2 and 3 (concentration 2)
5	WCIN	Wax + Oil 3 concentration2 (3000 µL/L)
6	WSP	Wax + Oil 2 concentration 2 (3000 µL/L)
7	NM	SP/CIN mediated synthesis of silver nanoparticle
8	W+SP/P	Wax + Oil 2 on paper
9	W+LG/P	Wax + Oil 1 on paper

Treatments 2 to 7 (240 boxes x 2 kg x 9 treatments) were applied by means of a pack-line sprayer. Treatments 8 and 9 consisted of absorbent paper as previously used in Trial 1. All fruits went through a commercial pack-line and were then packed in boxes. Five boxes of each treatment were transported to TUT and stored at 5 °C for 34 days prior to the extended 4 days storage at 20 °C, while five more boxes of each treatment were stored at 5 °C for 34 days in Levubu. All the remaining boxes for treatments 1 to 7 were exported to Europe where, fruits firmness, colour, percentage of decay, rind pitting and shrivelling, were recorded. Fruits stored at TUT were only assessed for the presence of pathogen infection.

Slow release mechanisms for active volatile components of EOs against green mould and sour rot

It was important that, as a forerunner to the analytical work on the headspace, the spearmint oil be properly characterised and the analytical methods validated.

Analysis of spearmint essential oil and its components

The GC method used for identification of the compounds in the EO was described above. The relative retention index obtained for each compound was compared to those obtained for authentic standards on the same stationary phase as well in the literature to confirm the identities. Authentic standards were used for the quantification of the EO components identified in the spearmint oil. A series of standards ranging from 1.00-100 µL/L were prepared in hexane and analysed using GC-FID to validate the method for each compound. The exact concentration of the main components, rather than the relative concentration, of individual components within the oil was determined. The LOD and LOQ for the identified terpenes were determined using GC-FID (Table 6).

Table 6. Essential oil composition of spearmint essential oil determined using GC-FID/MS

Compound Name	% Composition	RRI
α-Pinene	0.708	948
Camphene	0.603	943
β-Pinene	0.688	943
Myrcene	0.35	958
Limonene	11.12	1048
3-Octanol	1.109	979
β-Bourbonene	1.327	1339
Dihydrocarvone	0.696	1179
Borneol	1.987	1088
Carvone	76.48	1190

KI is the Kovats index of the individual components

Encapsulation of spearmint oil

Spearmint EO was encapsulated using zein, zein+chitosan and polylactide acid (PLA). Liquid-liquid dispersion was used to encapsulate the oils in the different material. All the material used to encapsulate the oil is biodegradable and environmental friendly.

Characterisation of encapsulated oil

Fourier-transform infrared spectroscopy was used to characterise the chemical structure of the EO, zein, PLA and encapsulated oils. For each sample 16 scans at a resolution of 4 cm⁻¹ were obtained.

Minimum inhibition concentration of essential oil and pure compounds

The MICs of the encapsulated EOs were determined as described.

Determination of vapour pressure of essential oil components using thermogravimetric analysis (TGA)

The EOs, terpenoids and encapsulated EOs were all analysed using a thermogravimetric analyser. Approximately 10.0 mg of the sample mass was weighed and the data was collected in the temperature range of 25 °C to 350 °C. The equipment recorded both TG and DTA data simultaneously at a heating rate of 10 °C/min. Dry nitrogen was used as a purge gas at a flow rate of 50 mL/min.

Design of a mobile GC sampling system

A system was designed and constructed as discussed in the results section.

Analysis of spearmint oil using the designed GC-sampling system

The designed GC-FID modular system was used to quantify the composition of the EO vapour phase using: direct headspace, solid phase micro-extraction (SPME) and thermal desorption (TD). Through initial optimisation of the instrument it was found that the optimum volume and equilibration time is 100 µL and 60 min, respectively. Concentrated spearmint oil was added to an amber vial and equilibrated at room temperature for 60 min. For direct headspace determination the sample was injected into the GC after the equilibration time. The SPME syringe was simultaneously inserted into the specially designed port and kept there for two minutes, where after the oil components were desorbed and analysed through insertion into the injection port of a different GC-FID. Tenax samples were simultaneously prepared by allowing the sample to run through the tube for two minutes at a constant flow rate. A comparison was done of the composition of the vapour phases to that of the liquid phase.

Results and discussion

A principal component analysis (PCA) model of all the 106 oil profiles comprised 1723 compounds. No clustering or groupings were evident on the scores plot, indicating that the chemical profiles were highly variable in composition. The samples were then divided into three groups determined by their antifungal activities i.e. active (Class 1; 28 samples), intermediate activity (Class 2; 42 oils) and inactive (Class 3; 36 oils). The constructed orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was not encouraging. It was then decided to remove the intermediate group and the remaining 64 samples were then classified as active (Class 1) or inactive (Class 2). The resulting OPLS-DA scores plot revealed two clearly separated groups representing the two classes of activity. A loadings plot allowed the peaks associated with good activity to be identified. However, the model statistics, particularly the predictive component Q^2 , was not satisfactory. The model was therefore further refined by only selecting compounds directly associated or poor and with good activity. Only 64 variables (compounds) were retained in the model and a new model was constructed that revealed the most active compounds. Limonene, which we know to have poor activity, was amongst these compounds, indicating incorrect identification. It is possibly due to the presence of high concentrations of inactive limonene in many of the active oils, together with other active components that skewed the results. In a further model, four oils, including *Citrus sinensis* were flushed out as the most active oils, which we found not be true in further investigations. The lack of corresponding compounds in sufficient numbers in the selected EOs is a stumbling block to model development. For the toxic medium work, three EOs were selected for the modification trials i.e. lemongrass, spearmint and cinnamon. The main compounds were determined so that these could be tested for activity on their own to determine whether they play a role in the activity of the oil (Table 7).

Table 7. Relative percentage peak areas of the six most abundant compounds (above 1%) that were identified in selected essential oils by GC–MS and quantified by GC-FID.

Essential oil	Percentage peak areas of compounds					
	1	2	3	4	5	6
Lemongrass	Geranial (37.0%)	Neral (32.6%)	Limonene (10.0%)	α-Terpineol (5.1%)	Geraniol (3.5%)	Citronellol (3.2%)
Spearmint	Carvone (76.5%)	Limonene (11.1%)	Borneol (1.99%)	β-Bourbonene (1.33%)	3-Octanol (1.11%)	-

Lemongrass was the most effective EO against sour rot, while cinnamon was the most effective against green mould (Table 8). A mixture of spearmint and lemongrass (1:1) yielded the same result as lemongrass on its own for sour rot. A mixture of cinnamon oil and lemongrass also gave the same result for green mould as cinnamon alone. In the study conducted by Regnier *et al.* (2014), it was reported that lemongrass inhibited the spores of sour rot following exposure to concentrations of 600 µL/L. while the combination 1:1 (v/v) of the mixture lemongrass/spearmint also resulted in total suppression of spore germination at 500 µL/L.

Table 8. Inhibitory effects of individual oils (cinnamon, lemongrass and spearmint) as well as their mixture at various concentrations on the mycelial growth of sour rot and green mould. Guazatine and imazalil were used as positive controls for sour rot and green mould, respectively. All results are expressed as percentage inhibition ± standard deviation (%) (N = 5). Boldface indicates oil concentrations that completely inhibited mycelial growth.

Test substance	Concentration (µL/L)	Pathogen inhibition (%)	
		Sour rot	Green mould
Guazatine	250	100	-
	500	100	-
Imazalil	250	-	100
	500	-	100
Lemongrass	100	10.1 ± 3.3	7.9 ± 3.3
	250	74.8 ± 1.2	8.8 ± 3.7
	500	100	31.0 ± 7.3
	750	100	100
	1000	100	100
Spearmint	100	1.2 ± 0.9	0.00
	250	10.1 ± 1.9	20.2 ± 3.0
	500	40.3 ± 1.3	35.9 ± 2.7
	750	70.2 ± 1.3	100
	1000	100	100
Cinnamon	100	0.00	1.1 ± 0.8
	250	0.00	35.9 ± 2.7
	500	47.7 ± 3.5	100
	750	100	100
	1000	100	100
Lemongrass/spearmint (5:5)	250	50.7 ± 2.4	42.4 ± 8.3
	500	100	53.1 ± 8.6
	750	100	100
	1000	100	100
Lemongrass/cinnamon (5:5)	250	13.5 ± 2.7	36.1 ± 2.1
	500	51.0 ± 8.9	100
	750	100	100
	1000	100	100
Spearmint/cinnamon (5:5)	250	34.4 ± 4.5	47.2 ± 3.4
	500	89.1 ± 1.5	67.3 ± 6.3
	750	100	100
	1000	100	100

The best outcomes on the assessment of the mixture of oils at different ratios (Table 9) were lemongrass/spearmint at a ratio of 7:3 and for ratios with an even higher relative concentration of lemongrass, while lemongrass/spearmint with at least 60% lemongrass and more gave the best results against green mould. Spearmint/cinnamon inhibited sour rot completely at a ratio of 3:7 and at ratios with even higher concentrations of cinnamon. Both green mould and sour rot were inhibited at a ratio of 5:5 and at higher

concentrations of lemongrass oil. Their does not seem to be a synergistic effect when the oils are combined because the results obtained with oil mixtures were no better than those of the oils alone.

Table 9. Inhibitory effects of oil mixture combined at various ratios 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8 and 1:9 against sour rot and green mould. All results are expressed as percentage inhibition \pm standard deviation (%) (N = 5). The total concentration was selected based on the results from Table 8 and corresponds to the minimum effective concentration of the appropriate mixture.

Combination	Lemongrass/Spearmint		Spearmint /Cinnamon		Lemongrass/Cinnamon	
	Pathogen inhibition (%)					
	Sour rot	Green mould	Sour rot	Green mould	Sour rot	Green mould
	Total concentration (μ L/L)					
	500	750	750	750	750	500
9:1	100	100	10.4 \pm 5.2	40.2 \pm 6.8	72.3 \pm 2.9	53.2 \pm 2.3
8:2	100	100	20.6 \pm 4.8	51.8 \pm 3.4	74.6 \pm 3.1	65.0 \pm 6.7
7:3	100	100	34.2 \pm 6.0	61.3 \pm 4.7	76.7 \pm 2.0	72.8 \pm 1.8
6:4	93.9 \pm 12.2	100	56.1 \pm 5.9	100	81.4 \pm 0.7	84.7 \pm 0.0
5:5	64.9 \pm 4.6	91.2 \pm 12.1	61.1 \pm 7.1	100	100	100
4:6	54.7 \pm 6.7	79.0 \pm 5.3	68.5 \pm 1.9	100	100	100
3:7	49.1 \pm 0.9	57.4 \pm 5.5	100	100	100	100
2:8	37.8 \pm 7.0	55.5 \pm 4.7	100	100	100	100
1:9	31.2 \pm 5.5	44.06 \pm 3.8	100	100	100	100

The results from the microtitre plate assay were different to those from the toxic medium assay when comparing the inhibitory concentrations. The determined MIC values were higher than the concentrations from the toxic medium assay. The MIC values for lemongrass and spearmint against sour rot were 1000 and 2000 μ L/L, respectively, while the MIC value against green mould for both lemongrass and spearmint was 4000 μ L/L. Other researchers (Thippeswamy et al. 2013) also reported that results of the toxic media and the microtitre plate for MICs were not the same. However, in their experiments, the toxic medium concentrations were higher than those from the microtitre assay.

Microtitre assay to determine the effect of oils against the pathogens

The antimicrobial activity of the oils was not clearly observed with the application of the INT. The change in colour was apparent in all the wells. However, the colour change was less intense as the concentration of the oils increased. In addition, different results were observed after every week that the assay was repeated. As a result of this inconsistency, the assay was carried out in such a way the experiment had four replicates (four microtitre plates for one pathogen); from which one plate was examined for spore germination and mycelial growth without being subjected to the INT colouring reagent. The results of the plates to which INT was added, were compared to microscope observations of the well contents and the results obtained by re-plated onto PDA (Figure 1).

Microscope observations revealed an inhibition of spore germination at 3000 μ L/L (Figure.1). This result was confirmed with the inoculation of the PDA plate and incubation for four days; whereby complete inhibition of the pathogen was observed at 3000 μ L/L (Figure 2). Since sour rot is a wound pathogen inhibition of germination is very important since spores that are distributed in the pack-house may flourish in wounds formed on the rind.

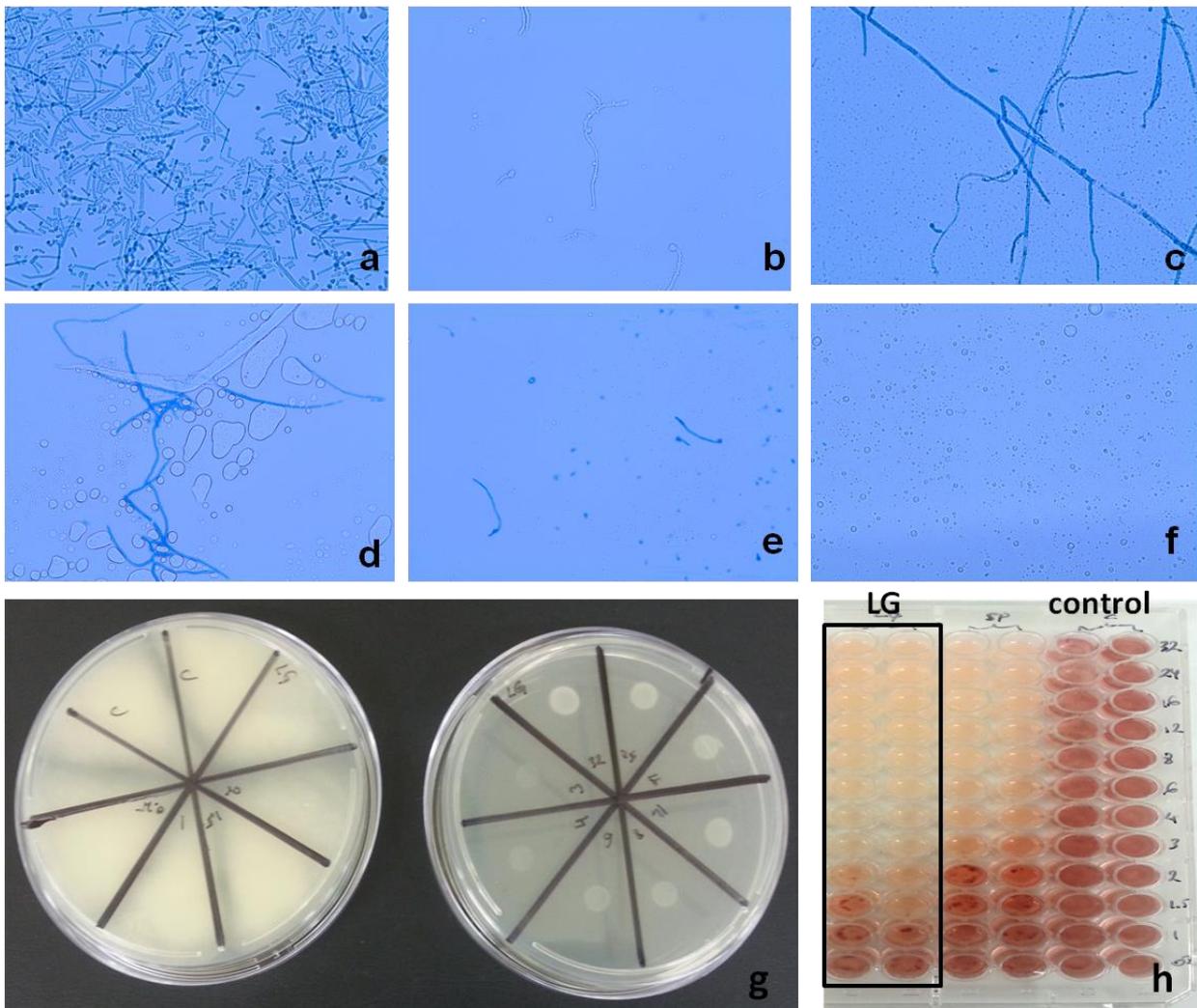


Figure 1. Micrograms obtained after exposing sour rot to lemongrass oil in microtitre wells a) Control sour rot b) 750 $\mu\text{L/L}$; c) 1000 $\mu\text{L/L}$; d) 1500 $\mu\text{L/L}$; e) 2000 $\mu\text{L/L}$; f) 3000 $\mu\text{L/L}$, Photographs of PDA plates after re-planting the well suspension without INT (g) and colour changes observed in the microtitre plate after the addition of INT (h). The concentration of the dilution ranged from 32000 to 750 $\mu\text{L/L}$.

The inoculation of the PDA with the suspension of each wells of the microtitre plate revealed that cinnamon oil provided the lowest concentration for the inhibition of both sour rot and green mould at concentrations 2000 $\mu\text{L/L}$ and 3000 $\mu\text{L/L}$, respectively (Table 10; Figure 2). The worst performance was demonstrated with spearmint which required high concentration such as 12 000 $\mu\text{L/L}$ to inhibit sour rot and 8000 $\mu\text{L/L}$ for green mould. The mixture lemongrass/spearmint performed better than the other mixtures (cinnamon/lemongrass and cinnamon/spearmint) against sour rot with a concentration of 3000 $\mu\text{L/L}$. The mixture of cinnamon/spearmint required a higher concentration (6000 $\mu\text{L/L}$) to inhibit green mould while the remaining mixtures inhibited the same pathogen at concentration 4000 $\mu\text{L/L}$.

Table 10. Concentrations of essential oils at which no growth was observed for individual oils and a mixture of oils using the microtitre assay. The plates were incubated for 24 hours prior to inoculation onto the PDA. The result was observed after 4 days of incubation. The concentrations ranged from 32 000 to 500 $\mu\text{L/L}$.

Essential oils	Concentrations for pathogens inhibition ($\mu\text{L/L}$)	
	Sour rot	Green mould
Cinnamon	2000	3000
Lemongrass	4000	4000
Spearmint	12 000	8000

Cinnamon/lemongrass (5:5)	4000	4000
Cinnamon/spearmint (5:5)	4000	6000
Lemongrass/spearmint (5:5)	3000	4000

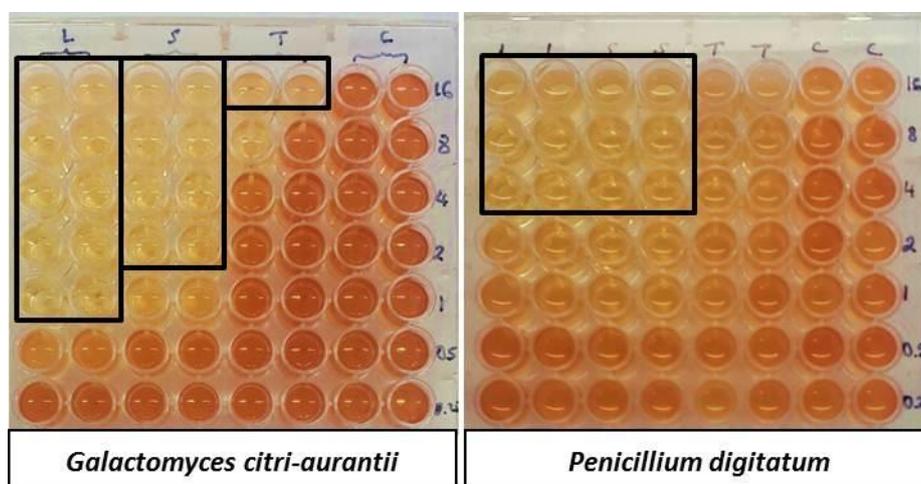


Figure 2. Microtitre assay to determine the minimum inhibitory concentrations of lemongrass and spearmint against sour rot and green mould at various concentrations from 16 000-250 $\mu\text{L/L}$. L= lemongrass, S = spearmint and C = control.

Evaluation of the ability of AgNO_3 and its combination with the oils to inhibit both pathogens

The results indicated that silver nitrate alone inhibited both pathogens at 0.10% (0.064% of Ag^+). However, silver nitrate at 0.05% (0.032% Ag^+), combined with lemongrass (100 $\mu\text{L/L}$) and spearmint (250 $\mu\text{L/L}$) oils, resulted in the complete inhibition of fungal growth of both pathogens, indicating a synergistic or additive effect of the two test substances. These results confirmed the ability of silver nitrate to inhibit fungal pathogens. The combination of oils and silver nitrate reduced the concentrations of both substances needed for the control of sour rot and green mould when compared to the individual oil results (Table 11) and silver nitrate alone.

Table 11. Inhibitory effects of selected essential oils, silver nitrate as well as mixture of oils and silver nitrate at various concentrations on the mycelial growth of sour rot and green mould. Guazatine and imazalil were used as positive controls for sour rot and green mould, respectively. All results are expressed as percentage inhibition \pm standard deviation (%) (N = 5). Boldface indicates concentrations that caused complete inhibition.

Test substance	Concentration	Pathogen inhibition (%)	
		Sour rot	Green mould
Silver nitrate	%		
	0.01 =0.0064% Ag^+	32.8 \pm 1.0	9.6 \pm 1.1
	0.05=0.032% Ag^+	37.4 \pm 1.4	27.3 \pm 4.7
	0.1 = 0.064% Ag^+	100	100
Silver nitrate + lemongrass	% + $\mu\text{L/L}$		
	0.01 + 100	35.5 \pm 11.0	42.7 \pm 8.0
	0.05 + 100	100	100
Silver nitrate + spearmint	% + $\mu\text{L/L}$		
	0.01 + 250	34.4 \pm 9.0	27.4 \pm 7.4
	0.05 + 250	88.5 \pm 23.1	94.4 \pm 11.2
	0.01 + 500	100	100
	0.05 + 500	100	100
Silver nitrate + cinnamon	%+ $\mu\text{L/L}$		

	0.01 + 250	100	100
	0.05 + 250	100	100
	0.01 + 500	100	100
	0.05 + 500	100	100

Synthesis and characterization of essential-oil encapsulated silver nanoparticles

The synthesis of nanoparticles was optimized at pH 10 where the colour change signalling nanoparticle formation was most marked. The samples were analysed using a Shimadzu UV-1800 spectrophotometer. The UV-spectrum of the resulting SNPs showed a prominent peak at λ_{\max} = 419.5 nm at pH 10 for lemongrass. This peak is associated with formation of EOs enhanced SNPs, and corresponds to the theoretical value of λ_{\max} = 420 nm. Prior to the characterisation of the synthesized nanoparticles, a blank composed of unheated silver nitrate and EO, which was interchanged depending on the oil used for formation of each of the sample of nanoparticles, was analysed and compared as a control. Scanning electron microscopy was used to obtain a visual record of the nanoparticles. This indicated the presence of SNPs. However, the technique for SNPs entrapment need to be revised since it is believed that some other substances, probably also sodium hydroxide, are also present in the solid generated. The antifungal assay of the freeze-dried SNPs and silver nitrate using the microtitre plate revealed that the synthesized SNPs have a weak inhibition towards both pathogens, while low concentrations of silver nitrate was very effective. There was no inhibition at 1000 μ L/L for SNPs against both pathogens. In contrast, silver nitrate inhibited both sour rot and green mould at 0.0156 μ L/L. All the nanoparticles were further characterized using FTIR. It was found that all the nanoparticles formed with different EOs had very similar spectra; there was a difference between the spectra of pure oils and the spectra of EO-enhanced nanoparticles due to formation of SNPs (Figure 3).

The spectra of all SNPs displayed similar absorptions. The band at 1639.5 cm^{-1} was characteristic for the EOs alone but almost disappeared after nanoparticle formation. The UV-spectrum of resulting SNPs showed a prominent peak at λ_{\max} = 419.5 nm at pH 10 as illustrated in the case of lemongrass (Figure 3). The peak is associated with formation of EO-enhanced SNPs, which is slightly lower than theoretical value of λ_{\max} = 420 nm. Prior to the characterisation of the synthesized nanoparticles, a blank composed of unheated silver nitrate and EO, which varied depending on the oil used for formation of each of the sample of nanoparticles, was analysed and compared to that of the prepared nanoparticle solution. The technique for SNPs entrapment need to be revised since NaOH crystals, originating from the addition of NaOH used to increase the pH of the solution, are visible between the nanoparticles (Figure 4).

Lemongrass pure oil

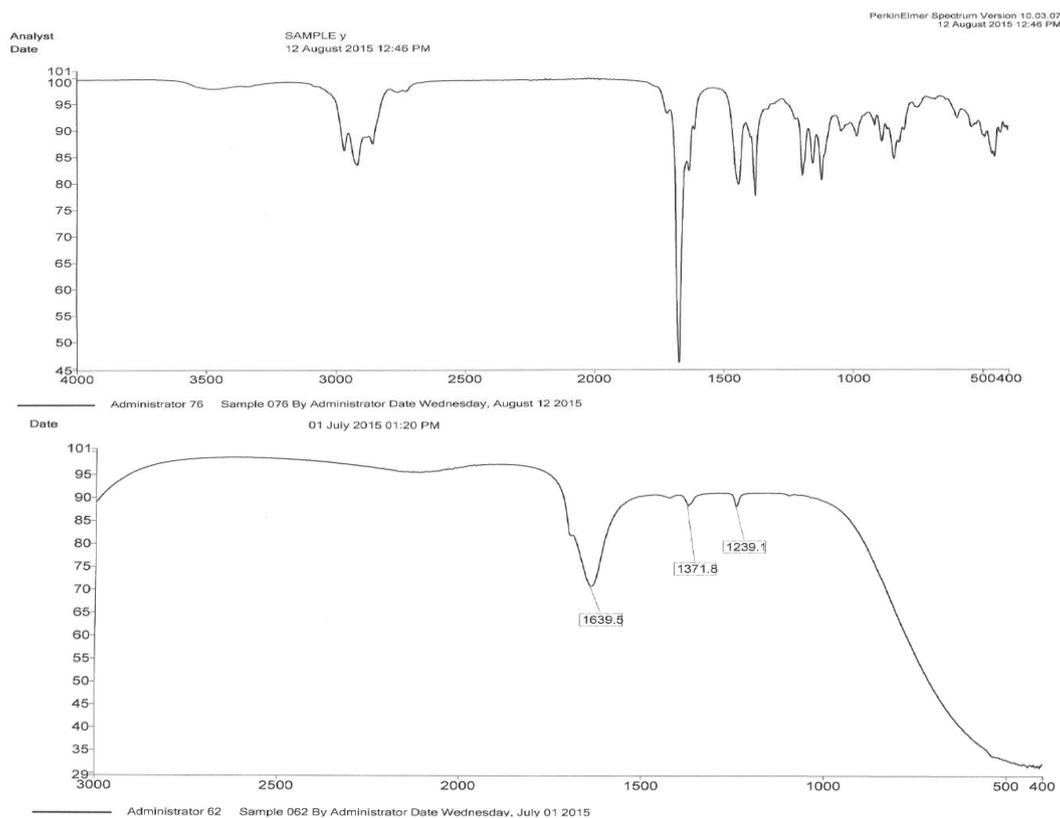


Figure 3. FTIR spectra of pure lemongrass (upper) and lemongrass enhanced SNPs at pH 10.

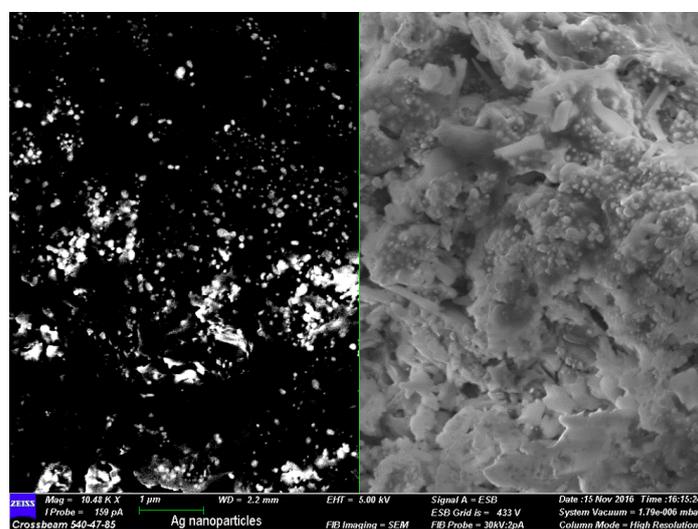


Figure 4. Scanning electron microscope illustration of SNPs using lemongrass as reducing and stabilizing agent. The white dots are indicative of nanoparticle formation.

All tested SNPs synthesized retarded the growth of green mould. After 24 hours of incubation, mycelial growth could already be observed on the control, although no growth was visible on the plates inoculated with the suspension containing SNPs and green mould at all the tested volumes of the SNP solutions (from 100 to 500 μ L) (Figure 4A and B). The results of the extended incubation period to 4 days revealed that the synthesized SNPs did not completely inhibit green mould, but prevented the sporulation of the pathogen (Figure 4C and D). The increase in the amount of added SNPs did not improve the inhibitory effect against the pathogen. This lack of a dose-response effect indicates that the concentration of EO may be too low in these particles and that an increase in the concentration of nanoparticles does not correspond to a large increase in EO

concentration. Attempts were made to increase the concentration of EO in the SNPs but this resulted in a failure to encapsulate the oil and instead it merely ended up as “free EO” in the solution.

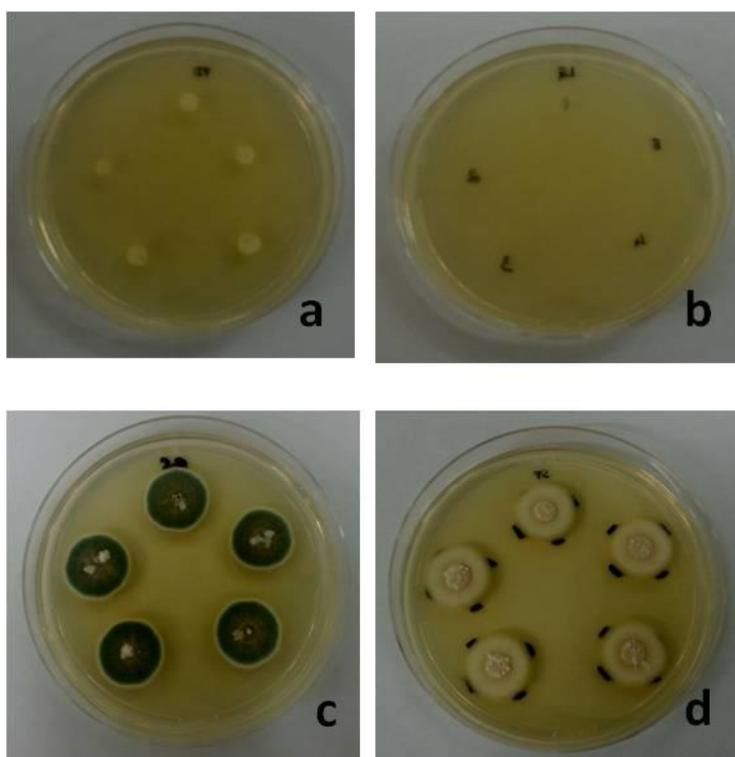


Figure 5. Radial mycelial growth of synthesized lemongrass-mediated-SNPs against green mould: a) control and b) SNP results after 24 hours of incubation; c) control and d) SNPs results after four days of incubation.

The synthesized SNPs were not effective against sour rot *in vitro*. Growth was observed on the plate after 24 hours incubation. There was no difference between the control and the tested SNP suspension, even when the plates were observed after 4 days of incubation (Figure 6). Microscope observations to evaluate the germination of spores after exposure to the EO-encapsulated SNPs revealed that the spores of green mould did not germinate, but that sour rot was not affected.

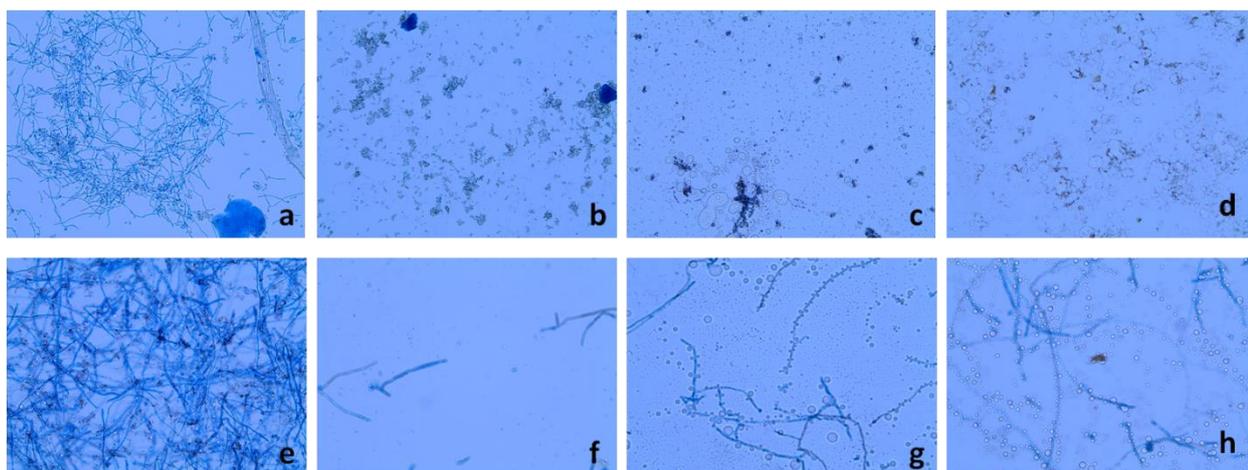


Figure 6. Effect of the lemongrass-mediated SNP suspension on the spore germination of green mould and sour rot after 24 hours of incubation: a) Control lemongrass; b) 100 µL SNPs against 100 µL of green mould suspension; c) 200 µL SNPs against 100 µL spores suspension; c) 300 µL SNPs against 100 µL spore suspension; e) control green mould; f) 100 µL SNPs against 100 µL of sour rot suspension; g) 200 µL SNPs against 100 µL spore suspension and h) 300 µL SNPs against 100 µL spore suspension.

Testing of oil-coated chitosan solutions

Chitosan alone inhibited the growth of the pathogens (Table 12; Figure 7). Complete inhibition of fungal growth was observed for the two highest concentrations (15 000 mg/L and 7500 mg/L) against both pathogens.

Table 12. Effect of chitosan and essential oils alone against citrus pathogens as determined by the macrotube assay. Results expressed as visible growth (+) and no visible growth (-)

Test substances	Concentration	Pathogen inhibition (%)	
		Sour rot	Green mould
		Test tube observation	Test tube observation
Chitosan	mg/L		
	15 000	-	-
	7500	-	-
	3750	+	+
	1875	+	+
Lemongrass	µL/L		
	15 000	-	-
	10 000	-	-
	7500	-	-
	5000	-	-
	3750	-	+
	2500	-	+
	1880	+	+
	1250	+	+
625	+	+	
Spearmint	µL/mL		
	15 000	-	-
	10 000	-	-
	7500	-	-
	5000	-	+
	3750	+	+
	2500	+	+
	1880	+	+
	1250	+	+
625	+	+	

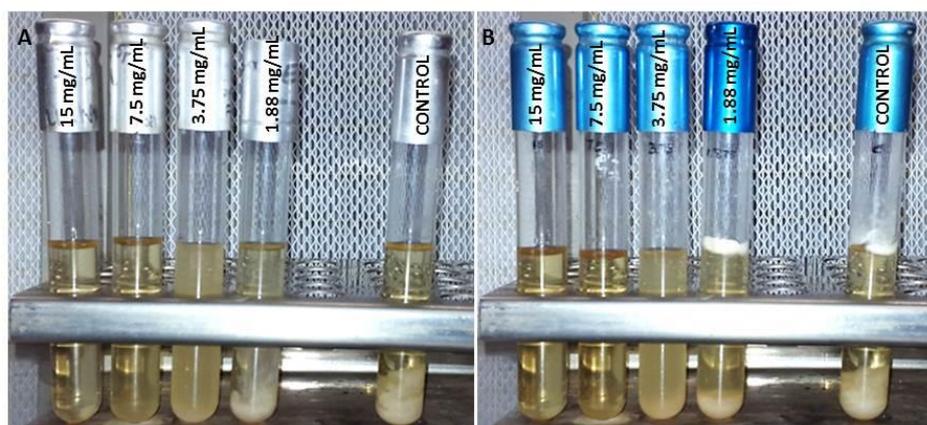


Figure 7. Macrotubes containing chitosan alone tested at 15 000, 7500, 3750 and 1880 mg/L against A) sour rot and B) green mould. Complete inhibition of fungal growth was observed for the two highest concentrations against both pathogens.

After coating the EOs with CHI, no visible growth was observed in the test tube, indicating the MIC values for lemongrass and spearmint at a concentration of 250 $\mu\text{L/L}$ and 500 $\mu\text{L/L}$ against sour rot, and 375 $\mu\text{L/L}$ and 750 $\mu\text{L/L}$ against green mould, respectively (Table 13).

The MIC value for CHI alone was 7500 mg/L against both pathogens. The MIC results of the macrotube assay also revealed that lemongrass performed better than spearmint against both pathogens. In this experiment, the inhibitory concentrations of lemongrass and spearmint were much lower than for the toxic media and the microtitre plate study. The MIC value determined for CHI was confirmed by the results of El-Mohamedy *et al.* (2015) in which green mould was inhibited by CHI at concentrations of 6000 and 8000 mg/L. The CHI-encapsulated EO also inhibited both pathogens at all tested concentrations (no turbid broth media in the tubes).

The CHI-encapsulated EO solutions at different concentrations had a considerable effect on the mycelial growth of both pathogens. It was observed that the mycelial growth was reduced over time, which can be an evidence of a slow release reaction of the inhibitory compounds present in the oil from the encapsulation structures. The same results were observed by Dos Santos *et al.* (2012).

Table 13. Effect of oil encapsulated in chitosan against citrus sour rot and green mould by means of the macrotube assay. Results expressed as visible growth (+) and no visible growth (-).

Test substances	Concentration	Pathogen inhibition (%)
		Sour rot
		Test tube observation
CHI + lemongrass	mg/L + μ L/L	
	7500 + 125	-
	3755 + 125	-
	7500 + 188	-
CHI + spearmint	mg/L + μ L/L	
	7500 + 250	-
	3755 + 250	-
	7500 + 375	-
CHI + spearmint	mg/L + μ L/L	
	7500 + 250	-
	3755 + 250	-
	7500 + 375	-

Test substances	Concentration	Pathogen inhibition (%)
		Green mould
		Test tube observation
Chitosan + lemongrass	mg/L + μ L/L	
	7500 + 250	-
	750 + 250	-
	7500 + 375	-
Chitosan + spearmint	mg/L + μ L/L	
	7500 + 375	-
	3755 + 375	-
	7500 + 500	-
Chitosan + spearmint	mg/L + μ L/L	
	7500 + 375	-
	3755 + 375	-
	7500 + 500	-

The CHI-encapsulated EO solutions at different concentrations had a considerable effect on the mycelial growth of both pathogens as determined in the broth medium (Table 14). It was observed that the mycelial growth was reduced over time which can be an evidence of a slow release reaction of the active compound from the EO from the coating structures. The same results were observed by dos Santos *et al.* (2012), meaning there was an increase in the reduction of the mycelial dry mass weigh over time.

Table 14. Effects of the combination CHI/EOs at different concentrations on mycelial growth of sour rot and green mould in broth media over three, five and seven days by means of mycelial dry mass technique. LG = lemongrass, SP = spearmint and CHI= chitosan

Combination (Concentrations in mg/L for CHI and in µL/L for the EOs)	Days of exposure		
	3	5	7
Sour rot	Percentage inhibition		
CHI (7500 + LG (188)	97.15	100	100
CHI (7500) + LG (125)	98.06	100	100
CHI (7500) + SP (375)	96.58	97.46	100
CHI (7500) + SP (250)	96.94	97.16	98.99
CHI (3750) + LG (188)	97.08	98.63	100
CHI (3750) + LG (125)	97.10	98.48	100
CHI (3750) + SP (375)	96.47	97.57	98.54
CHI (3750) + SP (250)	96.80	97.50	100
Green mould			
CHI (7500) + LG (375)	98.32	99.94	100
CHI (7500) + LG (250)	98.66	99.94	100
CHI (7500) + SP (500)	99.93	100	100
CHI (7500) + SP (375)	100	100	100
CHI (3750) + LG (375)	99.91	100	100
CHI (3750) + LG (250)	99.93	100	100
CHI (3750) + SP (500)	100	100	100
CHI (3750) + SP (375)	99.91	99.97	100

***In vivo* trials at CRI**

The outcomes of both inoculation trials were not satisfactory. The degree of decay was considerable for all the treatments applied, including the positive control, and the decay was not significantly different compared to the control. However, in the first trial that involved the dipping of fruit, a delay in sporulation was observed with the fruits infected with green mould and treated with spearmint oil. The deduction was made that EOs will not be successful in artificial inoculation trials, but that natural inoculation trials may work. For this reason we tested the modified and unmodified EOs in an industrial setting using fruit presenting with sour rot or green mould problems.

Laboratory *in vivo* pre-commercial pack-house trial

Trial 1

No significant differences were evident amongst the treatments, except for the application of lemongrass mixed with wax which provided the worst scenario (Figure 8). It was decided that the method of wounding was too severe for kumquat since the rind is much thinner than in the case of oranges. In the further experiments, only one quarter of the fruit were wounded and less penetration was applied during inoculation, while the remainder were only wounded and allowed to naturally infect.

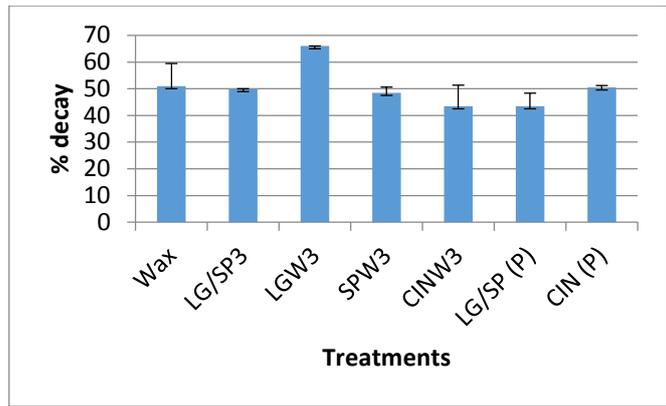


Figure 8. Trial 1 results on the decay percentage of sour rot infection growing on kumquats upon treatment with essential oils. The waxed fruit served as the control.

Trial 2

The results (Figure 9) indicated that the untreated kumquats performed better than those treated with various concentrations of CHI or EOs coated with CHI. This results indicated that there was an increase in decay with an increase in EO concentration.

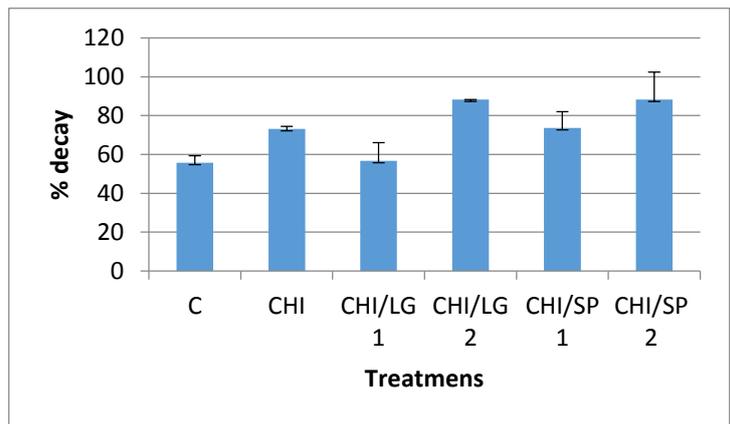


Figure 9. Results of Trial 2 indicating the percentage decay following inoculation of kumquat with sour rot after treatment with essential oils combined with CHI.

Trial 3: Effect of warm water dip on the quality of kumquats at different temperatures

Citrus is commonly treated by dipping in a warm bath to induce the immune response and so protect fruit against pathogens. Kumquat are also dipped in the packhouse at 37 °C. It was therefore decided to investigate the effect of different dipping temperatures on the phenolic content in the rind, since these compounds are associated with natural protection within the fruit.

The effect on decay was also investigated. There were no significant differences in the total phenolic content in the rinds of samples treated by dipping at different temperatures immediately after dipping or after 34 days of cold storage. This indicates that hot water treatment did not induce the release of phenolic compounds to boost the immune system of the kumquat. The incidence of decay on fruit stored for 14 days was too low to make any assumptions with regard to dipping temperature.

In vivo trials on kumquat (Levubu)

Trial 1

All treatments reduced the percentage of pathogen development under storage at room temperature with treatments CIN3 and SP3 being the most effective with 83 and 80% respectively (Figure 10). With regard to weight loss, treatment with cinnamon oil provided the best results with only 1% weight loss recorded.

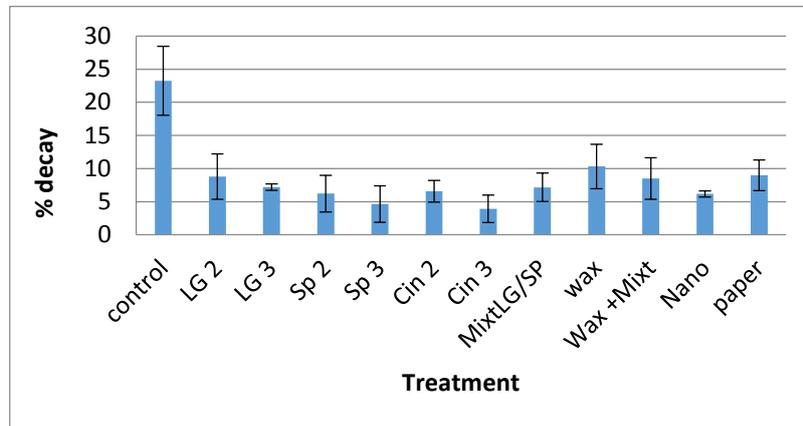


Figure 10. Effect of the treatment on pathogen development on fruit after 14 days storage at 18 °C (TUT) (Result expressed as percentage pathogen development per treatment)

After 34 days at cold storage, no significant difference between the control and the remaining treatments were observed (Figure 11). However, after 4 days at room temperature, treatments CIN2 and CIN3 reduced the decay by 85% and 82%, respectively (compared to the control) while the oil-amended wax was completely ineffective at controlling the pathogen development.

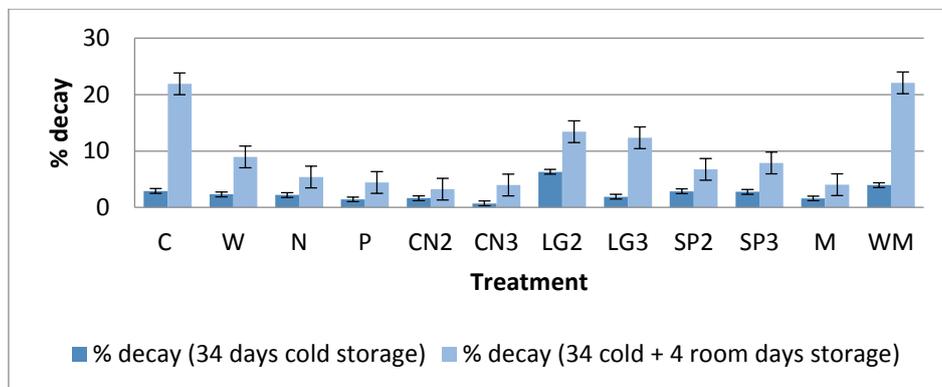


Figure 11. Effect of the treatment on fruit decay after 34 days storage at 5 °C and an extended 4 days at 20°C at TUT (Result expressed as percentage decay per treatment)

As illustrated in Figure 12, after cold storage in the pack-house, all treatments reduced the percentage of pathogen development with cinnamon EO at 3000 µL/L (CN3) (2.49%) and the SNPs (2.53%) performing the best compared to the control (13.62%).

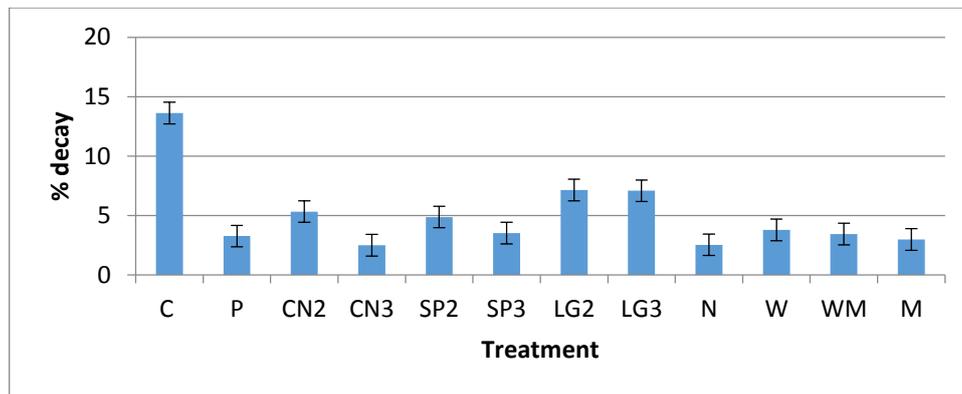


Figure 12. Effect of the treatment on pathogen development in fruit after 26 days storage at 5 °C in Levubu (Result expressed as percentage decay of fruit with pathogen development per treatment)

Trial 2

The dipping of the fruit with zein-CHI-EO or a mixture of spearmint and cinnamon oils at 8000 $\mu\text{L/L}$ (SP/CIN8) resulted in a decrease of the decay of 68% and 59%, respectively (Figure 13). Although the zein/CHI EO mixture was the most effective at controlling pathogen development it could not be applied using the in-line sprayer. Therefore, this treatment was applied manually with a brush. This could explain the efficacy of the treatment because the amount applied may have been higher. The inability of using the in-line spray under commercial conditions limits the potential use of this treatment. All treatments were less effective at controlling pathogen development during 10 days storage at room temperature when compared to the first trial. The quality of the fruit declines as the season progresses due to an increase in pathogen inoculum pressure. The pack-house also experienced greater losses of fruit upon export than within the previous period, corresponding to Trial 1. However, all treatments were ineffective at reducing the percentage of decay during cold storage for 34 days. Similar results were obtained for the boxes left in the cold room at the pack-house. The percentage weight loss demonstrated that the high level of decay was the major contributor of the weight loss.

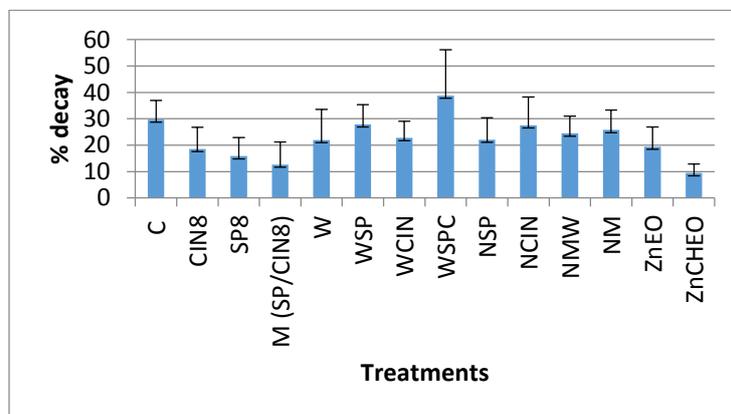


Figure 13. Effect of various treatments applied in the packhouse on fruit decay after 10 days storage at 20 °C in TUT

Trial 3

Once again, the experimental boxes brought back to TUT and stored for 34 days at 5 °C followed by 4 days at room temperature, displayed higher pathogen development than Trial 1. From all the treatments tested, only the boxes containing waxed fruit packed with spearmint oil impregnated paper displayed the best control of the pathogens with an overall percentage of decay of 10%. However, when the paper treatment was applied in the packhouse the results were considerably poorer (Figure 14). This could be as a result of the air circulation in the cold storage resulting in rapid evaporation of EOs from the paper. Treatment with spearmint

oil alone, cinnamon oil alone, nanoparticle or spearmint amended wax, resulted in a percentage of decay under 10%, in addition, the cinnamon oil treatment at 3000 $\mu\text{L/L}$ performed the best.

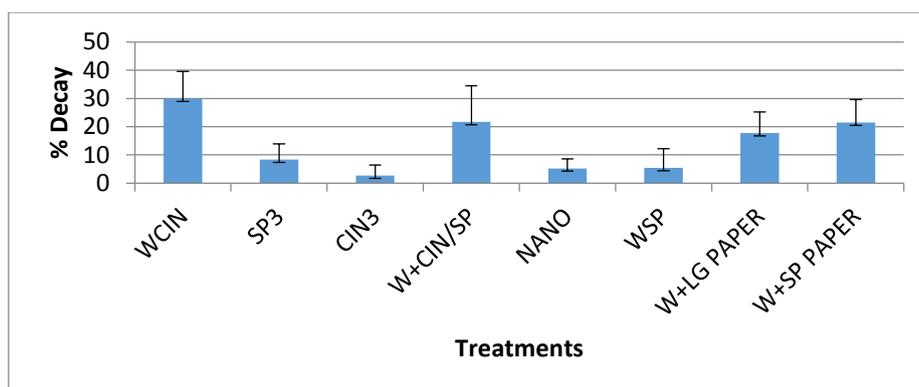


Figure 14. Effect of the treatment on fruits' decay after 34 days cold storage in Levubu

Finally, as summarized in Table 15, all treatments were able to control the decay during export to Rotterdam. This result was regarded by the exporter as a positive improvement, particularly since no rind pitting was observed with the spearmint treatment. Minimum rind pitting and shrivelling were observed.

Table 15. Fruit quality after air freight to Rotterdam evaluated by importer

Treatment	Firmness	Colour	Decay (<i>P. dig.</i>)	Rind pitting	Shrivelling
Chlorine Bath (control)	80% slightly sensitive/20% sensitive	light to dark orange	2%	1%	1%
Bath + Spearmint	80% slightly sensitive/20% sensitive	light to dark orange	-	-	1%
Bath + Cinnamon	100% slightly sensitive	light to dark orange	-	1%	-
Nano + mixture EO	80% slightly sensitive/20% sensitive	light to dark orange	1%	1%	1%
Wax + spearmint	80% slightly sensitive/20% sensitive	light to dark orange	2%	1%	1%

Although the cinnamon oil produced similar results than the spearmint, the strong taste and odour of cinnamon oil may limit its application to food. However, no off flavours or odours were perceived during the trial but a trained sensory panel must confirm this. In addition it is more expensive than the spearmint oil making it less economically viable. Overall, the treatments with spearmint oil alone without wax at 3000 $\mu\text{L/L}$ was the most effective with no decay and no rind pitting (Figure 15a) as compared to the control (Figure 15b). Spearmint oils was less effective against sour rot in the *in vitro* trials. This pathogen was not a major

concern for kumquat during the 2017 season and therefore spearmint oils controlled pathogen developed (primarily green mould) during the *in vivo* trials.



Figure 15. Boxes of kumquats after air freight to Rotterdam. a) Spearmint oil (3000 µL/L); b) Control (Chlorine bath at 37 °C) Photo courtesy of evaluators in Rotterdam

b

Slow release mechanisms for active volatile components of EOs against green mould and sour rot

Profiling and quantification of essential oils

Spearmint EO was found to be active against the targeted postharvest diseases. Therefore the identification of the components in the oil is crucial. The oil composition was determined using GC-FID/MS and 95% of the oil components were identified. The major component of the oil is carvone, with limonene being the second largest component.

The LODs and LOQs for the identified terpenes were determined using GC-FID (Table 2). Limits of detection (LOD) and regression values ranging from 0.12 – 1.30 $\mu\text{L/L}$ and 0.894 – 0.9994, respectively, for the pure standards were obtained from the calibration curves. Difficulties were experienced with the lower boiling point terpenes because they eluded with the solvent peak thus at low concentrations the peaks were difficult to detect. No authentic standards were available for some of the identified terpenes. The concentrations determined using GC-FID (Table 16) correlated with the percentage composition of the terpenes (Table 1). The spearmint oil analysed was found to contain 69.74 $\mu\text{L/L}$ of carvone and 14.61 $\mu\text{L/L}$ of limonene in 100 $\mu\text{L/L}$ of diluted oil. The smaller components in the oil ranged from 2.22 $\mu\text{L/L}$ to 0.78 $\mu\text{L/L}$ in concentration.

Table 16. Regression values, line formulae ($y= mx+c$) and limits of detection (LOD) and quantification (LOQ) for the terpenes present in spearmint essential oil as determined by GC-FID using standards

Compound name	Equation	R ²	LOD	LOQ	Concentration (in 100 $\mu\text{L/L}$)
α -pinene	0.0088x+ 0.4788	0.9456	0.84	1.69	1.02
Camphene	0.0435x+0.6751	0.894	1.3	2.77	0.78
β -pinene	0.0306x+0.4311	0.9645	1.67	3.55	0.88
Myrcene	0.017x- 0.0025	0.9963	0.12	0.65	0.52
Limonene	0.0099x - 0.0034	0.9902	0.739	2.46	14.61
Dihydrogecarvone	0.0203x-0.0072	0.9978	0.192	3.975	0.89
Borneol	0.0308x+0.024	0.9994	0.413	1.453	2.22
Carvone	0.0106x-0.0097	0.9917	0.679	2.26	69.74

Thermal analysis of the EOs

Understanding the thermal characteristics of the EO enables us to determine the applications of these in products/formulations. Figure 16 of the TGA data reveals that both the EOs and the terpenoids have the same thermal behavioural pattern. Spearmint EO and the terpenoids also exhibit mass loss in a single stage as indicated by the onset temperature (Table 17). The onset temperature indicates the temperature at which weight loss or evaporation of the volatiles occurs. From Table 17 it is evident that carvone is the least volatile in comparison to the EO and limonene, thus it has a slower volatilisation rate when compared to the two. This is due to the low vapour pressure of carvone in comparison to that of limonene.

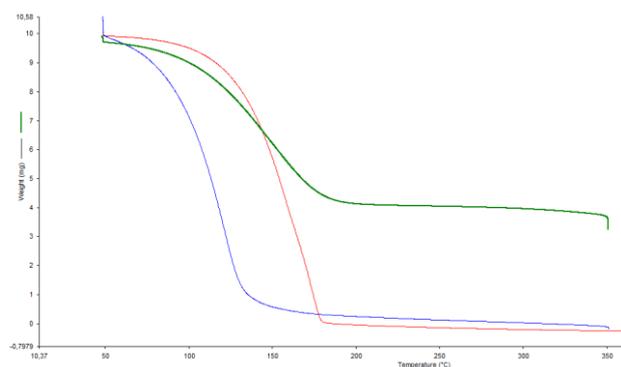


Figure 16. TGA profiles of spearmint oil (green). carvone (red) and limonene (blue). Where y axis = weight (mg) and x axis = temperature °C

Table 17. The onset temperatures of spearmint essential oil and two major components of the oil determined using a thermogravimetric analyser

Compound name	Onset temperature (°C)
Carvone	130.1
Limonene	92.24
Spearmint oil	101.5

Determination of MICs for individual oil components

This was done to ascertain which compounds in the EO contribute the most to the activity of the oil towards a specific pathogen. Table 18 indicates the MIC values for spearmint oil and that of the pure terpene. From these results it is clear that carvone, which the major component in the SM oil (liquid phase) is the most active against both the pathogens. Majority of the pure components are active against the target pathogens, with β -pinene and mycrene having no inhibition. The inhibitory effect of spearmint oil against the two pathogens is a result of a mixture of all these compounds. This test however is not a vapour phase analysis thus the activity of the pure components in the vapour phase will be evaluated and the results compared to see if there is correlation.

Table 18. Minimum inhibition concentration of spearmint oil and the pure compounds that make up the composition of the oil.

Treatment	Minimum inhibitory concentration (μ L/L)	
	Green mould	Sour rot
Spearmint	12 500	25 000
α - pinene	50 000	12 500
Camphene	12 500	25 000
β -Pinene	No Inhibition	No Inhibition
Mycrene	No Inhibition	50 000
Limonene	12 500	25 000
3-Octanol	3130	6250
Dihydrogencarvone	6250	25 000
Borneol	12 500	25 000
Carvone	3130	3130

Encapsulation of spearmint oil

Fourier-transform infrared spectroscopy was used to characterise the chemical structure of the EO, zein, PLA and encapsulated oils. Characteristic peaks which were present in the oil were evident in the zein and zein + CHI encapsulated oils (Figure 17), thus we can conclude that spearmint oil was encapsulated/adsorbed on the medium. Sharp peaks at 2955 cm^{-1} and 2957 cm^{-1} representing stretching from C-H groups are present in the oil and the encapsulated samples however these peaks are not present in the zein alone. The phenolic ring peak present at 1622 cm^{-1} is also present in the encapsulated oils and not in the zein only sample. This validates the presence of spearmint oil in the encapsulated samples. All the information present in the spectra indicates that the encapsulation was physical and not chemical, because no new peaks appeared after the process. Thus the structure of the EO was not changed within the encapsulation process. For the PLA encapsulated oil, no characteristic peaks were present (Figure 18) although TGA analysis proved that the characteristics of the encapsulated particles were very different from the EO alone.

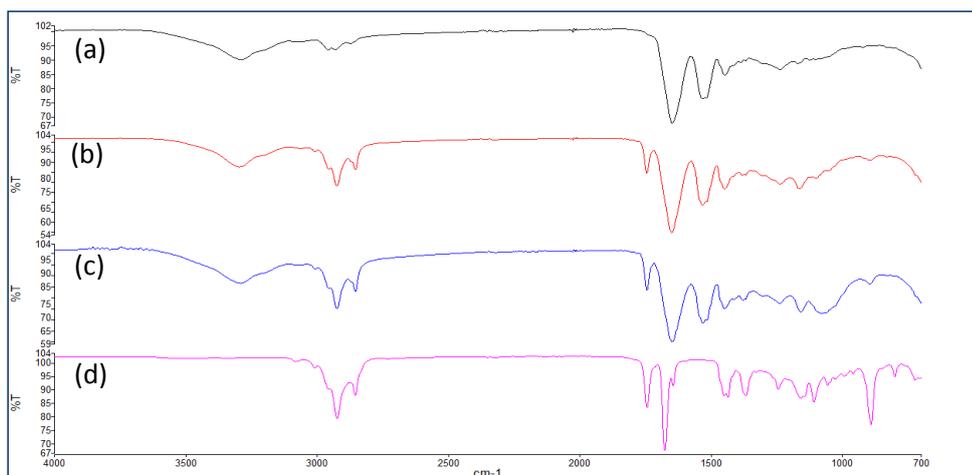


Figure 17. FTIR spectra of (a) zein only. (b) zein + spearmint oil. (c) zein + spearmint oil + chitosan (d) spearmint oil only

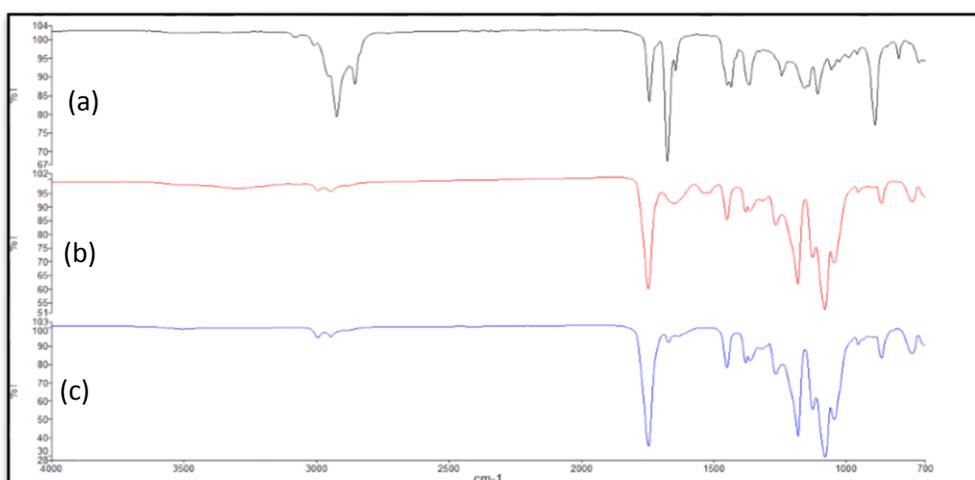


Figure 18. FTIR spectra of (a) spearmint oil. (b) PLA only and (c) PLA + spearmint oil

Thermogravimetric analysis of the encapsulated essential oils

The encapsulated samples were analyzed using a thermo gravimetric analyser to measure the rate of mass loss. This provides an indication of the rate at which Eos will be released from the encapsulating structures. A three stage mass loss was observed in the zein and zein + CHI encapsulated oils (Figure 19), two of these stages were attributed to the presence of EO. The mass loss at 150 °C was attributed to the presence of EO on the surface (adsorbed oil), mass loss from 300 °C – attributed to EO-encapsulated in the zein and zein + CHI medium. As shown in the thermograms (Figure 20) the EO possesses a one-step mass loss. The rate of mass loss depicted by the encapsulated PLA is much slower to that of the pure PLA. This can be attributed to the presence of EO, thus the encapsulation may improve the thermal stability of the EO.

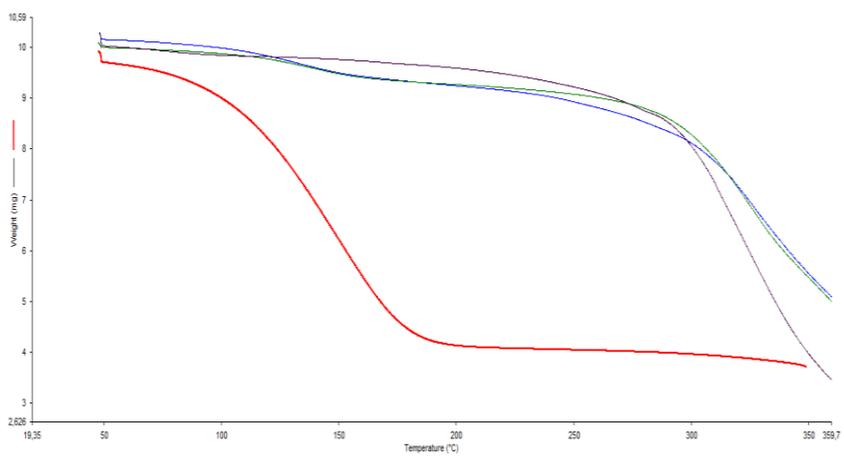


Figure 19. TGA profiles of spearmint oil (red), zein only (purple), zein + spearmint oil (blue), zein + chitosan + spearmint oil (green). Where y axis = weight (mg) and x axis = temperature °C

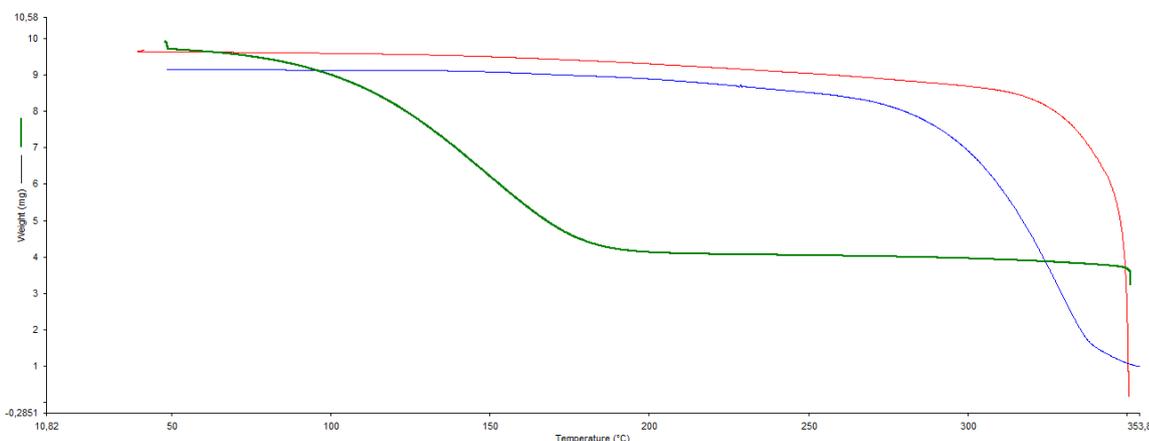


Figure 20. TGA profiles of spearmint oil (green), PLA only (blue) and PLA + spearmint oil (red). Where y axis = weight (mg) and x axis = temperature °C

Minimum inhibition concentration of essential oil and pure compounds

The MIC of the encapsulated EO was also investigated and the results are shown in Table 19. The zein + CHI encapsulated EO oil had the lowest MIC value thus was the most active against the two pathogens. Further work will also be done on the vapour phase analysis of these encapsulated oils. However, the zein + CHI medium appear to be the best candidate for slow release of the active components. More slow release vehicles will also be investigated.

Table 19. Minimum inhibition concentration of the encapsulated essential oils

Treatment	Minimum inhibitory concentration	
	Green mould	Sour rot
Zein	Some inhibition	Some inhibition
Zein + oil	200 mg/L	800 mg/L
Zein + oil + CHI	200 mg/L	200 mg/L
PLA	No Inhibition	No Inhibition
PLA + oil	160 μ L/L	160 μ L/L

The encapsulated EO particles performed much better than the EO alone. These particles seem a promising option for control of pathogens. The zein-CHI/EO mixtures yielded good results when tested on kumquat (see earlier results) but could not be sprayed due to blockage of the nozzles on the pack-line. These limitations would have to be addressed if needed.

Design of a mobile GC sampling system

The developed sampling system (Figures 21 and 22) fitted to a gas chromatograph is able to monitor the vapour pressure/concentration of EO/components at different temperatures and volumetric flow rates. The water bath temperature controller housing the sample will enable sample temperature to be set and measured with thermocouple TI. Pressure is measured with pressure transmitter PI. Gas mixture flows through the GC sampling valve, and this mixture is periodically injected on to GC column, separated, and analysed with the GC. The atmospheric gas mixture in a packing plant can be sampled and analysed as follows: the desired flow rate is set via metering valve, allowing the packing plant atmosphere to be sucked through the GC sampling valve and periodically, samples are then injected on to the column and analysed.

This data can then be compared and quantified with the thermal gravimetric analyser (TGA) vapour pressure data, solid phase microextraction (SPME) sampling device, or using thermal desorber (TD) sampler employing Tenax tube traps. Experiments under other atmospheres (e.g. synthetic air, N₂, He, etc) can be performed by coupling the desired gas to the system, flushing and then filling the system with the gas to the desired pressure.

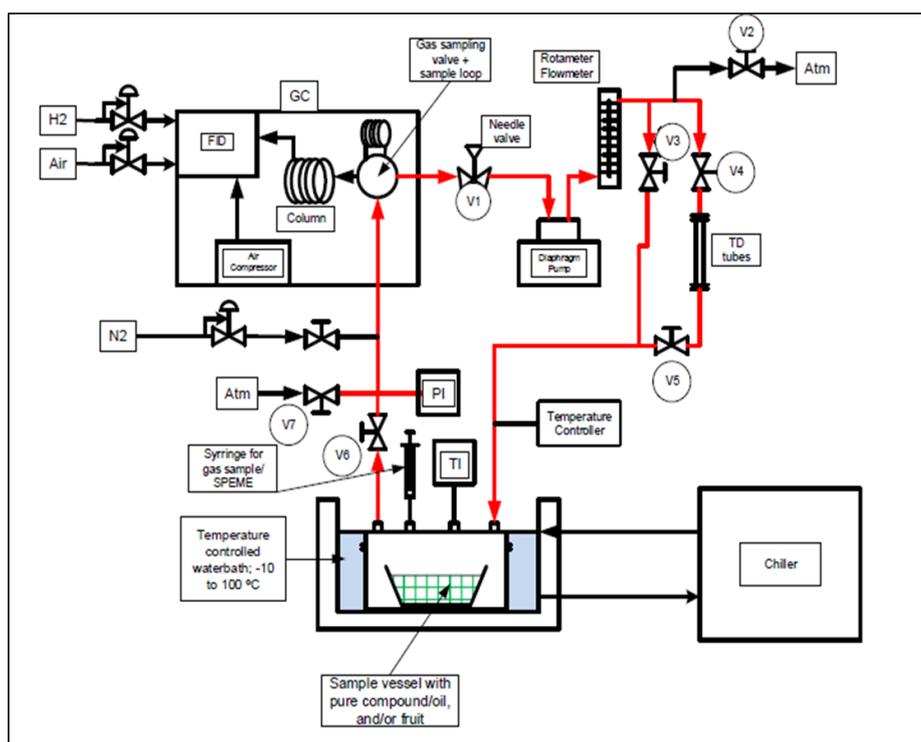


Figure 21. The developed sampling system fitted to a gas chromatograph which allows headspace samples of EOs to be collected using Tenax tube traps (that can be desorbed using thermal desorption) or by solid phase microextraction (SPME) or as direct headspace.

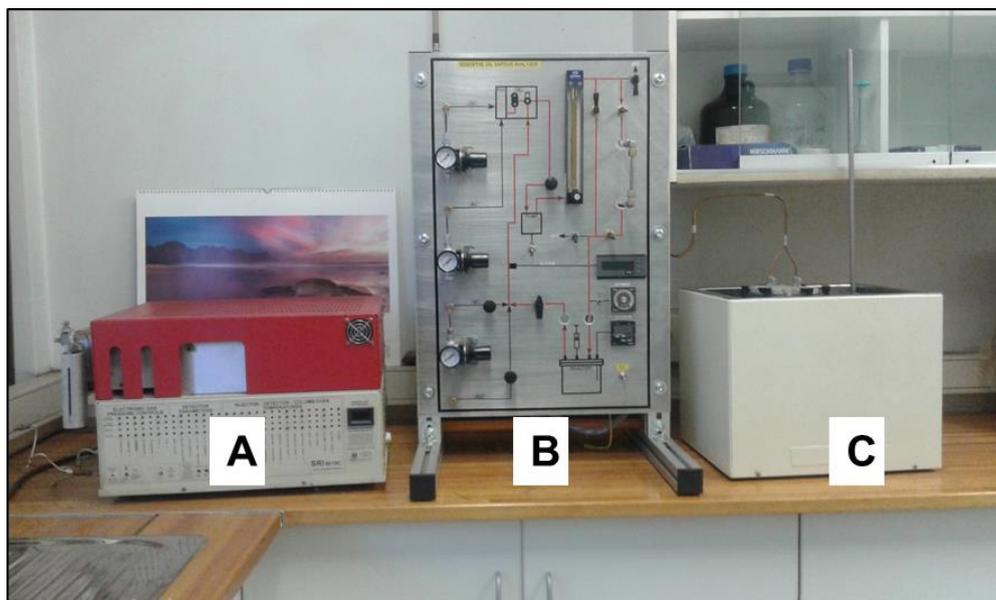


Figure 22. A photograph of the actual GC sampling system the liquid sample/fruit is placed in the sample vessel (C), which is thermally regulated and connected to a chiller. The carrier gas is pumped through the sample at a known flow rate; all the parameters can be adjusted using the control panel. The sample vessel has a port where a SPME needle can be inserted and headspace samples (gas samples/ gas mixtures) can be extracted using the needle. Thermal desorption sampling tubes can be coupled onto the control panel and gas flow can be directed through the TD tube at a desired flow rate and time. Solid phase micro extraction and TD analysis by external GC-MS and/or GC-FID allows for further correlations and quantification.

Analysis of spearmint oil using designed GC-sampling system

The designed GC-FID modular system was used to quantify the composition of the EO vapour phase using: direct headspace, solid phase micro-extraction and thermal desorption. A comparison was done of the composition of the vapour phase to that of the liquid phase (Table 20).

Table 20. Composition of spearmint oil headspace and liquid sampled using different techniques.

Compound	% Composition			
	Liquid	Headspace	SPME	Tenax
α -Pinene	0.71	10.1	5.60	6.53
Camphene	0.60	20.1	5.47	0.96
β -Pinene	0.69	3.23	4.68	1.60
Mycrene	0.35	5.73	0.27	2.19
Limonene	11.1	45.8	56.7	40.0
Carvone	76.5	2.63	10.4	1.54

Carvone was found to be the major component of spearmint oil in the liquid phase (76.5%), and it is the most active component in the oil according to the MIC results. However when using the GC modular system headspace techniques, which analyse the vapour phase of the oil, limonene (45.8%) was the major component while only 2.63% of the headspace comprises carvone. The composition of the oil components varies

according to headspace technique used, with direct headspace being the most accurate of the three methods. The results of SPME and Tenax indicate selective adsorptions of different components on the sorbets. Further verification of these results is in progress. The concentration of the active compound in the headspace is key to the activity of the EO when applied as a vapour.

Conclusions

- For identification of active multi-target EOs, the reductionist approach was more accurate. This approach did not reveal the same result as the chemometric approach. In the chemometric approach many of the compounds flushed out as markers for activity were not active. It was concluded that the chemometric approach may be biased towards compounds that have high concentrations in active oils, but which are not necessarily active.
- Different *in vitro* assays yielded different results. This explains why the results obtained by researchers are so variable. In addition, some pathogens are more virulent than others and are therefore less susceptible towards antifungal agents. Despite this, lemongrass continuously outperformed spearmint oil for control of both pathogens. Cinnamon oil was in some cases more active than lemongrass oil but may have more limitations in terms of taste and flavour considerations.
- Chitosan-coated EOs were more effective (lower MIC values) than the EOs alone, because CHI itself is also active. However, when combined with zein, the zein-CHI was effective on the fruit but blocked the nozzles of the sprayers on the pack-line and had to be manually applied.
- SNPs were prepared and characterised. TGA indicated that these SNPs are stable. However, they were not effective either *in vitro* or *in vivo*.
- A low-cost (mobile) headspace analysis system (headspace, tenax tubes and SPME) was successfully developed to investigate EO fumigant concentrations under conditions of non-homogeneous distribution.
- Headspace analyses indicated that an active EO does not guarantee an effective fumigant due to the huge differences in volatility of the components. For spearmint oil, the concentration of the main active component (carvone) dropped from 76.55 to 2.63% of the total composition following volatilisation. The temperature at which the EO is volatilised would further influence the concentration in the headspace. Under cold storage, even less carvone would be available in the headspace.
- An accurate measurement of the active EO constituents is crucial for the development of fumigants for pathogen control. Different measurement techniques yield vastly different answers and the correlation between them needs to be investigated.
- Although lemongrass consistently outperformed spearmint oil in the *in vitro* trials, this was not the case *in vivo*. Other researchers have indicated that *in vitro* and *in vivo* results do not always correspond and this was proved to be the case in this study. *In vitro* trials can only provide an indication of active oils but the physiology of the fruit plays a key role.
- EO-supplemented wax could possibly be applied to oranges, but are not suitable for kumquat. This is probably due to the large surface area of the small kumquat compared to the volume of the fruit. The physiology of the rind and fruit is therefore affected more by rind applications. For each fruit-type, the studies would therefore have to be optimized.
- In a realistic setting, it is difficult to obtain exactly reproducible results since many variables affect the results. For example, the quality of the fruit declines as the season progresses due to higher inoculum loads originating from the orchard pack-house. The temperatures and humidity upon harvest may differ from trial to trial-influencing the results.

- Pathogens on artificially inoculated fruit could not be controlled by EO application, yet the export trials (Trial 3) indicated that spearmint oil does provide protection compared to the control.
- The most important conclusion made from this study was that, when working with EOs as a possible antifungal agent, all trials must be done through natural infection in the packhouse. This is because the conditions in the packhouse are so different to those in a laboratory environment. For example, the draughts in the packhouse and particularly in the cold-storage facility are extreme and conducive to the evaporation of the EOS. This was particularly evident when working with paper application. These papers dried within minutes after wetting, while they remained wet for hours in the laboratory environment. Air is blown over the fruit on the packline following spray application, which also accelerates evaporation.

3.5.3 FINAL REPORT: Precision fungicide application for the control of postharvest diseases on citrus

Project 1126 (2014/2015-2016/17) by Catherine Savage (CRI), Wilma du Plooy (CRI), Cheryl Lennox (USPP) and Paul Fourie (CRI)

Summary

Postharvest fungicides can be applied to fruit using several methods, namely pre-packline drench application, dip, flooder, in-line aqueous spray, wax and bin drench treatment. Imazalil (IMZ) and thiabendazole (TBZ) are the most widely used remedies in citrus postharvest decay, providing effective curative control and sporulation inhibition of green mould (*Penicillium digitatum*), but provides practically no control against sour rot (*Galactomyces citri-aurantii*). These two pathogens cause discernible losses in the South African citrus industry. Previous work pointed out that all the potential parameters in an IMZ solution play a role in residue loading and influence each other, implying that one factor never ever acts in isolation. By understanding the interactions between treatment variables, potential precision application of the fungicide IMZ sulphate can be achieved. Imazalil residue loading on citrus fruit and the resultant green mould control achieved can be influenced and possibly even predicted. Application methods are constantly evolving, and in order to be proactive in the South African citrus industry, it was important to evaluate a new application method, such as the heated flooder, timeously. In this study, residues loaded from IMZ sulphate with respect to pH in conjunction with temperature and exposure time in aqueous treatments, particularly at pH 3 and 4, was investigated. The use of the heated flooder as an alternative aqueous application to the fungicide bath, and in conjunction with solution temperature and pH, was explored. Lastly, the survival of an indicator organism for biological contamination of the fungicide bath, *Rhizopus stolonifer* was studied at various water temperatures. Imazalil residue levels on citrus can be increased by increasing solution pH, temperature, concentration or exposure time. Most treatments gave excellent infection control and only a low residue was found to be necessary to cure or prevent a green mould infection. However, improved residue levels were closely linked to the level of sporulation inhibition achieved. Both the flooder and dip tank offered good green mould control. Contaminants that build up in solution can be eradicated at high temperatures.

Opsomming

Na-oes swamdoders kan met behulp van verskeie metodes aangewend word, naamlik voor-pakhuis stortbehandeling, dip, in-lyn spuit en stortbehandelings en in die waks. Die mees gebruikte chemikalië vir na-oes siektebeheer is tans imazalil (IMZ) en thiabendasool (TBZ), met goeie kuratiewe werking, sowel as inhibisie van *Penicillium* sporulasie, maar dit bied geen beskerming teen suurvrot (*Galactomyces citri-aurantii*) nie. Hierdie twee patogene veroorsaak aansienlike verliese in die Suid Afrikaanse sitrus industrie. Vorige werk het bevind dat al die potensiële veranderlikes in die fungisiede aanwending 'n rol speel in die die werking van IMZ, met geen enkele faktor wat uitgesonder kan word nie. Deur hierdie veranderlikes beter te verstaan, kan die presisie aanwending van IMZ behaal word. Imazalil residulading en gepaardgaande groenskimmelbeheer kan sodoende beheer en waarskynlik selfs voorspel word. Aanwendingsmetodes is voordurend besig om te verbeter en ten einde proaktief te wees in die Suid Afrikaanse industrie, was dit belangrik om nuwe tegnologie soos die vloedtoediener, betyds te evalueer. In hierdie studie is die residue wat met IMZ sulfaat gelaai word

in wateroplossing onder verskillende pH, temperature en blootstellingstye, veral by pH 3 and 4, ondersoek. Die verhitte vloedtoediener as 'n waterbehandeling en ten opsigte van pH en temperatuur, was vergelyk met die fungisiedebad. Laastens was die oorlewing van 'n indikator organisme van biologiese kontaminasie in die fungisiedebad, *Rhizopus stolonifer*, teen verskillende watertemperature ondersoek. Imazalilvlakke op sitrus kan verhoog word deur 'n toename in die oplossing se pH, temperatuurbeheer, konsentrasie en blootstellingstyd. Die meeste behandelings het uitstekende infeksiebeheer gegee, terwyl slegs lae residue nodig is om bestaande infeksie te genees of om voorkomende beheer te verkry. Verbeterde residuevlakke is egter baie nòù gekoppel aan goeie sporulasiebeheer. Die opbou van kontaminante kan beheer word met hoër temperature.

Introduction

A major problem in postharvest citrus disease management is the fungal disease *Penicillium digitatum* causing green mould. Imazalil (IMZ) and thiabendazole (TBZ) are fungicides widely used to combat green mould (Kellerman et al, 2014; Zhang and Timmer, 2007), with IMZ the most important of the two (Erasmus et al, 2013). Solution pH and temperature, and exposure time of the fruit to the solution, are important when using the sulphate form of IMZ. Research has increased our understanding of IMZ use (Eckert & Brown, 1986), but further variables needed to be investigated, along with an alternative application method.

The fungicide bath is the most common IMZ application method in South Africa (Erasmus et al, 2013). The ability of IMZ to control green mould was investigated in a cold bath of 10°C and compared to ambient temperature and 35°C baths. Solution temperature had no significant effect on IMZ's ability to cure 24 hr old green mould infections with all temperatures providing control above 80%. Sporulation inhibition and residue loading increased as solution pH, temperature, and exposure time increased. Sporulation inhibition was < 50% in pH 3 baths, irrespective of temperature, complete inhibition was obtained at 35°C and pH 6, but IMZ MRLs were exceeded at longer exposure times (> 45 s).

Imazalil's control of green mould infection and sporulation was tested in a heated flooder. Solution variables included the effects of pH (3; 4; 5; 6), temperature (45; 55; 65°C), and concentration (250 or 500 µg.mL⁻¹). Residues increased with increasing pH, temperature range and concentration. The majority average residues loaded were between 0.4 and 3.0 µg.g⁻¹. Treatments at pH 6 loaded higher residues at 55 and 65°C, where the Maximum Residue Limit (MRL) was almost always exceeded. The flooder loaded adequate residues, offering good curative and protective control. Sporulation inhibition of green mould was linked to residues, and complete inhibition was achieved at the higher residue levels. The flooder was shown to be an effective applicator of IMZ.

The survival of *Rhizopus stolonifer* was studied *in vitro* at various water temperatures (10°C to 65°C) for exposure times of 1 or 60 min, and after a pasteurization step. Sub-treatments included the addition of IMZ fungicide or green mould spores. Solution temperature had no significant effect on *Rhizopus* spore survival at temperatures below 35°C, but temperatures of 45, 55 and 65°C, particularly after a 60 min exposure, caused a significant reduction in *Rhizopus* spore viability. Complete *Rhizopus* eradication was achieved with 65°C and the pasteurization step. In order to control fungal contaminants in the fungicide bath, packhouses need to apply IMZ in heated solutions (*circa* 45°C) and/or pasteurize fungicide baths overnight.

Imazalil residue levels on citrus can be increased by increasing solution pH, temperature, concentration or exposure time. Most treatments gave excellent infection control and only a low residue is necessary to cure or prevent a green mould infection. Residue levels were closely linked to the level of sporulation inhibition achieved. Both the flooder and dip tank offered good green mould control. Contaminants that build up in solution can be eradicated at high temperatures.

Objectives

Part 1: See PROGRESS REPORT 2017

Part 2: Dip application

1. Investigate the interaction between pH, temperature and fruit exposure time in a heated IMZ sulphate solution
 - a. Four stainless steel tanks were fitted with temperature probes and propellers. The temperature for each tank solution were adjusted to a specific temperature with the IMZ sulphate at pH 3. Fruit were treated at the exposure time range curatively and protectively and residue samples were taken for each treatment. The pH level were amended to the next level until all planned ranges were treated.
 - b. Trial treatments consisted of combinations of:
 - i. Temperature: 25, 35, 45 and 55°C
 - ii. pH: 3,4.5 and 6
 - iii. Exposure time: 15, 30, 45, 90 and 180 s
 - c. Evaluation of parameters, using standard methodology
 - i. Sporulation inhibition rating
 - ii. Analysis of residue loading
2. Determining the effect of cooler (< 20°C) IMZ sulphate solutions on residue loading and green mould control
 - a. The equipment in Objective 1 were utilised
 - b. Trial treatments consisted of
 - i. Temperature: 10 and 25°C
 - ii. pH: 3, 6
 - iii. Exposure time: 15, 30, 45, 90 and 180 s
 - iv. Curative control, sporulation inhibition and residue loading were evaluated using standard methodology
3. Determining the effect of heated solutions on the survival of contaminants such as *Rhizopus*
 - a. This were an *in vitro* trial where *Rhizopus* were used as model contaminant.
 - b. *Rhizopus* were added to different solutions that represented the temperature and pH levels applied in Objectives 1 and 2. Aliquots of each solution were plated out after specific time increments. Survival were rated per plate.

Materials and methods

Part 1: See PROGRESS REPORT 2017

Part 2: Dip application

The work done on part two of this study used navels and Valencias. All the trials were done at two pH values (3 and 6), three temperatures (10, 20, 35 °C) and four exposure times (15, 45, 90, and 180 seconds). Curative control of *Penicillium* as well as sporulation was evaluated and fruit sent for residue determination.

Results and discussion

Objective / Milestone	Achievement
Dip application	
1. Investigate the interaction between pH, temperature and fruit exposure time in a heated IMZ sulphate solution	Variables investigated for the IMZ solution included pH (3; 4; 5; 6), temperature (45; 55; 65°C), and concentration (250 or 500 µg.mL ⁻¹). Solution temperature had no significant effect on IMZ's ability to cure 24 hr old green mould infections with all temperatures providing control above 80%. Sporulation inhibition and residue loading increased as solution pH, temperature, and exposure time increased.
2. Determine the effect of cooler (< 20°C) IMZ sulphate solutions on residue loading and green mould control	Sporulation inhibition was < 50% in pH 3 baths, irrespective of temperature, complete inhibition

	was obtained at 35°C and pH 6, but at these values, IMZ MRLs were exceeded at longer exposure times (> 45 s).
3. Determine the effect of heated solutions on the survival of contaminants such as <i>Rhizopus</i>	Solution temperature had no significant effect on <i>Rhizopus</i> spore survival at temperatures below 35°C, but temperatures of 45, 55 and 65°C, particularly after a 60 min exposure, caused a significant reduction in <i>Rhizopus</i> spore viability. Complete <i>Rhizopus</i> eradication was achieved with 65°C and the pasteurization step. In order to control fungal contaminants in the fungicide bath, packhouses need to apply IMZ in heated solutions (<i>circa</i> 45°C) and/or pasteurize fungicide baths overnight.

A complete report of the results from the dip tank applications are attached as Appendix 1 as the thesis of Catherine Savage.

Conclusions

Dip tank / Aqueous Fungicide Application

Imazalil's control of green mould infection and sporulation was tested in a heated flooder. Residues increased with increasing pH, temperature range and concentration. The majority average residues loaded were between 0.4 and 3.0 µg.g⁻¹. Treatments at pH 6 loaded higher residues at 55 and 65°C, where the Maximum Residue Limit (MRL) was almost always exceeded. The flooder loaded adequate residues, offering good curative and protective control. Sporulation inhibition of green mould was linked to residues, and complete inhibition was achieved at the higher residue levels. The flooder was shown to be an effective applicator of IMZ.

Imazalil control of green mould was investigated in a cold fungicide bath of 10°C and compared to ambient temperature and 35°C baths. Solution temperature had no significant effect on IMZ's ability to cure 24 hr old green mould infections, with all temperatures providing control above 80%. Sporulation inhibition and residue loading increased as solution pH, temperature, and exposure time increased. Sporulation inhibition was < 50% in pH 3 baths, irrespective of temperature; complete inhibition was obtained at 35°C and pH 6, but IMZ MRLs were exceeded at longer exposure times (> 45 s).

The survival of *Rhizopus stolonifer* was studied *in vitro* at various water temperatures (10°C to 65°C) for exposure times of 1 or 60 min, and after a pasteurization step. Sub-treatments included the addition of IMZ fungicide or green mould spores. Solution temperature had no significant effect on *Rhizopus* spore survival at temperatures below 35°C, but temperatures of 45, 55 and 65°C, particularly after a 60 min exposure, significantly reduced *Rhizopus* spore viability. Complete *Rhizopus* eradication was achieved with the pasteurization step at 65°C. For packhouses to control fungal contaminants in the fungicide bath, packhouses should apply IMZ in heated solutions (*circa* 45°C), and/or pasteurize fungicide baths overnight.

Technology transfer

All the work on the dip tank applications were completed and finally presented as a technical talk at the 2017 and 2018 packhouse workshops:

1. Catherine Savage, Wilma du Plooy, Cheryl Lennox, Arno Erasmus and Paul Fourie. 2017. Fungicide application using a flooder.
2. Wilma du Plooy, Catherine Savage, Cheryl Lennox and Paul Fourie. 2018. Fungicide bath and flooder application of postharvest fungicides.

Other presentations

1. Catherine Savage, Wilma du Plooy, Cheryl Lennox, Arno Erasmus and Paul Fourie. 2017. Hot or cold fungicide application? The effect of temperature on citrus green mould control and survival of *Rhizopus stolonifer*. SASPP, Champagne Sports Resort.

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APPENDIX

FURTHER OPTIMIZATION OF IN-LINE AQUEOUS APPLICATION OF IMAZALIL TO CONTROL CITRUS GREEN MOULD CAUSED BY *PENICILLIUM DIGITATUM*

by

CATHERINE SAVAGE

CHAPTER 2

Influence of pH and temperature in a heated flooder application on imazalil residue loading, citrus green mould control and sporulation inhibition

Abstract

Green mould (caused by *Penicillium digitatum*) is the largest contributor of loss due to postharvest decay of citrus fruit. Imazalil (IMZ) in the IMZ sulphate formulation is the most important fungicide in green mould management in South African citrus packhouses. Recent studies highlighted the importance of pH and temperature of IMZ sulphate solutions, as well as exposure time of fruit to the solution, but were focussed on dip application as the common IMZ application method in South Africa. Heated flooder (in-line drench or cascade) application is a novel means of IMZ application and studies on its optimal use and efficacy were needed. Variables that were studied included the effects of pH (3, 4, 5 and 6), temperature of the solution (45, 55 and 65°C at pH 6), and concentration (250 or 500 µg.mL⁻¹). Imazalil residue loading models were developed for the effects of pH and temperature. Levels loaded on Satsuma mandarin, lemon, navel and Valencia orange fruit increased with increasing pH range, increasing temperature range and increasing concentration, and were generally in the range between 0.4 and 3.0 µg.g⁻¹. The Maximum Residue Limit (MRL) of 5.0 µg.g⁻¹ was often exceeded at pH of 6 and temperatures of 55 and 65°C. At residue levels within the MRL, IMZ application by means of the heated flooder offered excellent curative control of 24-hour old infections, excellent protective control as well as sporulation inhibition. Sporulation inhibition of green mould was modelled on residue levels, and 90% inhibition was obtained at residue levels of 1.84 to 2.47 µg.g⁻¹.

Introduction

South Africa has a successful citrus industry and exported 115 million cartons during the 2015 season (Edmonds, 2016; PPECB, 2016). With production being situated in the Southern hemisphere, citrus is often supplied to markets in the Northern hemisphere who have opposite seasonality, with South Africa's main markets being Europe and Asia. Unfortunately, with markets geographically very far from the production areas, the fruit spend weeks being shipped, and not only need to arrive in a satisfactory condition, but should remain so during further storage (Hough, 1969; Pelser, 1977).

The largest contributor to loss in a shipment of citrus is fungal decay, with *Penicillium digitatum* Pers. Sacc. being the foremost cause (Eckert and Eaks, 1989). *P. digitatum* is an obligate wound pathogen that turns damaged tissue into a soft, water soaked lesion within 5 to 7 days. As the infection continues, white mycelia grow outwards from the initial point of infection and are followed by sporulation, also originating at the infection point (10 - 14 days later). The fungus is characterised by olive green spores, hence the disease's common name 'green mould' (Fawcett and Lee, 1926; Eckert and Brown, 1986; Eckert and Eaks, 1989). Growth is inhibited at storage and transport temperatures of 3 – 5°C. However, once in a favourable environment, the mould will continue to proliferate, with infected fruit decayed in a matter of 2 – 3 days (Eckert and Eaks, 1989). Imazalil (IMZ; 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H imidazole) (Siegel *et al.*, 1977; FAO, 2001) is the most effective fungicide against green mould, and is still widely used in the international citrus industry (Smilanick *et al.*, 1997; Erasmus *et al.*, 2011; Besil *et al.*, 2016). Imazalil is able to eradicate an infection that occurred prior to treatment (curative action), protect the fruit from infections that occur after treatment (protective action), and inhibit the formation of *P. digitatum* spores (sporulation inhibition) (Erasmus *et al.*, 2011; Sepulveda *et al.*, 2015). Sporulation inhibition is very important in combating the problem of soilage, a cosmetic problem whereby spores from infected fruit dusts the surface of healthy, sound fruit (McCornack and Brown, 1977; Eckert and Kolbezen, 1978; Eckert and Eaks, 1989). Such fruit is unmarketable until it has been cleaned and repackaged, thus adding cost to the value chain. The Maximum Residue Limit (MRL) for IMZ on citrus is 5 µg.g⁻¹ for the European Union and 10 µg.g⁻¹ for the USA (DAFF, 2008; AgrIntel, 2015). Despite concerns, MRL's are very rarely exceeded (Fernández *et al.*, 2001; Blasco *et al.*, 2006; Erasmus *et al.*, 2011). South African packhouses actually load much lower levels (≈ 1.0 µg.g⁻¹) (Erasmus *et al.*, 2011). An IMZ residue level of between 1.0 and 3.0 µg.g⁻¹ can offer adequate levels of control, but may fail to completely inhibit sporulation (Kaplan and Dave, 1979; Schirra *et al.*, 1996; Smilanick *et al.*, 1997; Erasmus *et al.*, 2011). Imazalil is available in two formulations, namely an emulsifiable concentrate (EC), and a sulphate salt form in either soluble powder (SP) or soluble granules (SG) (Pelser, 1980; Sepulveda *et al.*, 2015). The local industry uses the sulphate form in all aqueous applications at a registered concentration of 500 µg.mL⁻¹ (Pelser and La Grange, 1981; Fourie and Lesar, 2008; Erasmus *et al.*, 2011). The EC formulation is solely used in the wax application (Erasmus *et al.*, 2011; Njombolwana *et al.*, 2013a, b). South Africa favours the sulphate formulations because, unlike the EC formulation, it is more stable, needs less agitation and does not settle out of suspension, making it unavailable as a fungicide (Eckert, 1977; Altieri *et al.*, 2005; Erasmus *et al.*, 2011). While IMZ blends well into commercial fruit wax, such wax applications typically need a much higher concentration of IMZ than an aqueous application in order to offer a similar level of control (Brown, 1984; Njombolwana *et al.*, 2013a).

The most popular method of applying IMZ in South Africa is by means of a fungicide bath (dip tank) (Pelser and La Grange, 1981; Erasmus *et al.*, 2011). Imazalil sulphate solutions in these tanks differ widely in terms of temperature (12 - 45°C), solution pH (3 – 8), exposure time of the fruit (16 – 107 s) and concentration of the IMZ (typically 250 – 615 µg.mL⁻¹) (Erasmus *et al.*, 2011). Despite variations, the application method is very effective if fruit can be fully submerged during treatment (Eckert, 1977) and offers excellent curative control (Dore *et al.*, 2009; Erasmus *et al.*, 2011, 2013, 2015a, b). It is superior to application methods such as sprays, overhead brushes saturated with solution (Eckert, 1977; Pelser, 1977; Brown and Dezman, 1990; Bronkhorst *et al.*, 1993; Erasmus *et al.*, 2011) or Imazalil Thin Film Treatment (ITFT) (Altieri *et al.*, 2013).

Since the management of a fungicide bath can be problematic, especially considering the variation in dip application parameters (Erasmus *et al.*, 2011), there has been a need in the South African industry for a more manageable application method. An innovative approach used in some citrus producing regions, often referred to as a flooder or cascade, is an in-line drench of fruit with a heated and recirculated IMZ solution. The concept of fungicide application using recirculating sprays and cascades over rollers or brushes is not new (McCornack, 1970; Smoot and Melvin, 1970; Wild *et al.*, 1975; Brown, 1977). Recirculating systems are advantageous in

comparison to total loss systems as they retain the solution, thereby conserving fungicides and water. Furthermore, the solution temperature is more easily managed in a closed system. Higher volume application is often much more effective than low volume (Förster *et al.*, 2007; Kanetis *et al.*, 2008), but high volume applications cannot readily be considered in total loss systems, due to the vast volume of solution needed, and the economic and ecological footprints associated with that. In a recirculating system a smaller overall volume is used, but a greater volume of solution comes in contact with fruit, leading to better coverage, which results in increased decay control (Brown and Dezman, 1990; Kanetis *et al.*, 2008). One disadvantage of recirculating systems is that, because the solution is kept, contaminants can build up and the fungicide concentrations reduced (Eckert, 1977); however, these are the same issues seen in the fungicide bath and can be easily mitigated with proper packhouse management (Lesar, 2008; Lesar and Erasmus, 2014).

Smilanick *et al.* (2003) studied a heated drench application that applied fungicide solution onto fruit in a high volume, low pressure drench over rotating brushes. The elevated temperature (35°C) was found to significantly reduce green mould incidence at both treatment times examined (15 s and 30 s). The heated in-line drench, cascade or flooder differs from other drench applications, as well as the fungicide bath, in that the water is contained in a closed tank, and constantly circulated and heated. The weirs are covered aiding in heat retention and protection from outside contamination by dirt and debris. The fruit going through the in-line drench are rolled over rotating brushes, and pass through several laminar flows of high volume waterfalls of fungicide solution. This ensures complete coverage of the fruit compared to the fungicide bath where floating fruit may not have all sides suitably exposed to the fungicide. The JBT Heated Flooder (JBT FoodTech, Brakenfell, Cape Town, South Africa), as well as other similar in-line drenchers are becoming more common place in South Africa. Work done by Erasmus *et al.* (unpublished) shows that it has the potential to be an alternative to the fungicide bath application of IMZ, also offering superior green mould control. However, since the flooder is new to the South African citrus industry, various parameters still need to be investigated to ensure optimal application of IMZ. It is important to look at solution parameters such as IMZ concentration, solution temperature, and solution pH in conjunction with the application method, as these factors influence IMZ's ability to adhere to the fruit exocarp in the form of a residue (Brown and Dezman, 1990; Erasmus *et al.*, 2011, 2013). It has been found that IMZ's solubility in water is pH sensitive: since it is a weak base, it is most soluble at a low solution pH (FAO, 2001). Erasmus *et al.* (2011, 2013, 2015a) and Kellerman *et al.* (2016) have shown that solution pH and temperature play a crucial role in residue loading of IMZ sulphate. Both researchers have demonstrated that at pH 3, IMZ residue loading is low and stable regardless of other solution parameters but that at pH 6 and above, residue loading increased linearly to levels far exceeding the MRL. Historically, the fact that IMZ is pH sensitive was not considered, but addition of the IMZ sulphate form to neutral pH water drops the solution pH to around 3 due to the presence of the sulphuric acid molecule (FAO, 2001). South African water specifications are often outside of the ideal values and pH levels in packhouses are often higher, because different municipal water supplies vary in pH, and packhouses often source their water from rivers or dams, boreholes and rainwater (Savage *et al.*, unpublished). Effective management of IMZ application therefore depends on adjustment of the solution pH in order to maintain availability of the active, creating the potential to achieve the maximum activity from the product through simultaneous regulation of temperature (Erasmus *et al.*, 2011, 2013, 2015a).

Although hot water treatments by itself can be effective at high temperatures (Erkan *et al.*, 2005; Şen *et al.*, 2010), the addition of a fungicide always improves that control (Schirra *et al.*, 1997; Puawongphat *et al.*, 2008). Imazalil penetrates easier and persists longer in citrus rind when applied in a heated solution (Schirra *et al.*, 1996, 2010; Cabras *et al.*, 1999; Dore *et al.*, 2009). Dore *et al.* (2009) found that as concentration and temperature increased concurrently, residue loading increased and residue persistence remained much more stable. Finally longer exposure times also resulted in higher residues (Erasmus *et al.*, 2015a; Kellerman *et al.*, 2016).

The aim of this study was to determine the influence of pH and temperature in a heated flooder application on IMZ residue loading and subsequent green mould control and sporulation inhibition.

Materials and methods

Fruit

Export quality, freshly harvested lemon, Satsuma mandarin, navel orange and Valencia orange citrus fruit were collected from various packhouses in the northern citrus producing regions of South Africa (Mpumalanga and

Limpopo provinces) during the 2014 season. Eight trials were conducted, twice on each citrus variety. Fruit were hand selected for uniformity in size and quality. Upon arrival at the Citrus Research International laboratories in Nelspruit, South Africa, the fruit were washed with a 75 µg.mL⁻¹ chlorine high pressure spray over rotating brushes. This was done to clean the fruit, reduce spore load on the fruit coming from the orchard and to simulate normal packhouse procedure, where fruit is washed before being treated. Fruit were stored at 4°C for no more than 4 days before trials were conducted. Fruit were removed from cold storage 1 day before the trials commenced in order to reach ambient temperature (≈ 22°C) and to evaporate condensation.

***Penicillium digitatum* isolate and inoculation**

For all trials an IMZ sensitive isolate (STE-U 6560, Department of Plant Pathology, University Stellenbosch, South Africa) of *P. digitatum* was cultured on chloramphenicol amended potato dextrose agar (PDA+; Difco Potato Dextrose Agar, Becon, Dickinson and Company, Sparks, USA; Chloramphenicol, Chlorcol, 250 mg CAP 500, Adcock Ingram, Midrand, Gauteng, South Africa) at 25°C for 10 – 14 days. Cultures were flooded with sterile water amended with one drop of Tween 20 to a concentration of ≈ 0.01 µL.mL⁻¹ (Sigma-Aldrich, St. Louis, MO, USA). Conidia were dislodged from cultures using a sterilized hockey stick and filtered through a double layer of cheesecloth into 200 mL of the Tween 20 amended sterile water. Concentration of the spore suspension was adjusted to 1 x 10⁶ spores.mL⁻¹ by means of a spectrophotometer (absorbance of 0.100 at 425 nm; Cecil CE1011, Lasec, Midrand, Gauteng, South Africa) (Morris and Nicholls, 1978; Eckert and Brown, 1986). Spores were maintained in a uniform suspension by use of magnetic stirrers throughout inoculation.

Two methods of inoculation were used during trials. In order to test curative and protective ability of imazalil against infections, a custom made wounding tool was used. This tool had a flattened cylindrical tip that mimicked the cut stem of a citrus fruit and delivered a wound 1 mm in diameter and 2 mm deep through the flavedo into the albedo of the fruit. Wounds were made around the stem end of the fruit except in the case of lemons, which typically have a more oblong shape, when the wounds were made on the sides of the fruit. Four wounds were made on each fruit, in a square pattern with a distance of approximately 4 cm between each. There were 12 fruit in each replicate, with 3 replicates per treatment during a trial. Curative inoculations occurred 24 hours prior to treatment, while protective inoculations occurred 24 hours after treatment.

The second method of inoculation was to test the sporulation inhibition of imazalil. A sterile 0.60 x 25 mm gauge needle (NN*2325R, Terumo Corporation, Tokyo, Japan) was used to inject 0.2 mL of spore suspension between 1 and 2 cm deep into the shoulder on the stem end of a fruit. Only one point of entry was made per fruit. The sporulation inoculation method was chosen to ensure rot while still enabling the effects of the IMZ residue to be seen clearly on the surface of the fruit (Brown *et al.*, 1983; Brown and Dezman, 1990). There were 12 fruit in each replicate with 3 replicates per treatment during a trial. Sporulation inoculations were done approximately 30 min before treatment.

Residue analysis

Fruit samples for residue analysis consisted of two replicates of six uninoculated fruit added to the first and last replicate of each treatment. After treatment, they were stored in plastic bags at 4°C for no more than a week before being chopped and blended (Salton Elite Blenders, Amalgamated Appliance Holdings Limited, Reuven, South Africa) into pulp using distilled water to dilute the fruit into a soft, paste-like consistency. The dilution factor for each sample was recorded. The pulp was frozen into sub-samples that were couriered to an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Cape Town, South Africa) for IMZ (chloramizol) residue analyses. Acetonitrile was used for extraction, which was followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography tandem mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA) according to an accredited procedure. Residue data received from the analytical laboratory were recalculated using the dilution factor recorded when the pulp was blended.

Treatment conditions

All eight trials conducted had the same parameters, and fruit was treated using an experimental packline, which simulated commercial packlines. A custom-built, experimental unit of the heated flooder (JBT FoodTech, Brakenfell, Cape Town, South Africa) was used in all cases. The unit was 185 cm in length and 88 cm wide. Treatment involved fruit being moved along 14 rotating brushes, passing through five laminar waterfalls of the heated fungicide solution. The fruit were pushed at a controlled speed by several rotating metal sweepers. The experimental flooder has a tank capacity of 375 L.

Fruit batches for the curative, protective and sporulation inhibition treatments were treated with the IMZ solution applied using the flooder at 250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ at pH 3, 4, 5 and 6. At pH 6, the effect of solution temperature at 45, 55 and 65°C was evaluated. Imazalil sulphate (Imzacure, 750 $\text{g}\cdot\text{kg}^{-1}$ SG, ICA International Chemicals, Stellenbosch, South Africa) was added to a concentration of 250 $\mu\text{g}\cdot\text{mL}^{-1}$ and the temperature was raised to 45°C. The pH of the solution was measured with a portable pH meter (HI 98121 Waterproof pH/ORP & Temperature meter, Hanna Instruments, Morninghill, Johannesburg, South Africa) and the pH was adjusted with hydrochloric acid (32%; Merck (Pty.) Ltd., Modderfontein, Gauteng, South Africa) to a pH of 3. After treatment of the respective fruit batches for curative, protective and sporulation inhibition treatments, the pH of the IMZ solution was increased using sodium hydrogen carbonate (sodium bicarbonate; NaHCO_3 ; Saarchem uniLAB, Merck Chemicals (Pty) Ltd., Wadeville, Gauteng, South Africa) to 4, and another set of fruit batches was treated. The protocol was repeated twice more, at an IMZ solution of pH 5 and 6. After the pH 6 treatment at 45°C, the temperature was raised to 55°C and 65°C for subsequent treatments. The flooder was then completely drained, rinsed out twice with clean water, and refilled with water. The water was adjusted to an IMZ concentration of 500 $\mu\text{g}\cdot\text{mL}^{-1}$ and the same processes as explained with the 250 $\mu\text{g}\cdot\text{mL}^{-1}$ treatments were repeated. Untreated but inoculated fruit acted as controls for the treatments.

Incubation and treatment evaluation

After IMZ treatment with the flooder, the fruit followed a commercial packline set up by moving over 14 rotating brushes under an airknife (± 100 nozzels supplying a stream of air onto the fruit to remove moisture) and then moving through an unheated forced air drying tunnel. No wax coating was applied to the fruit. The dry fruit were packaged stem end facing upward on SFT13 nectarine trays (Huhta-maki South Africa (Pty) Ltd., Atlantis, South Africa) placed in lock-back stonefruit cartons (115 mm; Mpact, Epping, South Africa).

The cartons were then covered in clear polyethylene bags (50 MIC, Lanpack Manufacturers C.C., Woodstock, Cape Town, South Africa) with the end not sealed but folded under the carton. Four to six small (≈ 5 mm diameter) holes were made to reduce humidity and moisture build-up. The cartons were stacked and stored at ambient temperature ($\approx 22^\circ\text{C}$) for 4 days for curative and protective inoculations, and 10 days for sporulation inoculations.

Evaluations for curative and protective treatments were done by recording the number of infected wounds on each fruit. Early infection was visualised using a near-UV light (UV-A at 365 nm, Labino Mid-light, www.labino.com), which caused infected wounds to fluoresce bright yellow (Njombolwana *et al.*, 2013a). In the sporulation inhibition fruit batches, sporulation was evaluated for each individual fruit using a rating index of 1 – 6: 1 = complete sporulation inhibition i.e. completely white fruit; 2 = sporulating area was small ($\approx 20\%$); 3 = sporulating area larger than a quarter of the fruit, but smaller than half of the fruit ($\approx 40\%$); 4 = sporulating area larger than half of the fruit, but smaller than three quarters of the fruit ($\approx 60\%$); 5 = sporulating area larger than three quarters of the fruit, but smaller than the whole fruit ($\approx 80\%$); and 6 = sporulating area covering the whole fruit (= 100%). Fruit that showed no sign of infection was regarded as missing data points.

Residue loading in a commercial flooder

Residue loading following IMZ application with a commercial flooder was assessed in an operational packhouse in the northern part of South Africa. The flooder had 5 weirs and 16 rotating brushes. Immediately following the flooder, the packline had 16 donut sponge rollers, a drying tunnel and wax applicator. IMZ at 500 $\mu\text{g}\cdot\text{mL}^{-1}$ was applied to lemon (cv. Eureka) and early navel oranges (cv. Navelina) at temperatures of 25, 35 and 45°C. The pH was managed and adjusted to 3 or 6, and fruit samples were collected in various treatment combinations, namely after the flooder, after the donuts and after a wax coating application with a commercial citrus wax coating (PolyOrange polyethylene coating, 18% solids, Citrashine (Pty) Ltd., Johannesburg, South Africa) incorporated with either 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ (EC). Fruit were collected for residue analysis and prepared as described above.

Statistical analysis

For curative and protective infection data (percentage wounds infected per fruit) were normalised relative to the data for untreated control treatments; percentage control data were subsequently used. All data were subjected to analyses of variance (ANOVA) and Fisher's least significant difference test at 95% confidence interval to compare means. To demonstrate trends between residues loaded from treatments, or the levels of control achieved, values were regressed using an appropriate non-linear regression model. The coefficient of determination (R^2) was used to demonstrate goodness of fit. Where possible, the model was used to predict

either residue results or percentage control that would be achieved through treatment. XLSTAT (version 2016.7.01, Addinsoft, www.xlstat.com) was used for the analysis described. Regression models with reliable fits were used to determine residue levels that would be indicative of 50% or 90% control (Erasmus *et al.*, 2015b).

Results

Statistical analyses for the 250 and 500 $\mu\text{g.mL}^{-1}$ solutions were done separately. Results are presented as 'effect of pH' and 'effect of temperature'. The former is to determine the effect of pH at the range of 3, 4, 5 and 6 at a temperature of 45°C, while the latter determines the effect of temperature at pH 6, using a temperature range of 45, 55, and 65°C. To increase the robustness of the data, and because no meaningful interpretation could be drawn from batch differences, despite frequent statistical differences between them, batches of the same citrus types were combined. Citrus types were analysed separately as the type effect was always significant in combined analyses. Analysis of variance results are described below.

Imazalil residue loading

The effect of pH at a solution temperature of 45°C

Analysis of variance for imazalil (IMZ) residue data for all four citrus types treated at a concentration of either 250 $\mu\text{g.mL}^{-1}$ or 500 $\mu\text{g.mL}^{-1}$ showed that pH was a significant effect ($P < 0.026$). Therefore, data were regressed using a Three Parametric Logistic non-linear regression model with the function $Y = \text{pr}3 / (1 + \text{Exp}(-\text{pr}1 - \text{pr}2 * X1))$ and fitted with good R^2 values (0.635 – 0.865 for the 250 $\mu\text{g.mL}^{-1}$ treatment and 0.549 – 0.966 for 500 $\mu\text{g.mL}^{-1}$; Table 1). Residue levels increased as pH increased for both concentrations. Residue loading was greater and steeper for 500 $\mu\text{g.mL}^{-1}$ concentrations than 250 $\mu\text{g.mL}^{-1}$. For both concentrations, the highest residues levels were recorded on Valencia (0.83 and 4.04 $\mu\text{g.g}^{-1}$), the lowest on navel oranges (0.29 and 2.06 $\mu\text{g.g}^{-1}$), while intermediate and similar levels were recorded on lemon (0.48 and 3.18 $\mu\text{g.g}^{-1}$) and Satsuma mandarin (0.43 and 3.35 $\mu\text{g.g}^{-1}$; Fig. 1A and B).

The effect of temperature at a solution pH of 6

Analysis of variance for IMZ residue data for all four citrus types for either concentration, showed a significant effect for the temperature range ($P < 0.008$), except lemon at 250 $\mu\text{g.mL}^{-1}$, which presented a meaningful effect at a 90% confidence level ($P = 0.080$). Data were regressed using a Three Parametric Logistic non-linear regression model with the function $Y = \text{pr}3 / (1 + \text{Exp}(-\text{pr}1 - \text{pr}2 * X1))$ and fitted with good R^2 values (0.426 – 0.954 for the 250 $\mu\text{g.mL}^{-1}$ treatment and 0.722 – 0.952 for 500 $\mu\text{g.mL}^{-1}$; Table 2). Residue levels increased as temperature increased for both concentrations. Residue loading was greater and steeper for 500 $\mu\text{g.mL}^{-1}$ concentrations than 250 $\mu\text{g.mL}^{-1}$. Steady residue loading for all citrus types was shown with Valencia once again loading the highest (2.84 – 12.50 $\mu\text{g.g}^{-1}$), navel the lowest (1.10 – 5.23 $\mu\text{g.g}^{-1}$), and lemon and Satsuma mandarin acting more moderately and similarly (1.48 – 9.95 and 1.68 – 15.85 $\mu\text{g.g}^{-1}$ respectively), except where Satsuma mandarin loaded very high levels with the 500 $\mu\text{g.mL}^{-1}$ dose at 65°C, pH 6 (Fig. 2A and B).

Green mould control

The effect of pH at a solution temperature of 45°C

Curative control

The pH was not a significant effect for Satsuma mandarin and navel at 250 $\mu\text{g.mL}^{-1}$ and for lemon at 500 $\mu\text{g.mL}^{-1}$ ($P > 0.206$), but were meaningful at a 90% confidence level ($P < 0.091$) for the remainder of the treatments. The mean curative control achieved was consistently high > 89%, except for some lower pH treatments on Satsuma mandarin with control levels of 71.55 – 82.36% (Table 3). Regression analysis gave very poor fits (results not shown) and models could not be used to predict residue levels required to achieve 90% control.

Protective control

Analysis of variance for protective control data showed that pH was a significant effect for all citrus types ($P < 0.001$). All treatments expressed a level of control above 75% (Table 4), but on all citrus types protective control was generally significantly better at pH 5 and 6 (93.99 – 100%), compared with pH 3 and 4 (74.89 –

98.31%). Regression analysis gave poor fits and, residue levels required to achieve 90% protective control could not be modelled (results not shown).

Sporulation inhibition

Analysis of variance for sporulation inhibition data indicated that pH was a significant effect at both tested concentrations for all citrus types ($P < 0.0001$). Regression analysis was conducted on the combined residue data from 250 and 500 $\mu\text{g.mL}^{-1}$ treatments and sporulation inhibition data. The non-linear regression using the afore-mentioned three parameter logistic model presented excellent fits with $R^2 > 0.906$ for all but Satsuma mandarin ($R^2 = 0.573$; Table 5). Sporulation inhibition increased very quickly relative to IMZ residue levels (Fig. 3). The model predicted that IMZ residue levels between 1.29 and 2.47 $\mu\text{g.g}^{-1}$ were required to achieve 90% sporulation inhibition. Between 0.47 and 1.49 $\mu\text{g.g}^{-1}$ were predicted necessary for only 50% inhibition. Higher residue levels were required for lemon and Valencia orange (2.47 and 2.34 $\mu\text{g.g}^{-1}$ for 90% inhibition, respectively) compared with Satsuma mandarin and navel orange (1.84 and 1.29 $\mu\text{g.g}^{-1}$, respectively; Table 5).

The effect of temperature at a solution pH of 6

Curative control

Analysis of variance showed a significant temperature effect for lemon and navel in the 250 $\mu\text{g.mL}^{-1}$ solution ($P = 0.036$ and $P = 0.019$, respectively) while Valencia citrus was the only fruit type where temperature played a significant role at 500 $\mu\text{g.mL}^{-1}$ ($P = 0.006$). For all other treatments the temperature effect was insignificant ($P > 0.168$). The mean curative control achieved was high $> 77.69\%$ (Table 6), with more consistent control at 500 $\mu\text{g.mL}^{-1}$ (93.38 - 100%); Satsuma mandarin expressing the worst levels of control and only lemon demonstrating almost 100% control. At 500 $\mu\text{g.mL}^{-1}$ the best control levels were observed on lemon at 100%, but $> 90\%$ control was observed on the other citrus types. No clear trend could be seen of an increasing relationship between curative control and these high temperatures at pH 6.

Protective control

Analysis of variance indicated that temperature was not a significant effect, and in some cases ANOVA could not be performed due to the uniform 100% control achieved (ANOVA not shown). Protective control was never lower than 97.94% which was for Satsuma mandarin at 45°C, while consistently close to 100% protective control was observed on other citrus types (results not shown).

Sporulation inhibition

Analysis of variance for sporulation inhibition data indicated that temperature was a significant effect for the 250 $\mu\text{g.mL}^{-1}$ solutions ($P < 0.0001$). A trend of sporulation inhibition increasing as temperature increased was observed for the 250 $\mu\text{g.mL}^{-1}$ concentration. Very high levels of sporulation inhibition ($> 89.58\%$) were observed on all citrus types at 55°C, with levels close to 100% sporulation inhibition observed at 65°C. All types had the most inconsistent sporulation inhibition at 45°C (25.93 - 100%). At 500 $\mu\text{g.mL}^{-1}$, inhibition values ranged from 95.21 to 100% at 500 $\mu\text{g.mL}^{-1}$ (results not shown). Rind damage in the form of browning was noted on both lemon and Satsuma mandarin citrus types treated at 55 and 65°C at either concentration. Damage was higher at 65°C but was not quantified due to the mycelial growth on the fruit, which, although decreased and inconsistent, was still present and obscured the brown discolouration.

Commercial flooder

Analysis of variance for IMZ residue levels on lemon fruit showed that there were significant temperature x treatment ($P = 0.001$) and pH x treatment ($P = 0.031$) interactions however for simplicity, the 3-way interaction of pH x temperature x treatment ($P = 0.217$) is discussed. Imazalil residue data on navel fruit showed a 3-way interaction of pH x temperature x treatment ($P = 0.000$). From the mean values in Table 7 it can be seen that residue loading increased with increasing temperature and increasing pH. The use of donuts after the flooder treatment reduced the residue by an average factor of 1.5. The addition of a wax coating augmented with IMZ increased the residue in almost all cases, with the 2000 $\mu\text{g.mL}^{-1}$ IMZ wax application often resulting in higher levels of IMZ residue than the 1000 $\mu\text{g.mL}^{-1}$ IMZ wax application.

For lemon treated at 45°C in a 500 $\mu\text{g.mL}^{-1}$ solution, the commercial flooder loaded an average 0.86 $\mu\text{g.g}^{-1}$ after the flooder alone, and 0.37 $\mu\text{g.g}^{-1}$ after fruit had rolled over donuts at pH 3. At pH 6, the commercial flooder loaded 2.45 $\mu\text{g.g}^{-1}$, which decreased to 1.70 $\mu\text{g.g}^{-1}$ after donuts were used. For navel treated under

the same conditions, pH 3 solutions loaded $0.74 \mu\text{g.g}^{-1}$ and $0.34 \mu\text{g.g}^{-1}$ after the flooder and after donuts, respectively. At pH 6, the residue on navel after the flooder was $2.09 \mu\text{g.g}^{-1}$ and after donuts, $1.63 \mu\text{g.g}^{-1}$ (Table 7).

The residue loading model developed in this study predicted $0.71 \mu\text{g.g}^{-1}$ residue on lemon that had been brushed at pH 3 and 45°C , while the commercial flooder loaded $0.86 \mu\text{g.g}^{-1}$ before the donuts and $0.37 \mu\text{g.g}^{-1}$ after the donuts. For the pH 6 and 45°C solution on lemon, the model predicted a residue of $3.20 \mu\text{g.g}^{-1}$. The commercial flooder loaded $2.45 \mu\text{g.g}^{-1}$ before the donuts and $1.70 \mu\text{g.g}^{-1}$ after the donuts. The prediction for navel from a pH 3 and 45°C solution predicted $0.52 \mu\text{g.g}^{-1}$ residue while the commercial flooder loaded $0.74 \mu\text{g.g}^{-1}$ before the donuts and $0.34 \mu\text{g.g}^{-1}$ after the donuts. For the pH 6 and 45°C solution on navel, the model predicted a residue of $2.04 \mu\text{g.g}^{-1}$. The commercial flooder loaded $2.09 \mu\text{g.g}^{-1}$ before the donuts and $1.63 \mu\text{g.g}^{-1}$ after the donuts (Table 7; Table 8).

Discussion

The influence of heated flooder solution parameters on IMZ residue loading on citrus fruit was clearly determined in this study: higher levels of IMZ residue were loaded by increasing the solution temperature, pH and/or concentration of the IMZ sulphate solution. Based on residue levels, sporulation inhibition could successfully be modelled, but similar models could not be developed for curative and protective control due to the very high levels of control observed in this study. Maximum Residue Limits (MRL) were exceeded at high temperatures (55 and 65°C) at pH 6, and heat damage was also a concern on lemon and Satsuma mandarin. The results in this study demonstrate clear trends but also confirm previous studies that found that different citrus types react differently in their susceptibility to green mould, as well as the amount of IMZ residue loaded (Nadel-Schiffmann and Littauer, 1956; Schirra *et al.*, 2008; Kellerman *et al.*, 2016). Our study also indicated significant differences between different batches of the same citrus type, but these were regarded as a statistical block-effect in order to increase the robustness of the findings. Batch differences, due to variations in fruit maturity, climate and cultivar, are often observed in postharvest citrus pathology research (Njombolwana *et al.*, 2013a; Erasmus *et al.*, 2015a, b; Kellerman *et al.*, 2016). These are near impossible to avoid as postharvest research is altogether dependent on fruit that becomes available throughout a season. Citrus susceptibility to green mould increases as fruit matures (Smoot and Melvin, 1961). The susceptibility and maturity relationship may in part be due to the pH of the albedo tissue that has shown to decrease in lemons (Smilanick *et al.*, 2005), and where yellow lemons are more likely to decay compared to green ones (Eckert, 1995).

In this study, it was noted that residue levels increased significantly with an increase in solution pH. It is well documented that IMZ loads more residues with increasing pH, and more so when temperature and exposure time of the solution are increased too (Brown and Dezman, 1990; Schirra *et al.*, 1996; Smilanick *et al.*, 1997; Cabras *et al.*, 1999; D'Aquino *et al.*, 2006; Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016). Imazalil solutions at pH 3 have been shown to be very stable with low residue loading but at pH 6, residues are increased and easily exceeded the MRL of $5 \mu\text{g.g}^{-1}$ after a 45 s exposure time (Erasmus *et al.*, 2011; Kellerman *et al.*, 2016). A short exposure time (8 s) was used in this study and therefore at $500 \mu\text{g.mL}^{-1}$ the pH 6 treatment at 45°C gave a maximum residue of below the MRL at $\approx 4.04 \mu\text{g.g}^{-1}$. Generally the $250 \mu\text{g.mL}^{-1}$ dose loaded lower residue than the $500 \mu\text{g.mL}^{-1}$ solution; however, at pH 3 the average residues loaded were comparable (≈ 0.51 and $0.85 \mu\text{g.g}^{-1}$, respectively). It is possible that at a solution of pH 3, concentration, like exposure time, may not be a major influencing factor. A similar observation was made with treatments involving the cascade application (an application similar to the flooder) of IMZ sulphate that showed that very similar residues were loaded at $1000 \mu\text{g.mL}^{-1}$ treatment and at $2000 \mu\text{g.mL}^{-1}$ treatment (0.5 and $0.4 \mu\text{g.g}^{-1}$, respectively). In the cascade, IMZ was applied at ambient temperature and at pH 3 for both concentrations (Besil *et al.*, 2016).

The effects of concentration are more noticeable at high temperatures. In our study, the $500 \mu\text{g.mL}^{-1}$ treatment loaded much higher levels ($\approx 6.32 \mu\text{g.g}^{-1}$) than the $250 \mu\text{g.mL}^{-1}$ ($\approx 3.52 \mu\text{g.g}^{-1}$) solution, such that at 65°C the MRL was always exceeded regardless of citrus type. The effects of higher temperatures loading higher residues, especially in conjunction with an increase in concentration has been noted previously (Schirra *et al.*, 1996; Smilanick *et al.*, 1997; Cabras *et al.*, 1999; D'Aquino *et al.*, 2006; Kellerman *et al.*, 2016). It is important to note that the temperature range was conducted at a solution pH of 6 where it is known that exposure time is important to avoid exceeding the MRL as already discussed. We found that the MRL of $5.0 \mu\text{g.g}^{-1}$ was

exceeded at 65°C and pH 6 despite a short treatment time of 8 s, suggesting that temperature has a more compelling effect than time in terms of residue loading at high temperatures. It is feasible to use a lower IMZ concentration by increasing the solution temperature to achieve the same level of control as demonstrated by Schirra *et al.* (2010). They used the EC formulation of IMZ and observed a similar result as obtained in our study with the sulphate formulation in the flooder. However, further research on maintenance of fungicide mixtures must be conducted to determine the effects of large quantities of fruit moving through the fungicide solution and stripping the fungicide from the solution, as well as the reduction of available fungicide as organic matter builds up from the fruit coming in from the orchard.

Post treatment brushing removes excess fungicide solution to assist drying of fruit prior to wax coating application and packing (Pelser, 1977; Smilanick *et al.*, 1997; Erasmus *et al.*, 2015a). However, it also removes some of the IMZ active and this has been shown by lower residues in the commercial flooder evaluation. Our findings in the commercial packhouse using donuts, confirm reports following post-dip brushing (Erasmus *et al.*, 2015a). How much residue is removed was linked to the pH of the solution. The reaction of IMZ to the pH is due to the lipophilic nature of undissociated IMZ at the higher pH, with this compound then binding more strongly to the oily constituents in the citrus rind when in a pH 6 solution (Siegel *et al.*, 1977). Despite reductions ($\approx 75\%$ at pH 3 and $\approx 44\%$ at pH 6), good infection control was still observed in green mould management trials by Erasmus *et al.* (2015a).

The commercial flooder was not entirely comparable to the experimental unit in terms of the drying mechanisms. On the commercial unit fruit were moved over donuts, while the experimental unit had brushes and with an air-knife after treatment. In the commercial flooder trials, it was seen that fruit taken after the donuts, compared to fruit treated with the flooder alone, had significantly less IMZ residue. Regardless, the residues loaded after treatment were still usefully close to those predicted by the model. The differences in results can in part be attributed to batch differences as explained above, but more likely due to the difference in effect between brushes and donuts. Whether donuts or brushes are more acceptable in terms of disease control would need to be investigated in a future study.

At the recommended IMZ application regimes evaluated, very good control was observed (average curative control of 90.8% and protective control of 93.3%), despite harsh inoculation methods and ideal incubation conditions provided. In terms of disease management in a commercial packhouse, where conditions are less favourable and pathogen/disease incidence is kept as low as possible through sanitation practices, the control achieved here is considered very good.

Good curative control ($> 71.6\%$) was achieved across a wide range of IMZ residues ($> 0.40 \mu\text{g}\cdot\text{g}^{-1}$). Whole fruit residue data was poorly correlated with curative control, and can be attributed to the fact that residue loading is more concentrated in the wounded tissue where the curative fungicidal action is required (Dore *et al.*, 2009, 2010; Erasmus *et al.*, 2015a). Previous studies have indicated that dip application resulted in excellent curative control (Dore *et al.*, 2009; Erasmus *et al.*, 2011, 2015a; Njombolwana *et al.*, 2013a; Kellerman *et al.*, 2016), and was generally better than other aqueous applications such as drench or spray (Erasmus *et al.*, 2011; Kellerman *et al.*, 2014). This efficacy was attributed to more effective residue loading in wound sites by dip application (Erasmus *et al.*, 2015a), and is supported by reports that curative control improved with longer exposure time in fungicide dip-treatments (Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016). Exposure time (8 s) in the flooder is markedly shorter than typical dip treatments (16 – 107 s) (Erasmus *et al.*, 2011). Excellent curative control despite this brief exposure time can be attributed to the flooder's effective and uniform means of fungicide deposition by means of five water films over rotating brushes. A possible explanation could be that, due to the high volume of heated solution in contact with the fruit, control is improved (Kanetis *et al.*, 2008). Erasmus *et al.* (unpublished) found that the higher volume and longer exposure time of solution gained from 3 to 5 weirs resulted in more effective residue loading and subsequent green mould control. Additionally, the increased mechanical action of solution being brushed onto the fruit by the rotational action of the brushes, may be enabling improved coverage and penetration of wound sites.

Protective control in dip applications was previously found to be relatively poor when compared to curative control following dip treatments, or to protective control following IMZ application in wax coatings (Njombolwana *et al.*, 2013a). The excellent protective control that was seen following IMZ application using the heated flooder was therefore a surprising result. This attribute of the flooder was first reported by Erasmus *et al.* (unpublished) in comparison studies between the dip and flooder application. Improved protective control following heated flooder applications can be attributed to better and more uniform residue loading, as

discussed above. The residue levels corresponding to effective green mould control presented in this study correlate with various other studies that has shown that a residue of between 0.6 and 3.0 $\mu\text{g}\cdot\text{g}^{-1}$ is necessary to effectively control green mould infections (Kaplan and Dave, 1979; Schirra *et al.*, 1996; Smilanick *et al.*, 1997; Erasmus *et al.*, 2011).

Sporulation inhibition was good where residue levels were high, which, as seen in this study and others, is strongly correlated to pH with solutions of pH 6 giving better sporulation inhibition results (Hall, 1991; Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016). Using the sporulation inhibition model developed, sporulation inhibition could be predicted from residue levels and a residue of between 1.29 and 2.47 $\mu\text{g}\cdot\text{g}^{-1}$ was predicted to inhibit sporulation at 90%. These values, are similar to previous research indicating that a minimum residue of 2.0 $\mu\text{g}\cdot\text{g}^{-1}$, but not necessarily higher than 3.5 $\mu\text{g}\cdot\text{g}^{-1}$, is sufficient to control sporulation (Kaplan and Dave, 1979; Schirra *et al.*, 1996; Smilanick *et al.*, 1997; Erasmus *et al.*, 2011).

Previous studies have shown that heat treatments alone can be useful in disease control, with a reduction in decay seen with hot water dips only (McDonald *et al.*, 1991; Erkan *et al.*, 2005; Şen *et al.*, 2010). This may in part be due to heated dips (47 – 53°C) decreasing *Penicillium* spp. inoculum (Şen *et al.*, 2010). The addition of a fungicide invariably increases the control achieved to more than what is possible with heated water or ambient fungicide solution alone (Schirra *et al.*, 1997; Puawongphat *et al.*, 2008; Dore *et al.*, 2009). Our study consistently demonstrated very good levels of control, with improved residue loading and sporulation inhibition at higher pH and temperatures. A heated solution only was not included as treatment, and conclusions cannot be drawn whether improved sporulation inhibition could also be attributed to some synergistic effect of heat treatment. Despite reports that heat can have a beneficial effect on reducing decay, we observed rind injury, blemishes and discolouration on lemon and Satsuma mandarin fruit even after very brief exposure to heated solutions in the flooder at 55 and 65°C. This is an unacceptable risk, particularly with less hardy fruit such as lemons or soft citrus types. Temperatures above 55°C were seen to injure fruit in various studies (McDonald *et al.*, 1991; Palou *et al.*, 2001; Puawongphat *et al.*, 2008) and the damages inflicted resulted in higher incidence of decay (Şen *et al.*, 2010).

A sensitive strain of *P. digitatum* was used in this study. Erasmus *et al.* (2015b) demonstrated practical resistance, *i.e.* significantly diminished levels of control and loss of sporulation inhibition, when attempting to control IMZ resistant strains by means of IMZ dip treatments. The heated flooder application has demonstrated some superior benefits to dip application and further research is required to investigate whether improved IMZ application by means of the heated flooder will improve control of IMZ resistant strains.

The addition of the IMZ in wax treatment in the commercial trials, apart from the heated flooder treatments, was for practical industry purposes. It was important to confirm that the MRL would not be exceeded with the double application of IMZ, which is commonplace in South African packhouses (Erasmus *et al.*, 2011). It was also necessary to determine if the second application of IMZ would be necessary if adequate residue levels could be achieved with a heated solution as was seen by Smilanick *et al.* (1997). Njombolwana *et al.* (2013a, b) found that, whilst IMZ application via wax coatings gave relatively poor curative control, it supported IMZ dip application which gave excellent curative control but moderate protective control. Based on the results presented here, the flooder offers both curative and protective control, negating the need for an additional application of IMZ in the wax. The flooder will load sufficient IMZ residues for both infection and sporulation control if the right solution parameters are implemented. Nonetheless, should packhouses choose to remain with the double IMZ application strategy, it was seen from these initial tests that the MRL was not exceeded, even when applying the higher 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ in the wax coating.

Most research on IMZ application has shown increased residues were loaded when applied at increased temperature, pH, exposure time and concentration variables (Smilanick *et al.*, 1997; Erasmus *et al.*, 2011, 2013; Kellerman *et al.*, 2016). To date, all the work done on the sulphate form has been in dip trials and with limited attention paid to the effect of aqueous treatment over rotating brushes, as in the heated flooder. The effect of brushing during treatment seems to aid protective control of infections, while post treatment brushing was shown to decrease residues without compromising control of green mould. The flooder has been shown to offer excellent green mould control both in terms of curing established infections and protecting from infections occurring post treatment, as well as effective sporulation inhibition. This is a major advantage over the fungicide bath which lacks adequate protective action. The flooder is easier to manage and loads residues more precisely than fungicide dip applications. This is an important characteristic to be aware of as there is a current demand from supermarkets for fruit with residues up to 60% below the MRL. Whilst this requirement is driven by market forces, rather than scientifically based food safety concerns, producers and packhouses

are pressured to abide by these demands (Wilma du Plooy, personal communication, 2016). From a sustainable green mould control perspective, our results show that infection control should not be affected by markedly reduced IMZ residue levels relative to the MRL; however, it is not practically feasible to treat fruit in a packhouse within 24 hours after infection/harvest. Moreover, sporulation control will be compromised, leading to further problems of soilage, increased spore load and the risk of fungicide resistance development. In conclusion, we have demonstrated that heated flooder solution parameters can be manipulated to precisely load imazalil fungicide on citrus rinds to ensure adequate green mould control while maintaining residues below the MRL.

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Tables and figures

Table 1. Parameters and goodness of fit of non-linear regression using the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ of imazalil residue data determined on lemon, Satsuma mandarin, navel orange and Valencia orange treated with a heated flooder with imazalil concentrations of 250 and 500 $\mu\text{g.mL}^{-1}$ and a pH range of 3, 4, 5, and 6 at a solution temperature of 45°C.

Citrus Kinds	Model parameter values and goodness of fit			
	Pr1	Pr2	Pr3	R ²
250 $\mu\text{g.mL}^{-1}$				
Lemon	-4.417	0.574	4.581	0.834
Satsuma mandarin	-5.470	0.509	20.480	0.685
Navel	-4.315	0.512	4.940	0.865
Valencia	-3.572	0.562	6.299	0.635
500 $\mu\text{g.mL}^{-1}$				
Lemon	-7.904	0.561	293.462	0.966
Satsuma mandarin	-7.376	0.549	199.420	0.549
Navel	-7.145	0.487	139.047	0.963
Valencia	-6.149	0.425	150.564	0.696

Table 2. Parameters and goodness of fit of non-linear regression using the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ of imazalil residue data determined on lemon, Satsuma mandarin, navel orange and Valencia orange treated with a heated flooder with imazalil concentrations of 250 and 500 $\mu\text{g.mL}^{-1}$ and a temperature range of 45, 55 and 65°C at a solution pH of 6.

Citrus Kinds	Model parameter values and goodness of fit			
	Pr1	Pr2	Pr3	R ²
250 $\mu\text{g.mL}^{-1}$				
Lemon	-9.740	0.087	412.627	0.426
Satsuma mandarin	-7.606	0.069	146.821	0.731
Navel	-6.442	0.050	70.544	0.954
Valencia	-5.395	0.051	66.965	0.661
500 $\mu\text{g.mL}^{-1}$				
Lemon	-7.634	0.060	426.051	0.722
Satsuma mandarin	-10.306	0.095	997.916	0.842
Navel	-7.249	0.052	248.176	0.952
Valencia	-8.124	0.064	679.667	0.747

Table 3. Mean curative control levels (%) determined on lemon, Satsuma mandarin, navel orange and Valencia orange treated with a heated flooder with imazalil concentrations of 250 and 500 $\mu\text{g.mL}^{-1}$ and a pH range of 3, 4, 5, and 6 at a solution temperature of 45°C.

pH	Green mould control (%) ^a			
	Lemon	Satsuma mandarin	Navel	Valencia
250 $\mu\text{g.mL}^{-1}$				
3	93.33 b	72.18 b	89.17 a	91.26 b
4	97.18 ab	78.01 ab	93.25 a	92.53 ab
5	98.91 a	82.36 a	90.43 a	95.98 a
6	97.21 ab	79.17 ab	88.28 a	93.87 ab
500 $\mu\text{g.mL}^{-1}$				
3	100.0 a	75.17 b	89.51 b	93.55 b
4	99.63 a	71.55 b	91.44 ab	94.19 b
5	99.62 a	80.93 b	90.16 b	97.84 a
6	99.63 a	94.23 a	95.38 a	97.89 a

^a For each citrus type and IMZ concentration separately, means followed by the same letter do not differ significantly ($P > 0.05$)

Table 4. Mean protective control levels (%) determined on lemon, Satsuma mandarin, navel orange and Valencia orange treated with a heated flooder with imazalil concentrations of 250 and 500 µg.mL⁻¹ and a pH range of 3, 4, 5, and 6 at a solution temperature of 45°C.

pH	Green mould control (%) ^a			
	Lemon	Satsuma mandarin	Navel	Valencia
250 µg.mL⁻¹				
3	74.91 b	83.14 b	78.17 b	92.08 b
4	74.89 b	87.67 b	82.19 b	94.77 b
5	93.99 a	95.85 a	94.54 a	99.23 a
6	99.55 a	97.94 a	98.59 a	100.0 a
500 µg.mL⁻¹				
3	80.62 c	93.74 b	92.03 c	94.58 b
4	92.77 b	95.69 ab	95.25 bc	98.31 a
5	100.0 a	98.04 a	97.89 ba	99.65 a
6	100.0 a	99.31 a	99.63 a	100.0 a

^a For each citrus type and IMZ concentration separately, means followed by the same letter do not differ significantly ($P > 0.05$)

Table 5. Parameters, goodness of fit and effective IMZ residue levels for predicted 50 and 90% sporulation inhibition of *Penicillium digitatum* of non-linear regression using the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ of imazalil residue data measured on lemon, Satsuma mandarin, navel orange and Valencia orange after treatment with either 250 or 500 µg.mL⁻¹ imazalil sulphate at pH levels of 3, 4, 5, and 6 and temperatures of 45, 55 and 65°C.

Citrus type	Model parameter values and goodness of fit				Residue level (µg.g ⁻¹)	
	Pr1	Pr2	Pr3	R ²	90% control	50% control
Lemon	-3.925	2.686	96.021	0.943	2.468	1.492
Satsuma mandarin	-0.764	1.658	99.149	0.573	1.84	0.471
Navel	-4.385	5.217	98.482	0.966	1.2932	0.8464
Valencia	-3.173	2.326	99.265	0.906	2.3426	1.371

Table 6. Mean curative control levels (%) determined on lemon, Satsuma mandarin, navel and Valencia orange treated with the heated flooder at a concentration of 250 and 500 µg.mL⁻¹ for the temperature range of 45, 55 and 65°C at a solution pH of 6.

Temperature (°C)	Green mould control (%) ^a							
	Lemon		Satsuma mandarin		Navel		Valencia	
250 µg.mL⁻¹								
45	97.21	b	79.17	a	88.28	ab	93.87	a
55	98.90	ab	77.69	a	84.97	b	96.36	a
65	100.0	a	82.21	a	93.31	a	96.69	a
500 µg.mL⁻¹								
45	99.63	a	94.23	a	95.38	a	97.89	a
55	100.0	a	94.32	a	95.36	a	93.78	b
65	100.0	a	95.28	a	97.36	a	97.80	a

^a For each citrus type and IMZ concentration separately, means followed by the same letter do not differ significantly ($P > 0.05$)

Table 7. Mean imazalil residue levels determined on lemon and navel fruit treated with a commercial heated flooder at 500 µg.mL⁻¹ at a solution temperature of 25, 35 or 45°C and a solution pH of either 3 or 6.

pH	Temperature (°C)	IMZ residues (µg.g ⁻¹) ^a			
		Flooder alone ^b	Donuts ^b	Wax + 1000 µg.mL ⁻¹ IMZ ^b	Wax + 2000 µg.mL ⁻¹ IMZ ^b
Lemon					
3	25	0.92 ghijkl	0.38 n	0.72 jklmn	0.55 lmn
	35	0.98 ghijk	0.52 mn	0.88 hijklm	1.47 bcde
	45	0.86 ijklm	0.37 n	0.68 klmn	1.45 bcdef
6	25	1.29 cdefg	1.08 fghij	1.18 defghi	1.11 efghi
	35	1.51 bcd	1.10 efghi	1.25 cdefgh	1.46 bcdef
	45	2.45 a	1.70 b	1.56 bc	2.32 a
Navel					
3	25	0.66 jkl	0.41 lm	0.71 ijk	0.79 ghijk
	35	0.74 hijk	0.52 klm	0.85 ghij	1.86 abc
	45	0.74 hijk	0.34 m	0.64 jkl	1.86 abc
6	25	1.35 ef	1.04 g	1.05 fg	1.52 de
	35	1.37 e	1.02 gh	1.01 ghi	1.90 ab
	45	2.09 a	1.63 bcde	1.59 cde	1.79 bcd

^a For each citrus type treatment separately, means followed by the same letter do not differ significantly ($P > 0.05$)

^b Fruit were sampled after each of the flooder alone, over donut sponges, a 1000 µg.mL⁻¹ IMZ wax and a 2000 µg.mL⁻¹ IMZ wax treatment

Table 8. Parameters, goodness of fit and effective IMZ residue levels predicted at pH levels of 3, 4, 5 or 6 of non-linear regression using the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ of imazalil residue data measured on lemon, Satsuma mandarin, navel and Valencia oranges after treatment with IMZ at 500 in a heated flooder at a temperature of 45°C.

Citrus type	Model parameter values and goodness of fit					IMZ residue ($\mu\text{g}\cdot\text{g}^{-1}$)			
	Pr1	Pr2	Pr3	SSE	R^2	pH 3	pH 4	pH 5	pH 6
Lemon	-1.329	1.854	6.063	0.668	0.966	0.709	1.075	1.557	3.200
Satsuma	-0.297	0.949	6.029	6.976	0.604	0.309	1.030	1.990	6.000
Navel	-1.236	2.284	6.205	0.755	0.962	0.515	0.805	1.165	2.040
Valencia	-0.686	0.732	6.362	5.869	0.707	0.781	1.660	2.720	4.800

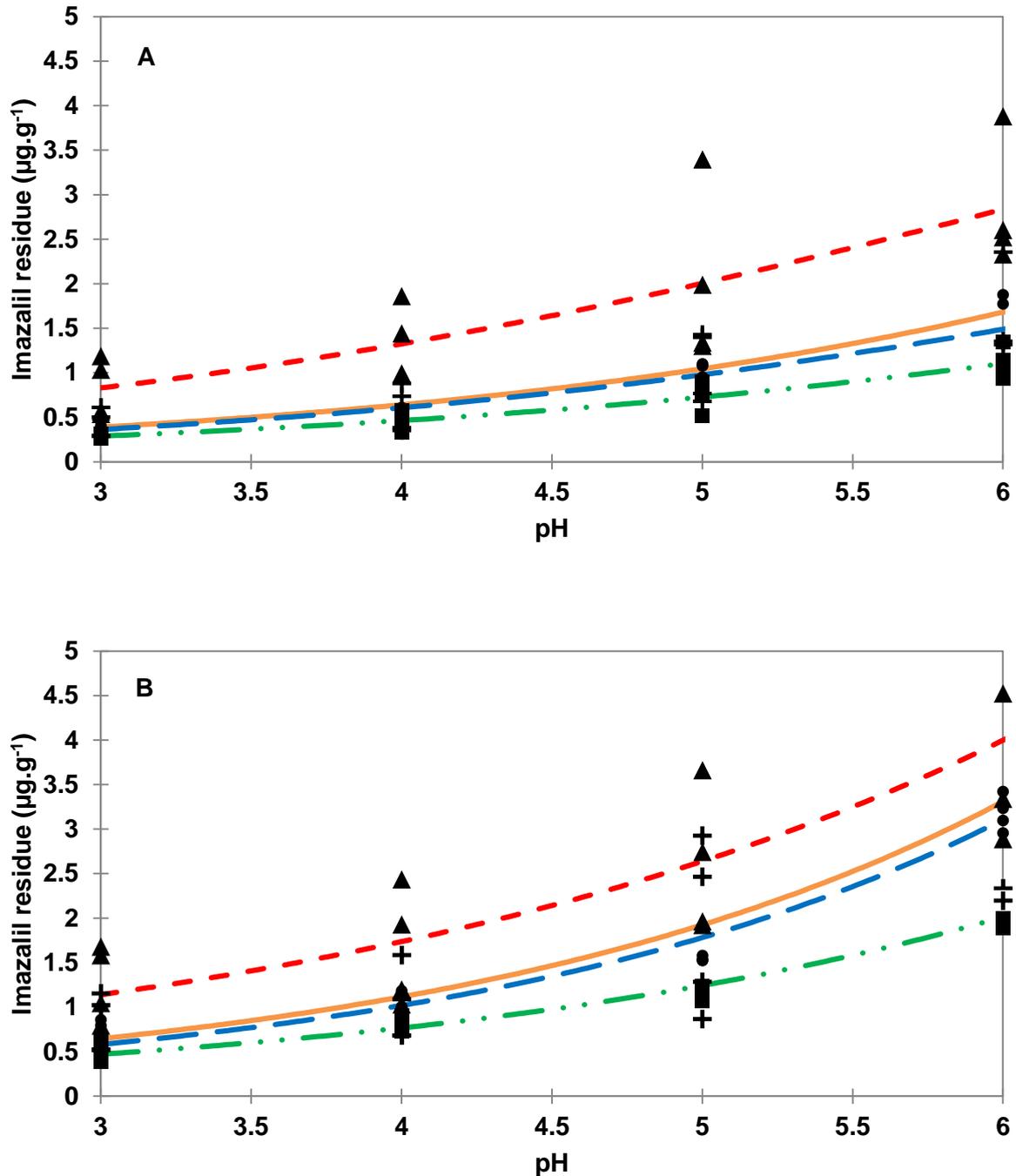
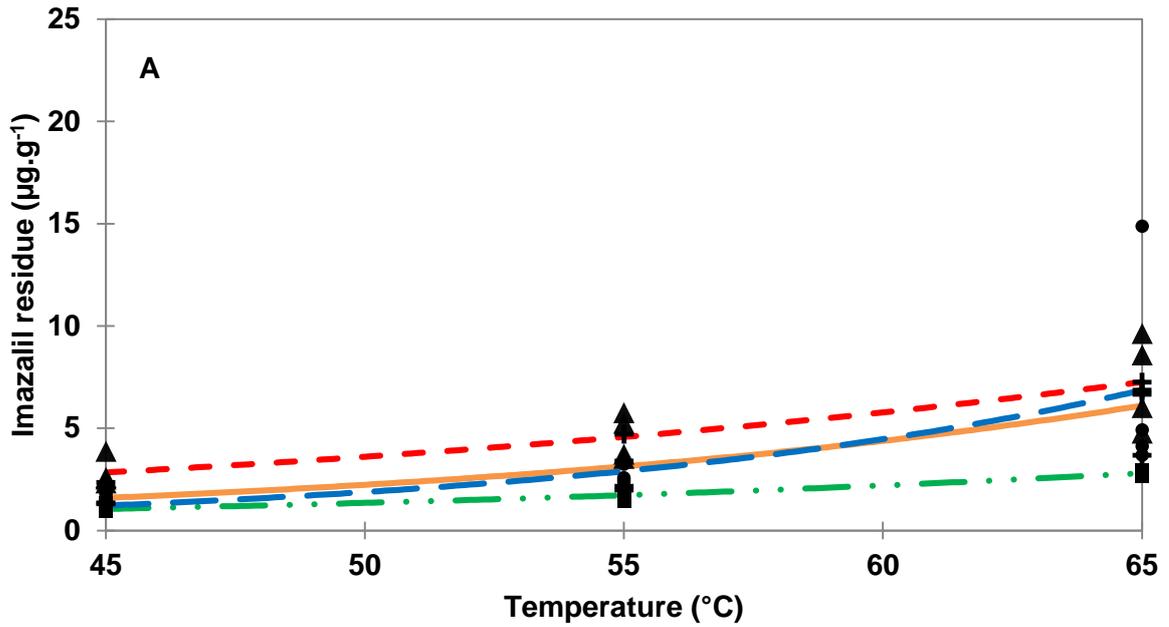


Figure 1. Mean imazalil residues values analysed on citrus fruit after treatment in imazalil solutions at 250 (A) and 500 µg.mL⁻¹ (B) at a pH range of 3, 4, 5, or 6 at a temperature of 45°C and non-linear regression models [$Y = pr3/(1+Exp(-pr1-pr2*X1))$] fitted indicating residue loading trends for lemon (●, blue long dash line, $R^2 = 0.834$ (A); 0.966 (B)), Satsuma mandarin (+, orange solid line, $R^2 = 0.685$ (A); 0.549 (B)), navel (■, green dash dot line, $R^2 = 0.865$ (A); 0.963 (B)), Valencia (▲, red short dash line; $R^2 = 0.635$ (A); 0.696 (B)) as influenced



by pH.

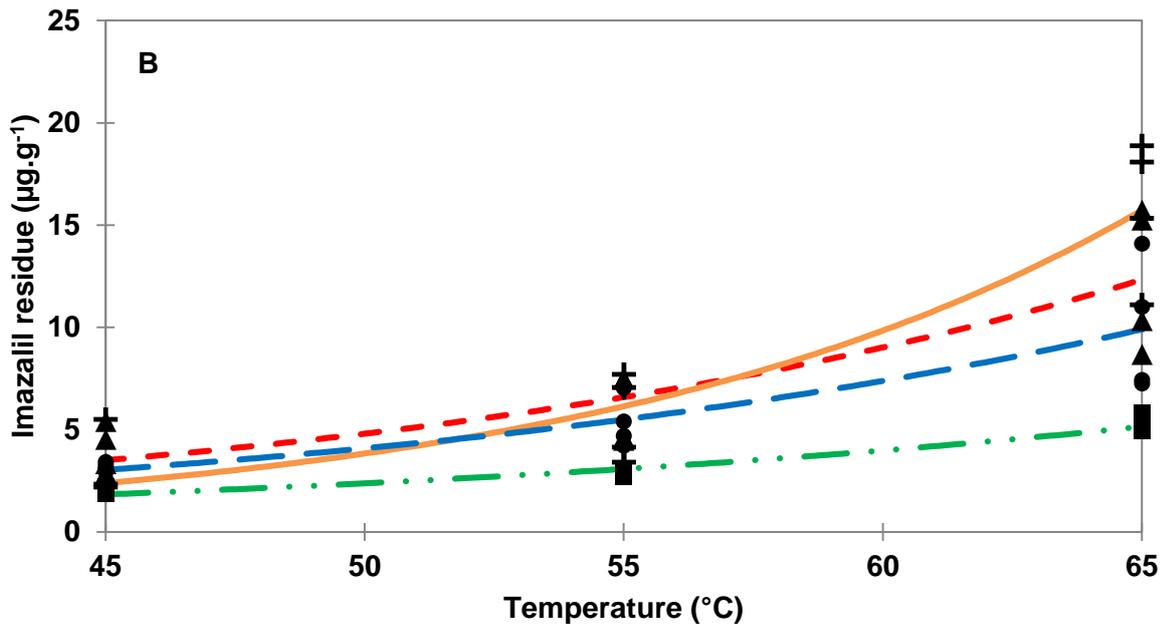


Figure 2. Mean imazalil residues values analysed on citrus fruit after treatment in imazalil solutions at 250 (A) and 500 µg.mL⁻¹ (B) at a temperature range of 45, 55, or 65 at a pH of 6 and non-linear regression models [$Y = pr3/(1+Exp(-pr1-pr2*X1))$] fitted indicating residue loading trends for lemon (●, blue long dash line, $R^2 = 0.426$ (A); 0.722 (B)), Satsuma mandarin (+, orange solid line, $R^2 = 0.731$ (A); 0.842 (B)), navel (■, green dash dot line, $R^2 = 0.954$ (A); 0.952 (B)), Valencia (▲, red short dash line; $R^2 = 0.661$ (A); 0.747 (B)) as influenced by temperature.

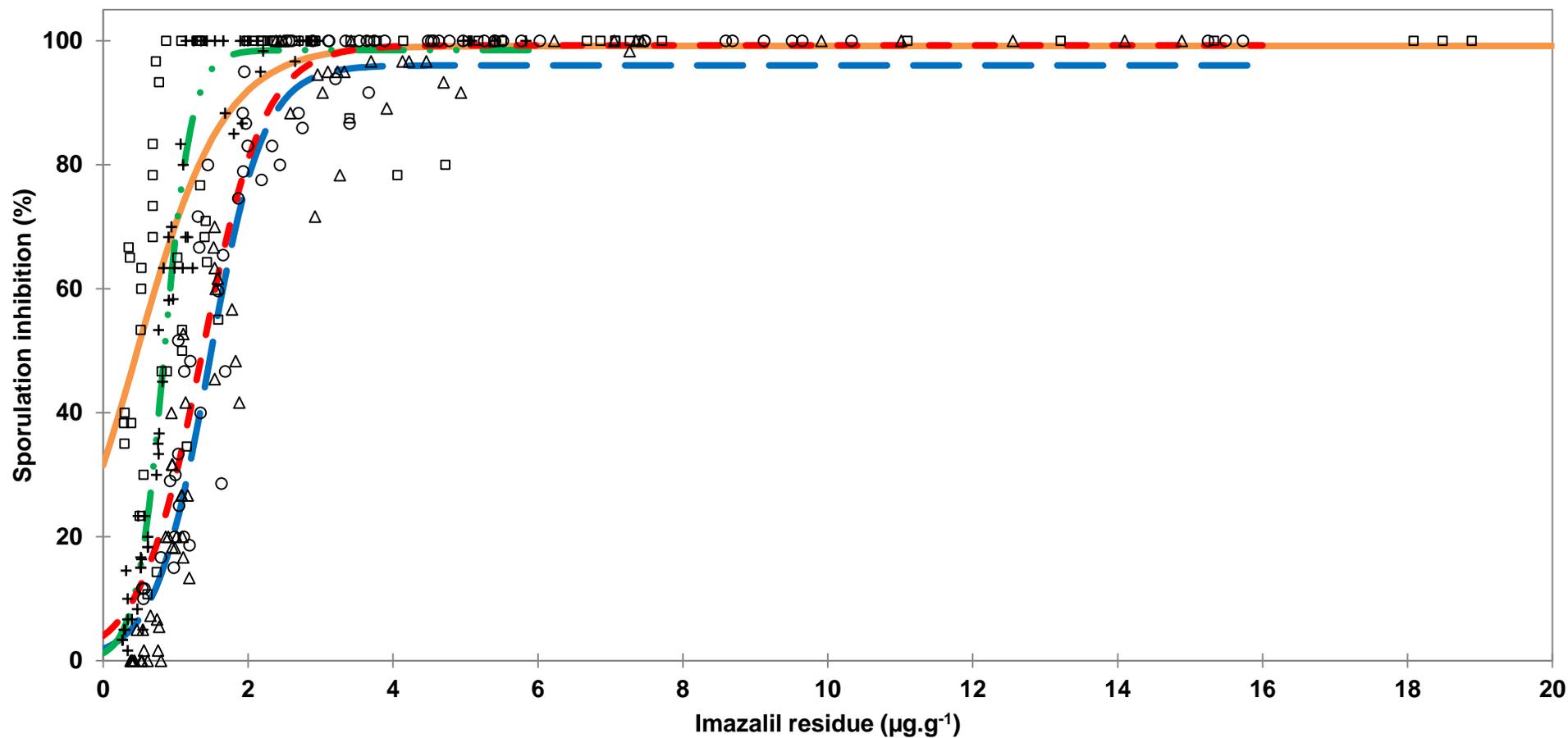


Figure 3. Mean percentage green mould sporulation inhibition on citrus fruit and non-linear regression models [$Y = pr3/(1+Exp(-pr1-pr2*X1))$] indicating sporulation inhibition trends for lemon (\circ , blue long dash line, $R^2 = 0.943$), Satsuma mandarin ($+$, orange solid line, $R^2 = 0.573$), navel (\square , green dash dot line, $R^2 = 0.966$), Valencia (Δ , red short dash line; $R^2 = 0.906$) as influenced by imazalil residue.

CHAPTER 3

The effect of pH, temperature and exposure time in imazalil dip applications on postharvest citrus green mould control and survival of *Rhizopus stolonifer*

Abstract

Citrus green mould (caused by *Penicillium digitatum*) is commonly controlled using imazalil applied by means of dip treatments in a fungicide bath. In South Africa, the fungicide bath is often heated to 35°C, as this has several benefits. However, heated solutions require high energy costs, and the cost of replacing the bath water and fungicides is high. Chemicals are therefore topped up to maintain concentration, and the bath is kept for extended periods of time. However, research has shown that adequate disease control can be obtained with fungicide dip treatments at lower temperatures. The aims of this study were to study the effects of pH, temperature and exposure time in imazalil dip applications on postharvest citrus green mould control, specifically curative control and sporulation inhibition, as well as to study the risk of contaminant build-up, in particular *Rhizopus stolonifer* spores. The ability of imazalil to control green mould was investigated in a cold bath of 10°C and compared to baths at ambient temperature and 35°C. In conjunction with these efficacy trials, the survival of *Rhizopus stolonifer* spores was studied *in vitro* at various water temperatures (10°C to 65°C) for exposure times of 1 and 60 minutes, as well as a simulated 1-hour pasteurization step at 65°C followed by an overnight cool-down period. Sub-treatments included the addition of imazalil fungicide or green mould spores, as would commonly be found in a citrus fungicide bath. The efficacy trials on navel, Valencia and mandarin fruit demonstrated that solution temperature had no significant effect on imazalil's ability to cure 24-h-old green mould infections with all temperatures providing control > 84% on oranges, and good but variable control on mandarin (52-76%). However, IMZ residue loading and sporulation inhibition increased as solution pH, temperature, and exposure time increased. Whilst sporulation inhibition was < 50% in pH 3 baths, irrespective of temperature and exposure time, complete inhibition was obtained at 35°C and pH 6, but maximum residue limits for IMZ were exceeded at longer exposure times (> 45 s). Solution temperature had no significant effect on *Rhizopus* spore survival at temperatures below 35°C, but temperatures of 45, 55 and 65°C, particularly after a 60-minute exposure, caused a significant reduction in *Rhizopus* spore viability (> 90%). Complete *Rhizopus* spore control was achieved following the 65°C pasteurization step.

Introduction

Citrus is an economically important crop in South Africa and a large volume of the produce is exported (DAFF, 2014; Edmonds, 2016; PPECB, 2016). The importance of maintaining fruit quality during shipping is crucial (Roth, 1967; Eckert and Eaks, 1989). The largest decay threat to fruit quality is green mould, a fungal disease caused by *Penicillium digitatum* Pers. Sacc. (Fawcett, 1927; Eckert and Eaks, 1989), an obligate wound pathogen (Kavanagh and Wood, 1967; Eckert and Eaks, 1989). Once infection occurs, the damaged tissue becomes soft and a water soaked lesion appears within 5 to 7 days, followed by white mycelial growth outwards from the infection point. Matured infection results in distinctive olive green sporulation 10 – 14 days later (Eckert and Brown, 1986; Eckert and Eaks, 1989).

Imazalil (IMZ; 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H imidazole) (Siegel *et al.*, 1977; FAO, 2001) is the most effective fungicide against green mould and used globally in the citrus industry (Smilanick *et al.*, 1997b; Erasmus *et al.*, 2011; Besil *et al.*, 2016). Imazalil has action against both infections and spore formation (Erasmus *et al.*, 2011). Additionally, the active's anti-sporulation properties are of high value as it decreases the problem of soilage (McCornack and Brown, 1977). Soilage occurs when green mould spores rubs onto and stick to the surface of an otherwise healthy fruit (Pelser, 1977; Eckert and Kolbezen, 1978; Barmore and Brown, 1982; Eckert and Eaks, 1989). Although the healthy fruit is still marketable it has to be cleaned prior to repacking, and has a high risk of rot if the fruit becomes wounded. The Maximum Residue Limit (MRL) for IMZ on citrus is 5 µg.g⁻¹ for the European Union and 10 µg.g⁻¹ for the USA (DAFF, 2008; AgriIntel, 2015). South African packhouses tend to load much lower levels (≈ 1 µg.g⁻¹) (Erasmus *et al.*, 2011). An IMZ residue

level of between 1 and 3 $\mu\text{g}\cdot\text{g}^{-1}$ is necessary to achieve adequate levels of control (Kaplan and Dave, 1979; Schirra *et al.*, 1996; Smilanick *et al.*, 1997b; Erasmus *et al.*, 2011).

South Africa uses the sulphate form of IMZ in all aqueous applications at a registered concentration of 500 $\mu\text{g}\cdot\text{mL}^{-1}$ (Pelser and La Grange, 1981; Erasmus *et al.*, 2011). The other available formulation, an emulsifiable concentrate (EC), is also used in South Africa, although it is solely applied via the commercial wax coating application (Erasmus *et al.*, 2011; Njombolwana *et al.*, 2013a, b). South African packhouses prefer the sulphate formulations in dip applications because, unlike the EC formulation: it is more stable, needs less agitation and is less likely to precipitate, in which case it then becomes unavailable (Eckert, 1977; Altieri *et al.*, 2005).

Treatment of citrus with IMZ sulphate occurs primarily in the fungicide bath (also known as the dip tank) (Pelser and La Grange, 1981; Erasmus *et al.*, 2011). Due to the individual nature of packhouses in South Africa, IMZ solutions differ widely in terms of concentration (usually 250 - 615 $\mu\text{g}\cdot\text{mL}^{-1}$), temperature (12 - 45°C), solution pH (3 – 8), and exposure time of the fruit (16 – 107 s) in the tank (Erasmus *et al.*, 2011). Despite variations, the fungicide bath is very effective if fruit can be fully submerged during treatment (Eckert, 1977) and offers excellent curative control (Dore *et al.*, 2009; Erasmus *et al.*, 2011, 2013, 2015a). The current recommendation for citrus packinghouses in South Africa is a solution temperature of 35°C, a pH of 3 (where exposure time is negligible) and the concentration maintained at 500 $\mu\text{g}\cdot\text{mL}^{-1}$ (Pelser, 1980; Erasmus *et al.*, 2011, 2013, 2015a; Lesar and Erasmus, 2014). Top-up procedures are in place for the industry so it is generally found that the concentration is satisfactory in most packhouses (Erasmus *et al.*, 2011; Lesar and Erasmus, 2014). In terms of recommendations for the other factors regarding IMZ sulphate solution (temperature, pH and exposure time) there has been a great advance in knowledge in recent years (Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016; Chapter 2).

The temperature recommendation of 35°C comes partly from work done with the EC formulation (Smilanick *et al.*, 1997b, 2003) as well as some other advantages of a heated solution: fruit coming from a warm fungicide bath will dry more quickly and easily in the subsequent drying tunnel (Pelser, 1977; Smilanick *et al.*, 1997a; Erasmus, personal communication, 2014); warm water is believed to be better at replacing air in wounds ensuring more effective fungicide delivery; and finally, that a warm, dry fruit appears to receive a more uniform wax coating than cold or wet fruit (Smilanick *et al.*, 1997a). Temperature is difficult to maintain adequately in the fungicide dip tank as these tanks typically do not have good circulation and colder fruit is constantly entering the solution. This difficulty as well as the high energy cost associated with heated fungicide bath, have led to the need to investigate the influence of temperature in fungicide dip tanks on green mould control. Research to date on the sulphate formulation has looked at temperatures of 25°C and up (Erasmus *et al.*, 2011; Kellerman *et al.*, 2016). This research, as well as work on the EC formulation, has highlighted the benefits of a heated IMZ solution where hot water not only offers some level of control (Erkan *et al.*, 2005; Şen *et al.*, 2010), but that the addition of a fungicide always improves that control (Schirra *et al.*, 1997; Puawongphat *et al.*, 2008). With regards to IMZ, it has been seen that the fungicide enters the citrus rind more easily and maintains a residue for longer when applied in a heated solution (Schirra *et al.*, 1996, 2010; Cabras *et al.*, 1999; Dore *et al.*, 2009).

Apart from temperature, Erasmus *et al.* (2011, 2013, 2015a) and Kellerman *et al.* (2016) have demonstrated that solution pH plays an immense role in residue loading of IMZ sulphate. This is due to the pH sensitive nature of IMZ's solubility in water: since it is a weak base, it is most soluble with a low solution pH (FAO, 2001). Exposure time is also very important when using a fungicide and residues usually increase as exposure time is lengthened (Brown and Dezman, 1990; Cabras *et al.*, 1999; Erasmus *et al.*, 2015a; Kellerman *et al.*, 2016). As with all IMZ research, formulation is important and it has been illustrated that the EC formulation of IMZ is much more affected by exposure time than the sulphate form. Both increased as time was increased however the EC formulation was recorded to load double the residues particularly after the initial 100 s (Sepulveda *et al.*, 2015). The pH is an important factor in this aspect, as the EC and sulphate formulations will once again act similarly at high pH solutions (Erasmus *et al.*, 2011, 2013).

An important concern about cooler fungicide dip tanks is the build-up of bacterium and fungal contaminants in the solution. Moreover, because of the cost involved when replacing both fungicides and water, the fungicide bath solution is often kept for extended period of time (Erasmus *et al.*, 2011) during which contaminants can build up (Eckert, 1977). It is a recommended practice to pasteurize fungicide solutions when fruit is absent in order to remove contaminants that have built up during treatment hours (Mildenhall *et al.*, unpublished; Morris,

1980; Smilanick *et al.*, 1997a), particularly in application methods where the solution is retained for long periods of time.

Rhizopus stolonifer (Fr.) Lind is a contaminant pathogen in South African packhouses (Lesar, 2013) that is very prevalent in fungicide baths (Mildenhall *et al.*, unpublished). The rots caused are soft and infection usually occurs through a wound, often in conjunction with other postharvest diseases. *Rhizopus* will decay an entire fruit within 48 hr under warm and moist conditions and is able to spread to fruit that it has had direct contact with (Lesar, 2013). *R. stolonifer* is a very hardy survivor on any decaying matter, and causes postharvest decay on many crops (Goos *et al.*, 1967; Yuan *et al.*, 1985). Due to its prevalence among citrus and its rapid, destructive nature, it was chosen as a model organism to study contamination in the bath. *R. stolonifer* grows optimally between 20 and 25°C while no growth was observed at 37°C (Yuan *et al.*, 1985). Pasteurization may eliminate the spores in a fungicide bath, and a heated solution may help keeping it under control, though these assumptions have never been investigated thoroughly. Morris (1980) demonstrated that the fungicides in the bath did not deteriorate even after extended periods at a temperature of 70°C.

The research done by Erasmus *et al.* (2011, 2013, 2015a) and Kellerman *et al.* (2016) have very clearly defined the improvement in IMZ residue loading and green mould control gained from a heated bath, as well as the linked effects of temperature to exposure time and solution pH. Despite this, the question remains if the same is true for a cool fungicide bath and if the elements of exposure time and pH play the same synergistic roles. Additionally, many of the presumed benefits of a heated bath have never been adequately studied, particularly that of the reduction or elimination of contaminants. The aim of this research was therefore to clarify the synergistic relationship of lower temperatures and all ancillary variables, as well as the efficacy of pasteurisation in contaminant control.

Materials and methods

Effect of temperature, pH and exposure time in imazalil dip application on control of green mould

Fruit

Export quality, freshly harvested navel orange, Valencia orange and mandarin (cv. Nadorcott) fruit were collected from various packhouses in the northern citrus producing regions of South Africa (Mpumalanga and Limpopo provinces) during the 2015 and 2016 seasons. Five trials were conducted, two each on navel and Valencia, and one on mandarin. Fruit were hand selected for uniformity in size and quality. Upon arrival at the Citrus Research International laboratories in Nelspruit, South Africa, the fruit were washed with a total loss ozone applicator that doses water with ozone at the spray nozzle (ArcAqua patented Ozone applicator; 24 L.min⁻¹ of Ozone at 2 g.h⁻¹ using 8 L.min⁻¹ tap water at 3 bar using four nozzles; ArcAqua (Pty) Ltd., Westlake Business Park, 7945, Cape Town, South Africa). During washing, fruit were pushed through over 6 rotating brushes and into an ambient air drying tunnel. The fruit were stored at 4°C for no more than 4 days before trials were conducted. Fruit were removed from cold storage 1 day before the trials were initiated in order to reach ambient temperature (\approx 22°C) and to evaporate condensation. Trials were conducted simultaneously for the first navel and Valencia fruit, although treated in separate solutions. The second rounds for each of navel and Valencia oranges, and the mandarin fruit, were all treated individually.

Penicillium digitatum isolation and inoculation

For all trials, an imazalil sensitive isolate (STE-U 6560, Department of Plant Pathology, University Stellenbosch, South Africa) of *P. digitatum* was cultured on streptomycin amended potato dextrose agar (PDA+; Difco Potato Dextrose Agar, Becon, Dickinson and Company, Sparks, USA; Steptomycin Sulphate, Ultrapure USBioAnalyzed, 725 μ g.mg⁻¹, USB Corporation, Cleveland, OH USA) at 25°C for 10 – 14 days. Cultures were flooded with sterile water amended with one drop of Tween 20 to a concentration of \approx 0.01 μ L.mL⁻¹ (Sigma-Aldrich, St. Louis, MO, USA). Conidia were dislodged from cultures using a sterilized hockey stick and filtered through a double layer of cheesecloth into 200 mL of the Tween 20 amended sterile water. Concentration of the spore suspension was adjusted to 1×10^6 spores.mL⁻¹ by means of a spectrophotometer (absorbance of 0.100 at 425 nm; Cecil CE1011, Lasec, Midrand, Gauteng, South Africa) (Morris and Nicholls, 1978; Eckert and Brown, 1986). Spores were maintained in a uniform suspension by use of magnetic stirrers throughout inoculation.

Two methods of inoculation were utilised during trials. In order to test the curative ability of imazalil against infections, a custom made wounding tool was used. This tool had a flattened cylindrical tip that mimicked the cut stem of a citrus fruit and delivered a wound 1 mm in diameter and 2 mm deep through the flavedo into the

albedo of the fruit. Wounds were made on the shoulder area around the stem end of each fruit, in a square pattern with a distance of approximately 4 cm between each. There were 12 fruit in each replicate with 3 replicates per treatment during a trial. Curative inoculations were done 24 hours before treatment. The second method of inoculation was to test the sporulation inhibition of imazalil. A sterile 0.60 x 25 mm gauge needle (NN*2325R, Terumo corporation, Tokyo, Japan) was used to inject 0.2 mL of spore suspension between 1 and 2 cm deep into the shoulder on stem end of a fruit. Only one point of entry was made per fruit. There were 12 fruit in each replicate with 3 replicates per treatment during a trial. Sporulation inhibition inoculations were done approximately 30 min before treatment.

Residue analysis

Fruit samples for residue analysis consisted of two replicates of six uninoculated fruit added to the first and last replicate of each treatment. After treatment, they were stored in plastic bags at 4°C for no more than a week before being chopped and blended (2.9 L Robot Coupe R2 Bowl Cutter Mixers, Bonanza Shop and Catering Equipment, Nelspruit, South Africa) into pulp using distilled water until the pulp was reduced to a pulp-like consistency. The dilution factor for each sample was recorded and the pulp was frozen into sub-samples that were couriered to an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Cape Town, South Africa) for IMZ (chloramizol) residue analyses. Acetonitrile was used for extraction which was followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography tandem mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA) according to an accredited procedure. Residue data received from the analytical laboratory were recalculated using the dilution factor recorded when the pulp was blended.

Treatment and incubation

The five trials conducted all had the same parameters unless stated otherwise. For each trial, six water baths containing 500 µg.mL⁻¹ imazalil sulphate (Imzacure, 750 g.kg⁻¹ SG, ICA International Chemicals, Stellenbosch, South Africa) were prepared. Three temperatures were investigated, namely 10°C, ambient (≈ 19 - 22°C) and 35°C. For each temperature, there were two baths, one containing a solution at pH 3 and the other at pH 6. The pH of the solution was measured with a portable pH meter (HI 98121 Waterproof pH/ORP & Temperature meter, Hanna Instruments, Morninghill, Johannesburg, South Africa) and was decreased using hydrochloric acid (32%; Merck (Pty.) Ltd., Modderfontein, Gauteng, South Africa) or increased using sodium hydrogen carbonate (sodium bicarbonate; NaHCO₃; Saarchem uniLAB, Merck Chemicals (Pty) Ltd., Wadeville, Gauteng, South Africa). Fruit were dipped for each treatment for 15 s, 45 s, 90 s or 180 s. In each case there were 3 replicates of each inoculation method. Uninoculated fruit were treated simultaneously for residue analysis.

After the fungicide treatments, the fruit followed a simulated commercial packline set up by moving over 14 rotating brushes passing under an airknife (±100 nozzels supplying a stream of forced air onto the fruit to remove moisture) and then moving through an unheated forced air drying tunnel. No commercial wax coating was added to the fruit at any point. The dry fruit were packaged stem end facing upward on SFT13 nectarine trays (Huhta-maki South Africa (Pty) Ltd., Atlantis, South Africa) placed in lock-back stonefruit cartons (115 mm; Mpact, Epping, South Africa). The cartons were then covered in clear polyethylene bags (50 µm, Lanpack Manufacturers C.C., Woodstock, Cape Town, South Africa) with the end folded under the carton but not sealed tightly. Four to six small holes (≈ 5 mm in diameter) were made to reduce humidity and moisture build-up. The cartons were stacked and incubated at ambient temperature (≈ 22°C) for 4 days before evaluating the curative and protective inoculations and 10 days for sporulation inoculations.

In the case of the first trials were conducted on navel and Valencia (described as 'delayed brushing'). The fruit were dipped in the solution, whereafter they remained in the crate for approximately 10 minutes before progressing through the rest of the packline. i.e. these fruit had a delayed brushing. In the case of the second trials on navel and Valencia (described as 'immediate brushing'), as well as that on the mandarin, the trial had been optimised so that fruit followed the full packline sequence without any delays. i.e. the fruit were subjected to immediate brushing.

Treatment evaluation

Evaluations for curative treatments were done by counting infected wounds out of four on each fruit. Early infection was visualised using a near-UV light (UV-A at 365 nm, Labino Mid-light, www.labino.com) which

caused infected wounds to fluoresce bright yellow (Njombolwana *et al.*, 2013a). Sporulation fruit were evaluated for each fruit using a rating index of 1 – 6, where fruit that showed no sign of disease was taken as missing data points: 1 = complete sporulation inhibition i.e. completely white fruit; 2 = sporulating area was small ($\approx 20\%$), 3 = sporulating area larger than a quarter of the fruit, but smaller than half of the fruit ($\approx 40\%$), 4 = sporulating area larger than half of the fruit, but smaller than three quarters of the fruit ($\approx 60\%$); 5 = sporulating area larger than three quarters of the fruit, but smaller than the whole fruit ($\approx 80\%$); and 6 = sporulating area covering the whole fruit (= 100%). Fruit that showed no sign of infection were regarded as missing data points.

Statistical analysis

For curative infection data (percentage wounds infected per fruit) and sporulation inhibition percentage data, were normalised relative to the data for untreated control treatments; percentage control data were subsequently used. Data were subjected to analyses of variance (ANOVA) and Fisher's least significant difference test at 95% confidence interval to compare means. To demonstrate trends between residues loaded from treatments, or the levels of control achieved, values were regressed using an appropriate non-linear regression model. The coefficient of determination (R^2) was used to demonstrate goodness of fit. Where possible, the model was used to predict either residue results or percentage control that would be achieved through treatment. XLSTAT (version 2016.7.01, Addinsoft, www.xlstat.com) was used for the analysis described. Regression models with reliable fits were used to determine residue levels that would be indicative of 50% or 90% control (Erasmus *et al.*, 2015b).

***In vitro* effect of solution temperature on *Rhizopus* spores in the fungicide bath**

Treatment

A *Rhizopus stolonifer* isolate was obtained from an infected citrus fruit and identified based on its morphological characteristics (Yuan *et al.*, 1985; Lesar, 2013). It was plated onto Rose Bengal (Rose Bengal Chloramphenicol Agar, Neogen, Michigan, USA) and PDA+ media to observe its growth. After initial assessment of the media, Rose Bengal media, offering slightly retarded growth, was used for all the cultures. *Rhizopus* spore suspension was created by flooding the lid of a petri dish with $0.01 \mu\text{L}\cdot\text{mL}^{-1}$ Tween 20 (Sigma-Aldrich, St. Louis, MO, USA) amended water. A scalpel was used to gently remove the sporangia of the *R. stolonifer* culture from the mycelia on the pink coloured Rose Bengal media. The spore mass was placed in the water and gently agitated to release the sporangiospores. The suspension was prepared as described for the *P. digitatum* above using the spectrophotometer. The solution was then diluted through a dilution series to 10^{-3} spores. mL^{-1} . Based on a previous investigation evaluating dilutions from 10^0 to 10^{-20} , the 10^{-3} spores. mL^{-1} dilution factor was determined to be the best for colony counts and used in all following trials. The trials were repeated three times. In each case, 15 mL of *R. stolonifer* spore suspension was dispensed into test tubes as follows: *R. stolonifer* spore suspension only; *R. stolonifer* spore suspension and a 10^3 spores. mL^{-1} green mould spore suspension; *R. stolonifer* spore suspension and $500 \mu\text{g}\cdot\text{mL}^{-1}$ imazalil sulphate. Each of these suspensions in test tubes was held in water baths for 1 minute and then 60 minutes at temperatures of 10, 15, 20, 25, 35, 45, 55, and 65°C . The suspensions treated at 65°C for 1 hour were kept in the bath overnight as the bath cooled down (± 12 hr); this simulated a pasteurization step as would occur in a packhouse. After each time period, 300 μL was plated out onto Rose Bengal media. There were five replicates for each treatment.

Statistical analysis

Rhizopus stolonifer data consisted of counts of the colony forming units (CFU). The data were normalised relative to the untreated control counts and percentage control data were subsequently used. Data were subjected to analyses of variance (ANOVA) and Fisher's least significant difference test at 95% confidence interval to compare means. To demonstrate trends between the control achieved at the treatment temperatures, where possible, values were regressed using an appropriate non-linear regression model. The coefficient of determination (R^2) was used to demonstrate goodness of fit. XLSTAT (version 2016.7.01, Addinsoft, www.xlstat.com) was used for the analysis described.

Results

Effect of temperature, pH and exposure time in imazalil dip application on control of green mould

Imazalil residue loading

Analysis of variance showed that there was a significant citrus type interaction ($P < 0.0001$) when all the citrus types were included; this interaction was ascribed to the delayed brushing in the first trials, and the data were analysed separately. No type interaction was observed for the delayed brushing trials with navel and Valencia oranges ($P = 0.989$). A significant interaction between temperature x pH x exposure time ($P < 0.0001$) was observed. The second set trials with navel and Valencia fruit (immediate brushing) also demonstrated no citrus type interaction between the batches ($P = 0.743$), as well as a significant interaction between temperature x pH x exposure time ($P = 0.005$; Table 1). The mandarin trial was analysed separately.

Navel and Valencia

At pH 3, there was no significant differences between fruit treated at any of the temperatures (10°C, ambient or 35°C) and for any period of time (15 – 180 s) in the experiment. A range of 0.76 – 1.36 $\mu\text{g}\cdot\text{g}^{-1}$ for the delayed brushing and 0.57 – 0.96 for the immediate brushing trial were determined (Table 2), although a trend toward higher residue levels at higher temperatures could be observed. At pH 6, residue levels were significantly higher as temperature and exposure time increased, except for 10°C where the effect of exposure time was not significant, and residue levels were not significantly different from the pH 3 treatments in most cases. Residue levels in the delayed brushing trial were generally higher than those in the immediate brushing trial, especially at higher pH and temperatures and longer exposure times. In fact, residue levels on fruit in the delayed brushing trial at pH 6, 35°C and exposure times 45, 90 and 180 s exceeded the MRL.

Mandarin

Analysis of variance of the residue data from mandarin fruit showed that there was a temperature x pH x exposure time interaction ($P < 0.0001$; Table 3). Similar to the oranges, residue levels following treatment at pH 3 did not often differ significantly and ranged from 0.38 – 0.66 $\mu\text{g}\cdot\text{g}^{-1}$, with trends indicating marginally higher residues loading at higher temperatures and longer exposure times (Table 4). At pH 6, residue levels were generally significantly higher than those measured following pH 3 treatments, and ranged from 0.48 – 4.74 $\mu\text{g}\cdot\text{g}^{-1}$. Residue levels increased significantly as temperature and exposure time increased. Residue levels were generally comparable to those of the immediate brushing navel and Valencia trial. Residue analysis also unexpectedly revealed that the mandarin fruit used in this trial had been drenched prior to collection for the trial. Average drench fungicides present were 2,4-D (0.01 $\mu\text{g}\cdot\text{g}^{-1}$), pyrimethanil (1.25 $\mu\text{g}\cdot\text{g}^{-1}$), and thiabendazole (0.13 $\mu\text{g}\cdot\text{g}^{-1}$). Importantly, these residue levels did not appear to be affected by the IMZ treatments in any way (results not shown).

Green mould control

Curative control

Analysis of variance of the combined data set showed significant interactions with citrus type (and batch), and these were subsequently analysed separately. ANOVA for data from navel (delayed brushing) indicated a significant effect for temperature only ($P = 0.004$); for Valencia (delayed brushing) and for navel (immediate brushing) exposure time was significant ($P = 0.009$ and 0.004 , respectively); for Valencia (immediate brushing) a significant interaction for temperature x pH x exposure time was observed ($P = 0.0026$); and for mandarin a significant interaction for temperature x pH was observed ($P = 0.034$; Table 5). Despite these significant effects, average control levels were > 99% for navel and Valencia in the delayed brushing trial, and > 97 and > 84% respectively for navel and Valencia in the immediate brushing trial (results not shown). Mandarin control was variable across treatments and generally lower (52 - 76%) with only the lowest value (52% at pH 3 at 35°C) differing significantly from other treatments (results not shown).

Sporulation inhibition

Analysis of variance of the combined data set showed significant interactions with citrus type (and batch), which was ascribed to differences between the delayed and immediate brushing and mandarin trials. These were therefore analysed separately.

Navel and Valencia

Analysis of variance for data from the two trials using navel and Valencia fruit showed that there was a significant temperature x pH x exposure time interaction ($P < 0.0001$ and < 0.0001 , respectively; Table 5). Sporulation inhibition levels varied significantly, but generally increased as temperature, pH or exposure time increased (Table 6). Higher levels of sporulation inhibition were generally observed in the delayed brushing trial when compared to the immediate brushing trial; in the latter, 100% sporulation inhibition was only at the maximum of all parameters, whereas fruit in the delayed brushing trial exhibited 100% sporulation inhibition for exposure times at 35°C and with a solution pH of 6 (Table 6). Non-linear regression for sporulation inhibition data against IMZ residue data using a Three Parametric Logistic model with the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ gave good fits at an R^2 value of 0.682 for navel and 0.693 for Valencia. The model predicted that IMZ residue levels between 1.91 – 2.26 $\mu\text{g.g}^{-1}$ were required to achieve 90% sporulation inhibition, while between 1.06 and 1.24 $\mu\text{g.g}^{-1}$ were predicted necessary for 50% inhibition (Table 7). Sporulation inhibition increased steeply relative to the residue level, with 100% inhibition possible below the MRL (Fig. 1).

Mandarin

Analysis of variance of sporulation inhibition data from mandarin showed a significant temperature x pH x exposure time interaction ($P < 0.0001$; Table 5). Treatments at pH 3 did not differ significantly, but sporulation inhibition levels were very low (0.1 – 3.1%; Table 6). At pH 6, sporulation inhibition levels ranged from 3.2 to 79.5%, and significantly increased as temperature and exposure time increased (Table 6). Non-linear regression for sporulation inhibition data against IMZ residue data using the model described previously gave very good fits at an R^2 value of 0.919. The highest level of sporulation inhibition predicted was 77% at a residue of 2.40 $\mu\text{g.g}^{-1}$. For 50% sporulation inhibition, an IMZ residue level of 1.06 $\mu\text{g.g}^{-1}$ was predicted (Table 7; Fig. 1).

***In vitro* effect of solution temperature on *Rhizopus* spores in the fungicide bath**

Analysis of variance of percentage control data indicated significant trial effects (results not shown), but since all treatments were conducted identically, the trials were regarded as a statistical block effect to increase the robustness of the analysis.

The pasteurization step at 65°C resulted in 100% control (except for one replicate with 99.7% control), regardless of treatment (results not shown).

Analysis of variance of percentage control data obtained using the solution containing *Rhizopus* spores indicated a significant temperature x exposure time interaction ($P < 0.0001$; Table 8). The data allowed for non-linear regression using the model described above, which gave good fits with R^2 values of 0.767 and 0.656 for 1 and 60 minutes respectively (Table 9). The model predicted greater and faster control from a 60 minute exposure time than a 1 minute exposure time. Means indicated that 100% control was achieved at temperatures $> 45^\circ\text{C}$ for 60 minutes but the model predicted that only at 65°C would 100% control be seen at either exposure time (Fig. 2).

Analysis of variance of percentage control data obtained using the solution containing *Rhizopus* spores and *P. digitatum* spores indicated a significant temperature x exposure time interaction ($P < 0.0001$; Table 8). Non-linear regression gave poor fits for the data ($R^2 < 0.400$) and regression models were not presented. Treatments for a minute, ranging from 10 to 45°C showed minimal *Rhizopus* spore control with percentages between 9 and 31%. The trend was not clear between these temperatures, but at 55°C the control increased to 59% and 100% of the spores were controlled at 65°C. Similar to the *Rhizopus* solution alone, the 60-minute exposure time provided significantly higher levels of control in most cases. Finally, 93% control was achieved at 45°C and 100% at both 55 and 65°C (Table 10).

Analysis of variance of percentage control data obtained using the solution containing *Rhizopus* spores and IMZ fungicide indicated a significant temperature x exposure time interaction ($P = 0.0038$; Table 8). Non-linear regression gave poor fits for the data ($R^2 < 0.4$) and regression models were not presented. Control levels

were generally markedly higher (> 77%) than observed for other suspensions, especially at cooler temperatures. Complete control was achieved at 65°C for 1 minute and at 45, 55, and 65°C for 60 minutes (Table 10).

Discussion

Previous studies highlighted the importance and effect of solution temperature in conjunction with solution pH and exposure time on IMZ residue loading and subsequent green mould control (Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016; Chapter 2). Findings from this study confirm results from these studies, and contribute to our understanding of IMZ residue loading, green mould control and particularly sporulation inhibition following dip-treatment in cooler IMZ solutions. Additionally, my study demonstrated the pasteurisation effects of heated solutions and highlights the risk of contaminant build-up in cooler solutions.

Imazalil residues levels generally increased as either pH, temperature, or exposure time increased. At pH 3 solutions, neither the exposure time of the fruit to the solution, nor the temperature of the solution had any significant effect on IMZ residue loading. These results are a confirmation of work by Erasmus *et al.* (2011, 2013, 2015a) and Kellerman *et al.* (2016) who have shown that a pH 3 solution confer very stable loading with relatively low levels of IMZ that are below the MRL of 5 µg.g⁻¹. In their studies, they demonstrated that solutions with a pH of 6 or higher, increased residues to levels above the MRL with exposure times longer than 45 s in warm baths (*circa* 35°C, as recommended for the South African citrus industry). The same was noticed with our study but an interesting difference was that at the cooler temperatures, particularly of 10°C, pH 6 acted very similarly to the pH 3 treatments, and demonstrated lower and more stable residue loading trends.

The effect of brushing on IMZ sulphate residues on citrus has only recently been demonstrated by Erasmus *et al.* (2015a). Brushing of citrus is standard procedure in all citrus packhouses following IMZ aqueous application. In commercial packhouses, anything from 8 to 52 brushes can be operational post IMZ treatment (Erasmus *et al.*, 2011). In this study, fruit were always brushed, but the time between dip-treatment and brushing differed. The effect of this can be seen with the residues loaded where fruit subjected to delayed brushing loaded higher residues than fruit exposed to immediate brushing. Navel and Valencia oranges loaded similar residues as was also seen in Erasmus *et al.* (2011, 2013) but not in Erasmus *et al.* (2015a) or in Chapter 2. Mandarin fruit loaded lower residues but followed the same trends as the oranges in the immediate brushing trial, as this fruit kind was also immediately brushed. The implications of the brushing effect on residues were highlighted at 35°C as residues in the delayed brushing trial exceeded the MRL of 5 µg.g⁻¹, while fruit with immediate brushing, treated identically otherwise, loaded about 70% less residue (maximum of 3.13 µg.g⁻¹ for delayed brushing compared to a maximum of 11.56 µg.g⁻¹ for immediate brushing). The reduction in residues is likely because of the removal of the excess solution carrying the active, removing the lipophilic IMZ before it has time to adhere and permeate the rind. At similar treatment conditions, pH 6 solutions loaded higher residues. This is due to the lipophilic nature of undissociated IMZ, where this compound then binds more strongly to oily constituents in the citrus rind (Siegel *et al.*, 1977; Brown and Dezman, 1990).

The effect of increased residue loading with increasing exposure time and higher temperature is apparent both here and in previous studies (Brown and Dezman, 1990; Schirra *et al.*, 1996, 2010; Smilanick *et al.*, 1997b; Cabras *et al.*, 1999; Puawongphat *et al.*, 2008; Sepulveda *et al.*, 2015) with the effect of the conjunction of a high solution pH even more so (Erasmus *et al.*, 2015a; Kellerman *et al.*, 2016; Chapter 2).

Delayed or immediate brushing did not seem to have an effect on curative control, as no clear differences were noted between fruit with different brushing treatments, and overall high levels (> 84%) of curative control were achieved regardless of citrus type or treatment parameters. Fairly low residue levels (≈ 0.5 µg.g⁻¹) will still exert curative control if treatment is applied timeously. The observation is explained by loading of residues in wound tissue, where higher residue levels were loaded in wounds than surrounding unwounded tissue (Dore *et al.*, 2009, 2010; Erasmus *et al.*, 2015a). Whole fruit residue levels are therefore poor predictors of curative control. However, sporulation inhibition was clearly linked to residue levels as was also observed previously (Erasmus *et al.*, 2011, 2013, 2015a; Kellerman *et al.*, 2016; Chapter 2). From the analysis of the results it was seen that overall an increase in sporulation inhibition was achieved with an increase in treatment parameters and this was linked to the residues loaded onto the fruit. This relationship is explained by a logistic curve, with a residue of around 2.0 µg.g⁻¹ required to achieve 90% sporulation inhibition, and a residue of approximately 1.0 µg.g⁻¹, will achieve 50% sporulation inhibition, and still be sufficient for good curative control,. These findings correlate strongly with those of Erasmus *et al.* (2011, 2013, 2015a) and Kellerman *et al.* (2016).

Compared to the findings in Chapter 2 using the heated flooder, Valencia residues were very similar, needing just above 2.0 and 1.0 $\mu\text{g}\cdot\text{g}^{-1}$ for 90 and 50% sporulation inhibition, respectively. Navel fruit indicated that lower residue levels were necessary in the heated flooder application with 1.29 and 0.85 $\mu\text{g}\cdot\text{g}^{-1}$ for the respective control. This difference might be attributed to batch effects, and direct comparisons will have to be made to elucidate whether it can be ascribed to superior IMZ application in the heated flooder and/or a synergistic effect of temperature.

Drench chemicals such as thiabendazole and pyrimethanil convey some anti-sporulation activity, but markedly less than IMZ, where the sporulation inhibition is patchy, comprised of several smaller zones of inhibition (Kellerman *et al.*, 2014; Christie, 2016). Residue analysis revealed that the mandarin fruit had been drenched before collection. These residues must have exerted some protective control against the curative inoculation prior to treatment as the decay levels in the untreated control fruit decay were lower (average 81%) than near 100%, as is commonly expected following artificial inoculations. Despite the lowered decay influencing this efficacy trial negatively, the putative 20% control conferred from drench chemicals indicates how important the IMZ application in the packhouse is for effective green mould management (Smilanick *et al.*, 2006; Kellerman *et al.*, 2014).

In this study, it was observed that the control of *Rhizopus* spores were possible in solutions > 45°C given sufficient exposure time (1 hour). For a shorter exposure time, higher temperatures are required, pointing to the importance of carefully considered sanitation protocols. Packhouse sanitation and careful handling of the fruit, as well as maintaining the cold chain are the best ways to prevent *R. stolonifer* infections (Lesar, 2013). Heat (> 35°C) has been demonstrated to be detrimental to *Rhizopus* (Miller *et al.*, 1959; Baker and Smith, 1970; Margosan *et al.*, 1997), and pasteurization of a fungicide bath is a useful step to decrease contamination in the fungicide bath. The addition of IMZ drastically decreased the presence of *Rhizopus* spores. This study demonstrated that complete eradication of *Rhizopus* spores occurred at 55 and 65°C for the hour long exposure time. If exposure time is short (one minute), only a temperature of 65°C, with either green mould spores or IMZ present in the solution, will offer 100% control. It is important to note that sensitive citrus types such as lemon could experience heat damage at these high temperatures, therefore a good alternative is to pasteurize the bath each night after packing. The results of this study showed 100% control at 65°C for the pasteurization step.

Residue levels and the subsequent sporulation inhibition of green mould can be optimized by increasing the pH, temperature or exposure time of an IMZ bath solution. Care should be taken to not exceed the MRL, particularly with longer exposure times in pH 6 solutions; however, it was apparent from this study that if sufficient and timely brushing is applied, infection control is not compromised yet residues remain below the MRL. It is known that the fungicide bath offers excellent curative control (Dore *et al.*, 2009; Erasmus *et al.*, 2011, 2015a; Kellerman *et al.*, 2016), and this was confirmed for cold solutions.

A cold temperature bath could be considered a viable treatment option; however, residues and other benefits may be compromised in this case, particularly sporulation inhibition as a result of reduced residue loading. Heating the bath to more than 45°C may be necessary to achieve a greater level of benefits such as the eradication of *Rhizopus* spores. Additionally, hot water treatments may have other benefits such as a reduction of chilling injury (McDonald *et al.*, 1991; Schirra *et al.*, 2000; Erkan *et al.*, 2005), increasing lignin accumulation around wound sites to help prevent green mould development, or an increase in phytoalexin concentrations that aid in *P. digitatum* inhibition (Nafussi *et al.*, 2001).

Further benefits are that warm fruit assists in drying off excess water before the wax application, which in turn leads to a more uniform wax coating (Pelser, 1977; Smilanick *et al.*, 1997a; Erasmus, personal communication, 2014). For these reasons, it is not recommended to treat fruit in a cold fungicide bath. Brushing after aqueous treatments in a packhouse is crucial to reduce the risk of exceeding the MRL. However, brushing also needs to be considered in postharvest research where misleading residue results and subsequent observations in residue loading and disease control could be skewed due to the lack of brushing in experimental designs. A final recommendation from this study is that, in order to control fungal contaminants in the fungicide bath, packhouses need apply imazalil in heated solutions (*circa* 45°C) and/or pasteurize fungicide baths overnight.

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Tables and figures

Table 1. Analysis of variance for imazalil residue data as analysed from navel and Valencia fruit (delayed and immediate brushing) after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

Source	Navel & Valencia (delayed brushing)			Navel & Valencia (immediate brushing)		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	47	14.762	<0.0001	47	0.961	<0.0001
Temperature	2	108.737	<0.0001	2	5.635	<0.0001
pH	1	138.705	<0.0001	1	18.708	<0.0001
Exposure time	3	10.254	<0.0001	3	0.878	<0.0001
Fruit kind	1	1.814	0.016	1	0.382	0.013
Temperature*pH	2	91.483	<0.0001	2	2.903	<0.0001
Temperature*Exposure time	6	7.934	<0.0001	6	0.167	0.016
Temperature*Fruit kind	2	0.396	0.269	2	0.227	0.025
pH*Exposure time	3	7.936	<0.0001	3	0.792	<0.0001
pH*Fruit kind	1	0.862	0.093	1	0.028	0.488
Exposure time*Fruit kind	3	0.100	0.796	3	0.014	0.860
Temperature*pH*Exp.time	6	7.550	<0.0001	6	0.328	0.000
Temperature*pH*Fruit kind	2	0.556	0.161	2	0.029	0.604
Temp.*Exp.time*Fruit kind	6	0.203	0.657	6	0.009	0.986
pH*Exposure time*Fruit kind	3	0.279	0.424	3	0.026	0.710
Temp.*pH*Exp.time*Fruit kind	6	0.043	0.989	6	0.053	0.480
Error	48	0.293		48	0.057	
Corrected Total	95			95		

^a DF = Degrees of freedom

^b MS = Mean sum of squares

^c P = Probability

Table 2. Mean imazalil residue data as analysed from navel and Valencia fruit (delayed and immediate brushing) and mandarin fruit after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

pH	Temp. (°C)	Imazalil residues (µg.g ⁻¹) ^a							
		Exposure time							
		15 s	45 s	90 s	180 s				
Navel & Valencia (delayed brushing)									
3	10	0.76	h	0.97	efgh	0.77	gh	0.87	gh
	Ambient	0.88	fgh	1.03	efgh	1.17	efgh	1.13	efgh
	35	1.10	efgh	1.16	efgh	1.19	efgh	1.36	efgh
6	10	1.09	efgh	1.21	efgh	1.24	efgh	1.33	efgh
	Ambient	1.47	efgh	1.54	efg	1.68	e	1.65	ef
	35	3.57	d	5.91	c	9.00	b	11.56	a
Navel & Valencia (immediate brushing)									
3	10	0.57	j	0.61	j	0.57	j	0.68	hij
	Ambient	0.58	j	0.62	ij	0.68	hij	0.72	hij
	35	0.96	gh	0.73	hij	0.88	ghij	0.75	hij
6	10	0.89	ghij	0.85	ghij	0.95	ghi	1.17	efg
	Ambient	1.09	fg	1.35	ef	1.44	de	1.72	d
	35	1.40	def	2.22	c	2.75	b	3.13	a
Mandarin									
3	10	0.38	kl	0.43	jkl	0.49	hijkl	0.48	hijkl
	Ambient	0.37	l	0.43	jkl	0.45	jkl	0.51	hijkl
	35	0.49	hijkl	0.46	ijkl	0.66	ghij	0.61	ghijk
6	10	0.48	hijkl	0.70	ghi	0.62	ghijkl	0.75	efg
	Ambient	0.58	e	0.72	fgh	0.94	ef	0.99	e
	35	1.54	d	2.39	c	2.99	b	4.74	a

^a For delayed and immediate brushing and mandarins separately, means followed by the same letter do not differ significantly ($P > 0.05$; LSD = 0.770 and 0.339, 0.238 respectively)

Table 3. Analysis of variance for imazalil residue data as analysed from mandarin fruit (immediate brushing) after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

Source	Mandarin		
	DF ^a	MS ^b	P ^c
Model	23	2.110	<0.0001
Temperature	2	7.124	<0.0001
pH	1	11.337	<0.0001
Exposure time	3	1.072	<0.0001
Temperature*pH	2	5.789	<0.0001
Temperature*Exposure time	6	0.508	<0.0001
pH*Exposure time	3	0.715	<0.0001
Temperature*pH*Exposure time	6	0.494	<0.0001
Error	24	0.013	
Corrected Total	47		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

Table 4. Analysis of variance for curative infection control data as analysed from navel and Valencia fruit (delayed and immediate brushing) and mandarin fruit after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

Source	Navel (delayed brushing)			Valencia (delayed brushing)			Navel (immediate brushing)			Valencia (immediate brushing)			Mandarin		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	23	14.971	0.059	23	8.950	0.187	23	52.994	0.006	23	885.976	<0.0001	23	4830.265	<0.0001
Temp.	2	54.885	0.004	2	13.724	0.146	2	277.470	<0.0001	2	5478.615	<0.0001	2	14574.652	<0.0001
pH	1	26.346	0.103	1	2.973	0.518	1	31.032	0.290	1	997.746	0.013	1	13507.013	0.001
Exp. time	3	4.824	0.691	3	27.879	0.009	3	123.735	0.004	3	522.801	0.022	3	15519.612	<0.0001
Temp.*pH	2	8.740	0.414	2	5.084	0.490	2	6.150	0.801	2	66.111	0.665	2	4208.101	0.034
Temp.*Exp. time	6	1.915	0.979	6	7.043	0.431	6	18.085	0.688	6	351.552	0.043	6	1270.608	0.405
pH*Exp. time	3	20.065	0.109	3	0.991	0.936	3	12.472	0.717	3	766.000	0.003	3	550.891	0.720
Temp.*pH*Exp. time	6	17.079	0.112	6	6.070	0.529	6	15.349	0.767	6	388.404	0.026	6	667.984	0.777
Error	834	9.908		839	7.118		830	27.707		837	161.676		838	1234.225	
Corrected Total	857			862			853			860			861		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

Table 5. Analysis of variance for green mould sporulation inhibition data as analysed from navel and Valencia fruit (delayed and immediate brushing) and mandarin fruit after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

Source	Navel & Valencia (delayed brushing)			Navel & Valencia (delayed brushing)			Mandarin		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	23	64178.755	<0.0001	23	53681.888	<0.0001	23	30446.225	<0.0001
Temperature	2	144397.890	<0.0001	2	129500.943	<0.0001	2	88017.518	<0.0001
pH	1	1129038.928	<0.0001	1	810263.301	<0.0001	1	301624.971	<0.0001
Exposure time	3	2300.934	<0.0001	3	8443.770	<0.0001	3	6713.469	<0.0001
Temperature*pH	2	15877.533	<0.0001	2	47085.884	<0.0001	2	82695.719	<0.0001
Temperature*Exposure time	6	509.146	0.018	6	1808.749	<0.0001	6	1609.802	<0.0001
pH*Exposure time	3	925.982	0.003	3	6571.842	<0.0001	3	5241.858	<0.0001
Temperature*pH*Exposure time	6	939.800	<0.0001	6	2280.992	<0.0001	6	1597.466	<0.0001
Error	1677	198.946		1700	349.009		835	72.661	
Corrected Total	1700			1723			858		

^a DF = Degrees of freedom

^b MS = Mean sum of squares

^c P = Probability

Table 6. Mean green mould sporulation inhibition data as analysed from navel and Valencia fruit (delayed and immediate brushing) and mandarin fruit after dip application in imazalil solutions of 500 µg.mL⁻¹ IMZ at temperatures of 10, ambient (≈ 19 - 22) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

pH	Temp. (°C)	Sporulation inhibition (%) ^a							
		Exposure time							
		15 s	45 s	90 s	180 s				
Navel & Valencia (delayed brushing)									
3	10	26.67	hi	24.06	hij	28.33	h	23.61	ij
	Ambient	16.94	kl	20.83	jk	16.11	l	22.00	ij
	35	50.56	g	56.00	f	53.14	fg	50.06	f
6	10	67.25	e	68.41	e	66.29	e	80.00	cd
	Ambient	78.87	d	84.17	c	78.59	d	89.14	b
	35	100.0	a	100.0	a	100.0	a	100.0	a
Navel & Valencia (immediate brushing)									
3	10	18.06	l	24.23	jk	24.17	jk	16.62	l
	Ambient	21.94	jkl	38.89	gh	20.56	jkl	18.89	kl
	35	25.59	ghi	33.89	hi	32.78	i	38.61	ghi
6	10	41.94	fg	41.71	fg	52.50	e	45.56	f
	Ambient	58.06	e	64.72	d	75.83	c	80.00	bc
	35	81.94	b	94.72	a	97.50	a	100.0	a
Mandarin fruit									
3	10	0.10	j	0.19	ij	0.53	ij	0.41	ij
	Ambient	0.09	j	2.67	hij	1.57	hij	1.35	hij
	35	0.14	j	1.58	hij	1.34	hij	3.10	hij
6	10	3.23	hij	4.65	gh	8.40	g	20.21	f
	Ambient	4.17	hi	39.34	d	24.96	e	51.66	c
	35	67.67	b	79.52	a	79.52	a	79.52	a

^a For delayed and immediate brushing and mandarins separately, means followed by the same letter do not differ significantly ($P > 0.05$; LSD = 4.647, 6.114 and 3.955 respectively)

Table 7. Parameters, goodness of fit and effective IMZ residue levels for predicted 50 and 90% sporulation inhibition of non-linear regression using the function $Y = \frac{pr3}{1+\text{Exp}(-pr1-pr2*X1)}$ of imazalil residue data measured on navel, Valencia and mandarin fruit after treatment with 500 $\mu\text{g.mL}^{-1}$ IMZ at temperatures of 10, ambient ($\approx 19 - 22$) or 35°C and pH levels of 3 or 6, at exposure times of 15, 45, 90 or 180 s.

Citrus type	Model parameter values and goodness of fit				IMZ residue $\mu\text{g.g}^{-1}$	
	Pr1	Pr2	Pr3	R^2	90%	50%
Navel	-2.126	1.791	102.459	0.682	2.292	1.1607
Valencia	-3.071	2.935	95.203	0.693	2.018	1.0807
Mandarin	-6.272	6.511	77.225	0.919	2.4 for 77%	1.05669

Table 8. Analysis of variance for *Rhizopus stolonifer* control data as analysed from colony forming unit counts for solutions containing spore suspensions of *Rhizopus* only, *Rhizopus* and green mould, and *Rhizopus* and imazalil (500 µg.mL⁻¹) held in water baths of temperatures of 10, 15, 20, 25, 35, 45, 55 or 65°C at exposure times of 1 minute or 60 minutes.

Source	<i>Rhizopus</i> only			<i>Rhizopus</i> and green mould			<i>Rhizopus</i> and imazalil		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	15	18279.351	<0.0001	15	15506.228	<0.0001	15	576.024	<0.0001
Temp.	7	22934.014	<0.0001	7	20928.412	<0.0001	7	1064.093	<0.0001
Exp. time	1	79662.661	<0.0001	1	65550.402	<0.0001	1	160.518	0.126
Temp.*Exp. time	7	4855.644	<0.0001	7	2934.877	<0.0001	7	147.314	0.0038
Error	224	228.917		224	454.411		224	67.971	
Corrected Total	239			239			239		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

Table 9. Parameters and goodness of fit of non-linear regression using the function $Y = pr3/(1+Exp(-pr1-pr2*X1))$ of spore suspensions of *Rhizopus* only, held in water baths of 10, 15, 20, 25, 35, 45, 55 or 65°C for either 1 minute or 60 minutes.

Exposure time	Model parameter values and goodness of fit			
	Pr1	Pr2	Pr3	R ²
1 minute	-10.852	0.090	13603.132	0.767
60 minute	-1.981	0.042	157.153	0.656

Table 10. Mean percentage *Rhizopus* spore control for spore suspensions of *Rhizopus* only, *Rhizopus* and green mould, *Rhizopus* and imazalil (500 µg.mL⁻¹) held in water baths of 10, 15, 20, 25, 35, 45, 55 or 65°C for either 1 minute or 60 minutes.

Exposure time (s)	Temperature (°C)	Control (%) ^a		
		<i>Rhizopus</i> only	<i>Rhizopus</i> and green mould	<i>Rhizopus</i> and imazalil
1 minute	10	7.40 e	12.65 gh	90.93 cde
	15	6.60 e	23.18 fgh	85.06 e
	20	12.19 e	31.79 ef	94.06 bcd
	25	15.03 e	24.87 fg	94.35 abcd
	35	9.15 e	9.50 h	90.14 de
	45	14.02 e	26.52 fg	96.53 abc
	55	30.08 cd	59.19 bc	96.94 ab
	65	94.25 a	100.0 a	100.0 a
60 minutes	10	43.03 b	49.20 cd	93.01 bcd
	15	35.59 bcd	67.51 b	77.11 f
	20	37.96 bc	65.13 b	96.14 abc
	25	26.46 d	43.46 de	97.07 ab
	35	45.69 b	33.30 ef	97.77 ab
	45	91.38 a	93.51 a	100.0 a
	55	100.0 a	100.0 a	100.0 a
	65	100.0 a	100.0 a	100.0 a

^aMeans in each column followed by the same letter do not differ significantly ($P > 0.05$; LSD = 10.887, 15.339 and 5.932, respectively)

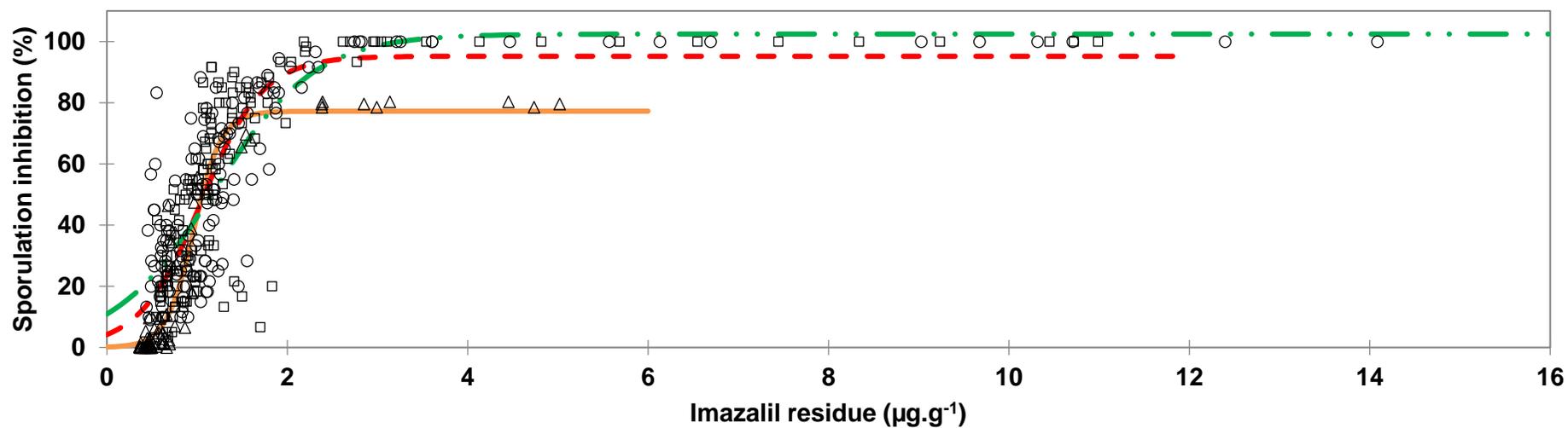


Figure 1. Mean imazalil residues values analysed on citrus fruit after treatment in imazalil solutions at 500 µg.mL⁻¹ at a pH of 3 or 6 at a temperature range of 10, ambient (≈ 19 – 22°C) or 45°C and non-linear regression models [$Y = pr3/(1+Exp(-pr1-pr2*X1))$] fitted indicating residue loading trends for navel (○, green dash-dot-dot line, $R^2 = 0.682$), Valencia (□, red dashed line, $R^2 = 0.693$), and mandarin (△, orange solid line, $R^2 = 0.919$), for sporulation inhibition.

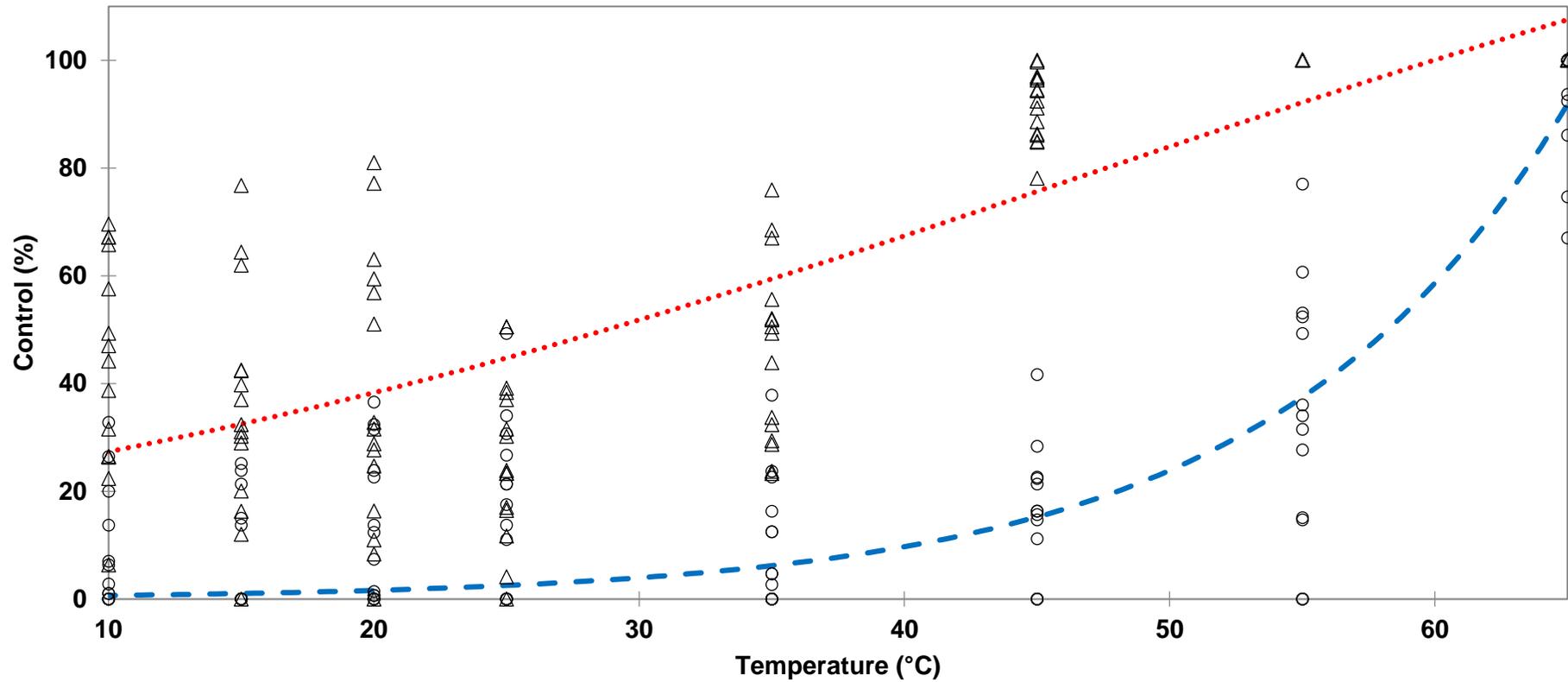


Figure 2. Mean percentage control of *Rhizopus stolonifer* colony forming units (CFU) at a temperature range of 10, 15, 20, 25, 35, 45, 55, 65°C for either 1 or 60 minutes' exposure time, and non-linear regression models [$Y = pr3/(1+Exp(-pr1-pr2*X1))$] fitted indicating control for 1 minute (\circ , blue dashed line, $R^2 = 0.767$) and 60 minutes (Δ , red dotted line, $R^2 = 0.656$) of *Rhizopus stolonifera*

3.5.4 PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided

Project 123 (Ongoing) by Wilma du Plooy, Catherine Savage and Paul H. Fourie (CRI)

Summary

Several products were evaluated, with varying results – the most successful were an azoxystrobin formulation against *Penicillium digitatum*. Products combining hydrogen peroxide and acetic acid were once again investigated as alternative sanitizers in the fungicide bath. The sensitivity of pyrimethanil and stability of propiconazole in the presence of the active was confirmed. Phytochemical reactions to some liquid formulations is a danger. A powder formulation of PAA was successfully evaluated, and as a result of the CRI contribution to the development of this product, the CRI will receive royalties from the sale of this product. Water sanitation is still very prominent, and several products with regards to this aspect was offered for testing.

Opsomming

Twee ringtoetse is onderneem en verskeie produkte was getoets. Daar was weereens klem gelê op sanitasie produkte met PAA en ozoon as aktiewe molekules. PAA lyk of dit goed verenigbaar is met na-oesprodukte, maar die korrosiwiteit daarvan is 'n bekommernis. Baie wisselvallige resultate word met osoon behaal in pakhuse, terwyl dit baie goed werk onder laboratoriumtoestande. Fitochemiese reaksies was waargeneem by hoër konsentrasies van PAA. Die poeier formulering van PAA het verdere ondersoek gehad rondom die oplosbaarheid van die produk. 'n Aantal produkte wat gebaseer is op bioflavonoïede was ondersoek. Geeneen van hierdie produkte het noemenswaardige sukses behaal as saniteermiddels nie.

Introduction

This project offers an ongoing industry service to evaluate potential new postharvest disease control products or options, as well as to conduct *ad hoc* experimentation. Products are mostly submitted from private companies, or projects/products are selected by the researchers involved. Given limited time and resources, requests are screened based on industry priorities. Below are brief reports of the activities in the project during the 2017/18 report year.

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides, this specifically include seeking actives for the control of <i>Phytophthora</i> brown rot and sour rot	None of products tested were effective against the pathogens. <i>Phytophthora</i> brown rot is a time and resource intensive pathogen in terms of inoculation and current resources does not allow for further testing of products against this pathogen.
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	Several trials were conducted. No viable alternatives have been found as yet.
3. Assist CRI DC with packhouse resistance testing	Swabs are either collected by extensionists visiting packhouses, or sent to the DC. Most do not indicate shifts in sensitivity, however, there were detections. The implicated packhouses were consulted on remedial action.
4. Analytical lab focus – ring test with the aim to reduce variability	Two tests were conducted. In the second test a German laboratory was included. Once again, three had acceptable results, with the PPECB laboratory failing to detect correct levels of the spiked fungicide they were presented with. The German laboratory had consistent results, but under-reported the concentrations of 2,4-D and TBZ.

Alternative products

A number of GRAS chemicals and alternative postharvest products were evaluated, in particular focussing on sour rot and green mould. The results were highly variable (Appendices 1-5)

Packhouse sanitation

Products offered as water sanitation options in citrus packhouses were evaluated. PAA is an effective sanitiser, but several reports of phytotoxicity or “burn” was received from soft citrus packers in particular. A powder formulation that negate this problem has been registered and is being introduced as a commercial option. We did several tests with this product (Appendix 6, 7).

Ring tests

Hearshaw and Kinnes. Microchem and Hortec all managed to detect the spiked fungicides within an acceptable range of the concentrations presented to them. The PPECB laboratory, however, failed the test. A further test will be done early in the 2018 season, which will be presented without the laboratories knowing that it is part of a ringtest. This will be done to prevent a “best-foot-forward” effect whereby non-standard levels of precision are achieved.

Resistance monitoring

Swabs from actively working packhouses are tested regularly throughout the season. This service will be continued and expanded in 2018. A few incidences of sensitivity shifts in pathogen resistance was detected and the packhouses concerned consulted about the issue. Suitable measures to curb this problem was suggested in cooperation with the extensionists.

Technology transfer

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 include seeking actives for the control of sour rot
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
3. Seek effective products and technologies for water sanitation in citrus packhouses
4. Analytical lab focus – ring test with the aim to reduce variability
5. Assist CRI DC with packhouse resistance testing

APPENDIX 1

Report of Pilot Trial: Agapan

On behalf of: AgraForUm SA
Researchers: Wilma du Plooy
Fruit type: Navels
Origin: Nelspruit area
Date: 01 August 2017

Objectives

Agapan is a concentrated blend of humic acid and bioflavonoid compounds. Alternative postharvest remedies are of particular importance with increasing pressure on conventional chemical control measures currently available. Not only in terms of acceptability (perceived human toxicity, emotional connotations, phytotoxicity, resistance development) but also in terms of market pressures exploiting the factors that determine acceptability. Although natural products and alternative remedies are often not successful on their own, it is hoped that a synergistic relationship can alleviate some of the market pressure experienced by the fruit producers.

Protocol

Navels were collected from a reputable commercial packhouse. They were washed with ozone, with excess water removed using a roller bed of standard brushes where after they were air dried, and stored at ≈ 23 °C for one day before the trial commenced.

1. For each trial, four repeats with 12 fruit per repeat were used.
2. The fruit was inoculated with a strain of *Penicillium digitatum* (PD) previously isolated from navels.
3. Inoculations were done by piercing the rind using a probe with a 2 mm² flat point. The probe is dipped in a 10⁶ PD spore suspension for each wound made. Four equidistant wounds are made on the shoulder area of the stem end of each fruit, resulting in 4 small piercings per fruit.
4. Treatments were:
 - Treated control (Imazalil at 500ppm).
 - Treatment – Agapan at 0.1% solution (prediluted)
 - Treatment – Agapan at 0.5% solution (v/v)
 - Treatment – Agapan at 1% solution (v/v)
 - Untreated control

Results

Agapan is a locally developed blend of humic acid and bioflavonoid compounds. A concentrate as well as a prediluted formulation (1%) was offered for the study. The fruit was inoculated with a strain of *Penicillium digitatum* (PD) previously isolated from navels.

- In the control group an average disease development of 97 % was observed, whilst the IMZ control had only 2.5% of rot. This confirms that the techniques used were appropriate.
- None of the Agapan solutions were able to exert any control over inoculated PD in any of the trialled fruit.

Discussion

In this trial Agapan did not prove to be an effective stand-alone product. The high failure rate makes it improbable that it will have a synergistic effect. In addition this product has a very strong and offensive smell, which complicates the use thereof in a fresh fruit environment.

Conclusion

No further work in a postharvest application will be done with this product.

APPENDIX 2

Pilot Trial: TESTING COMMERCIALLY AVAILABLE CITRUS COATINGS - COMPARISON TRIAL

On behalf of: CRI
Researchers: Wilma du Plooy and Thabang Mngwenya
Fruit type: Valencia
Origin: Burgersfort, Letsitele
DATE: September - October 2017

Objectives

The performance of fruit coatings is dependent upon many external variables, many of which are out of the control of the wax producers. Such variables include, but is not limited to, the internal packhouse environment (temperature, air flow humidity), fruit type and size, moisture on the fruit surface at the time of application, brush types and speed, maintenance of said brushes, and maintenance of the wax once a drum is opened. The postharvest disease management division of the CRI is of the opinion that every individual packhouse need to thoroughly test a formulation to ensure that the specific wax will perform optimally on that individual line.

Towards the end of the 2017 season, trials were run in two separate packhouses to evaluate the performance of commercially available waxes in these two facilities. They were in geographically distinct areas, with the first house packing soft citrus and the second house packing Valencia.

During the first trial, only a small number of formulations (four) from two manufacturers were used, as this was not a full commercial trial and more towards proving a point. However, increasing interest were expressed in this work when it became known that the purpose was not to pit waxes against each other as part of a marketing campaign. In the second trial therefore, a total of nine formulations from five manufacturers were used. This trial is by no means a full scientific investigation, and will need to be repeated with a far more stringent protocol. Even as a tool for extension work, more trials in 2018 will be needed.

Materials and Methods

1. The trial was run at two packhouses in two different production areas, using commercially available waxes.
 - a. Naranja Packhouse in Burgersfort has a very modern line, and a short applicator unit followed by heated drying. They were packing clementines at the time of the trial.
 - b. In the Naranja trial four waxes were used, with an untreated control.
 - c. Letaba Packhouse in Letsitele has an older line, and more brushes on the applicator unit, also followed by heated drying. They were packing Valencias at the time of the trial.
 - d. In the Letaba trial nine waxes were used, with an untreated control.
2. The packlines were running normally, with the commercial wax to be tested switched out in the applicator unit. The unit was then run for twenty minutes to allow the feeding lines, nozzles and brushes to be rinsed from the previous wax and saturated with the wax being tested.
3. Fruit was randomly collected from the line after wax application and drying, without colour or size sorting (jumble pack), to allow observation of the wax performance over a range of fruit quality.
4. Five 16-17 kg boxes were collected for ambient storage (26°C both Naranja and Letaba), and a duplicate set collected for cold storage at 5°C (Naranja) and 8°C (Letaba).

Results

TRIAL 1

Materials and Methods

- All the waxes contained 18% solids.
- Each wax that was tested was fed into the wax applicator on the commercial packline during normal operation. The line was run for 20 minutes with a change-over of the formulation, to clean out any previous formulation from the pipes, and to wet the brushes with the test formulation.
- For each formulation tested and an unwaxed control, thirty boxes were packed. Soft citrus fruit were collected randomly from the line after wax application and drying, without sorting or sizing. This was done to limit the number of cartons used.
- Collected fruit were jumble packed in open display cartons to a mass between 16 and 17 kg per box. The boxes were individually weighed and the starting mass noted.
- One set of fifteen boxes was kept in ambient conditions, and the second set placed in cold storage (5°C).
- For this first trial, due to logistical constraints, only weight loss was considered.
- The trial ran for a period of 45 days (six weeks and three days). Once a week, the boxes were removed from the respective storage area, weighed at a mass balance in the packhouse and returned to storage (six observations per treatment per temperature).
- Waxes 1-3 were polyethylene based formulations, with wax 4 a carnauba based formulation.

Results

Ambient temperature (26°C) average weight loss per formulation applied, after 45 days (figure 1):

Control = 15.77% / 2.70 kg

Wax 1 = 11.58% / 1.93 kg

Wax 2 = 12.88% / 2.10 kg

Wax 3 = 12.87% / 2.21 kg

Wax 4 = 12.11% / 2.00 kg

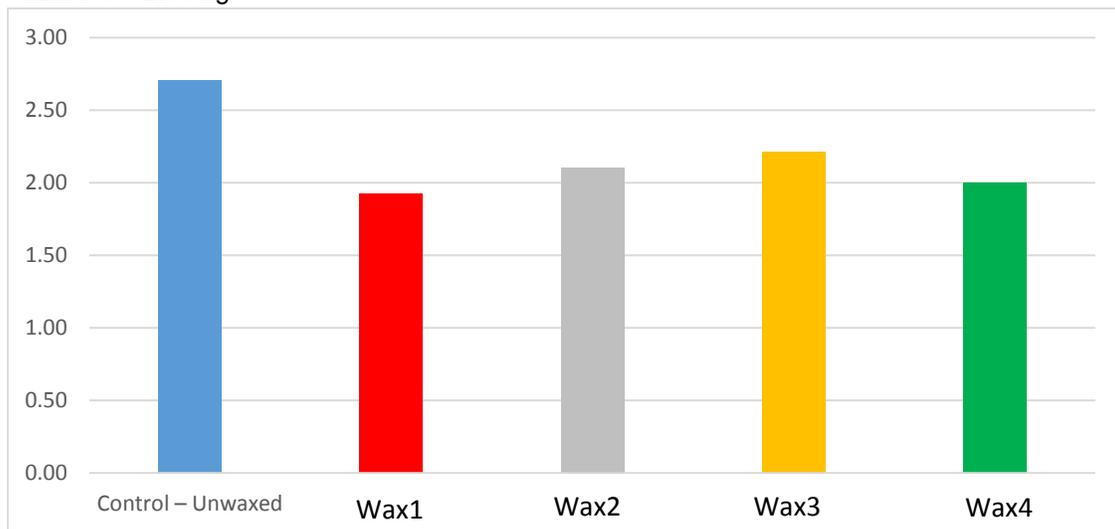


Figure 1. Average weight loss of each treatment at the end of 45 days at 26°C.

Cold storage (5°C) average weight loss per formulation applied, after 45 days (figure 2):

Control = 8.09% / 1.42 kg

Wax 1 = 5.84% / 0.98 kg

Wax 2 = 6.09% / 1.00 kg

Wax 3 = 6.20% / 1.05 kg

Wax 4 = 0.74% / 4.50 kg

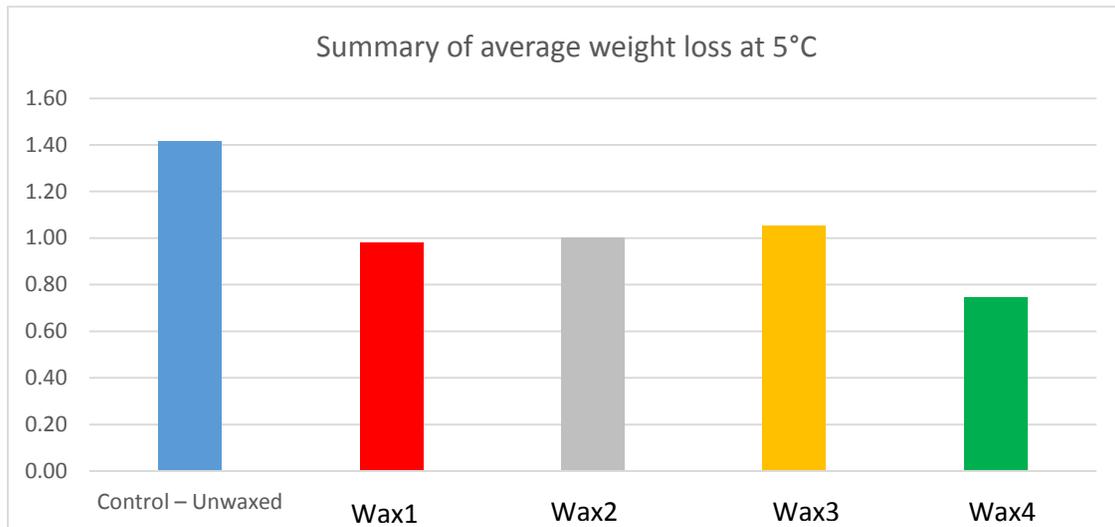


Figure 2. The total weight loss of each treatment at the end of 45 days at 5°C.

FURTHER REMARKS

- Although no weekly observation regarding the glossiness or powdering of the waxes were made, it was noted that the natural carnauba seemed to have the lowest gloss at onset, but that the starting gloss was maintained throughout the days in storage.
- The weekly removal, and subsequent return of the boxes from cold storage every week was a serious and repetitive break in the cold chain. This was purposefully decided on in order to stress the fruit and coatings to the maximum.
- At the end of this trial, it was decided to abandon ambient testing, as the number of fruit that starts to rot after four weeks makes collection of data difficult, with an unacceptable margin of error.

TRIAL 2

Materials and Methods

- All the waxes contained 18% solids.
- Each wax that was tested was fed into the wax applicator on the commercial packline during normal operation. The line was run for 20 minutes with a change-over of the formulation, to clean out any previous formulation from the pipes, and to wet the brushes with the test formulation.
- For each formulation tested and an unwaxed control, five boxes were packed. Valencia fruit were collected randomly from the line after wax application and drying, without sorting or sizing.
- Collected fruit were jumble packed in open display cartons to a mass between 16 and 17 kg per box. The boxes were individually weighed and the starting mass noted.
- All five boxes were placed in cold storage.
- For this first trial weight loss, gloss and powdering were all noted and considered.
- The trial ran for a period of six weeks and two days (44 days). Except in week one, the boxes were removed from the cold storage area twice a week, weighed at a mass balance in the packhouse and returned to storage (11 observations per treatment in total).

Results

Weight loss

A total of nine formulations from five different manufacturers were tested. They all contained 18% solids, and were divisible in two groups: polyethylene based coatings and carnauba based coatings.

Cold storage (8°C) average weight loss per polyethylene based formulation applied, after 44 days (Figure 3):

- Control 1,88 kg / 11,26%
- Wax 1 0,86 kg / 5,24%
- Wax 2 1,22 kg / 7,46%
- Wax 3 1,38 kg / 8,13%
- Wax 6 1,23 kg / 7,41%
- Wax 7 1,35 kg / 8,18%
- Wax 9 1,34 kg / 8,15%

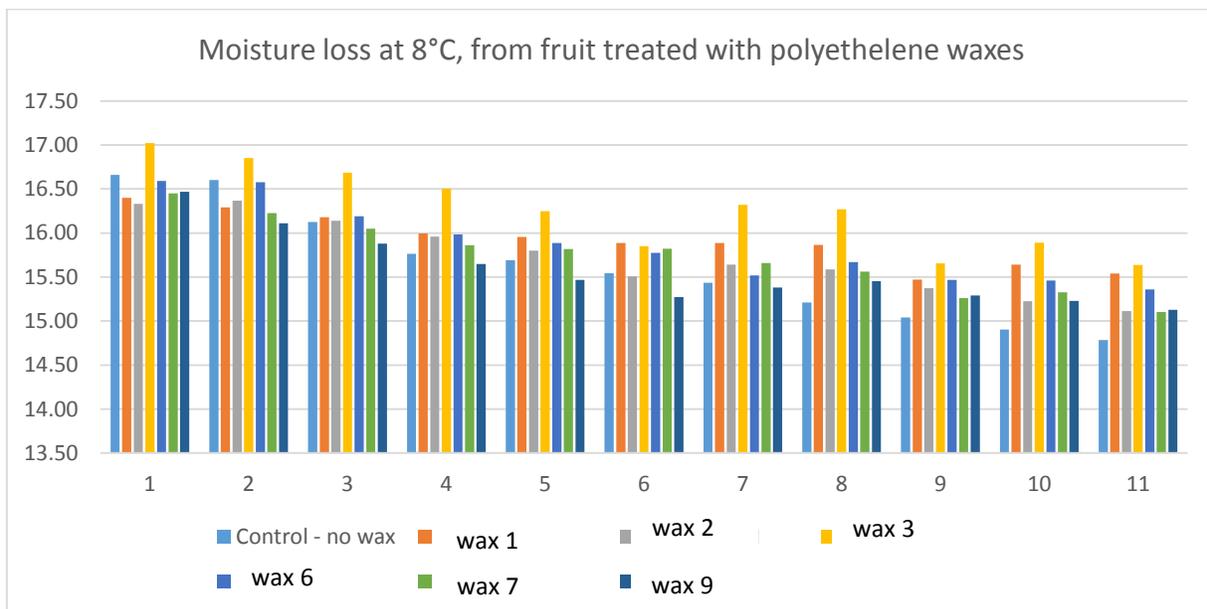


Figure 3. Week-by-week monitoring of weight loss by the different polyethylene based formulations kept at 8°C for 44 days, given as the average weight of 5 boxes per treatment per observation round.

Cold storage (8°C) average weight loss / carnauba-based formulation applied, after 44 days (Figure 4):

- Control 1,88 kg / 11,26%
- Wax 4 1,46 kg / 8,80%
- Wax 5 1,47 kg / 8,97%
- Wax 8 1,53 kg / 9,20%

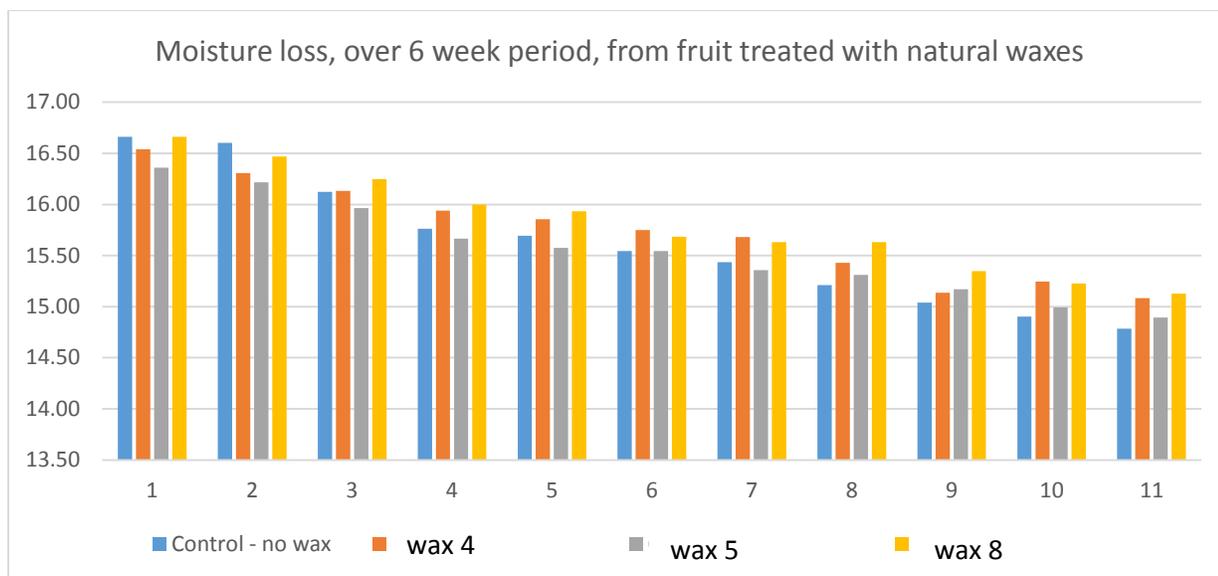


Figure 4. Week-by-week monitoring of weight loss by the different carnauba based formulations kept at 8°C for 44 days, given as the average weight of 5 boxes per treatment per observation round.

At the end of the 44-day trial, the average % moisture loss for all the polyethylene formulations were than the less average % moisture loss for the carnauba-based formulations (Figure 5). This is a normal occurrence, as the inherent qualities such as water and gas permeability for the different base ingredients are very different.

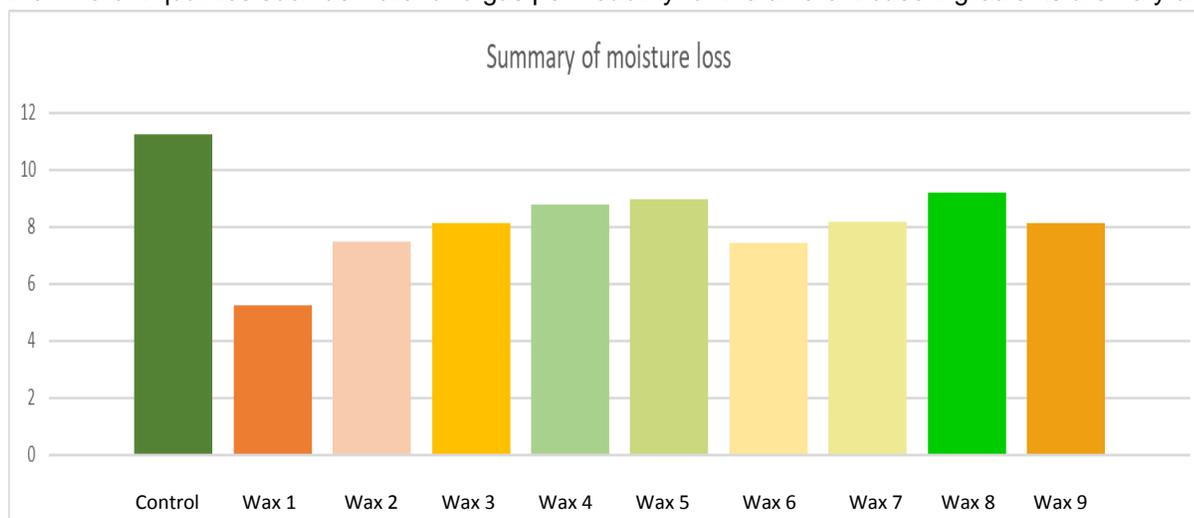


Figure 5. Summary of average moisture loss (%) on Valencia stored at 8°C, for all the formulations tested.

Loss in glossiness

The loss in gloss (figure 6) was measured on a scale from 5 – 1, with 5 being highly glossy and 1 being very dull. The observations were not qualified using any equipment, but was done by the same person who has received sensory training in minimising subjective observation.

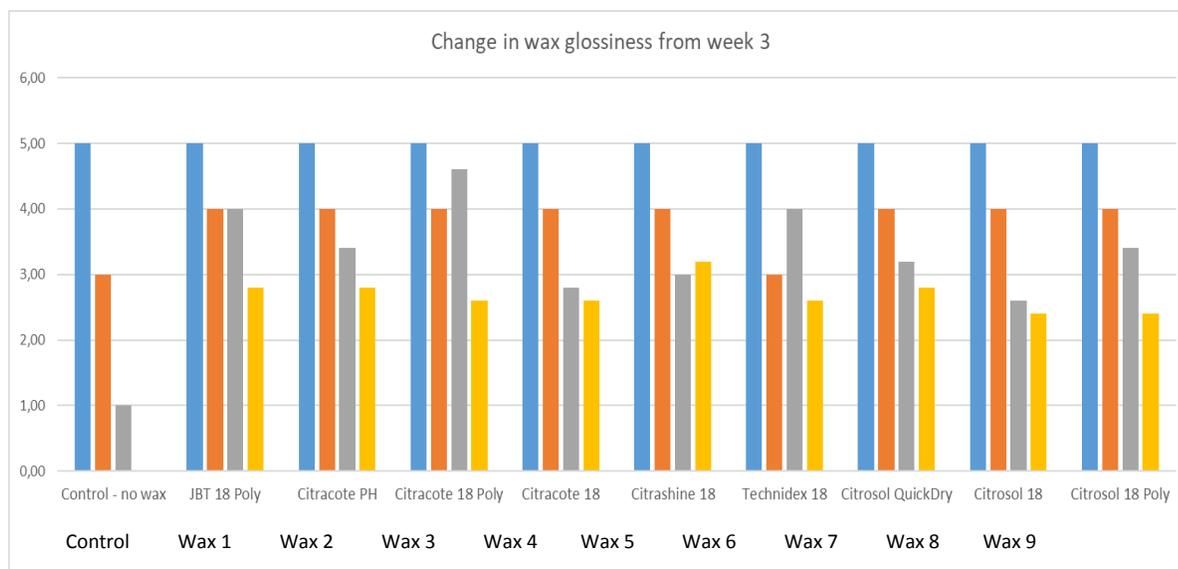


Figure 6. Summary of the week-by-week monitoring of loss in glossiness by the different formulations kept at 8°C for 44 days.

Increase in powdering

Every fruit in every box was inspected twice a week (except in week 1), and any perceptible powdering noted. All fruit were returned and recounted in the following round of observations. This numerical increase in powdering is represented by the final figure for each treatment in Figure 7.

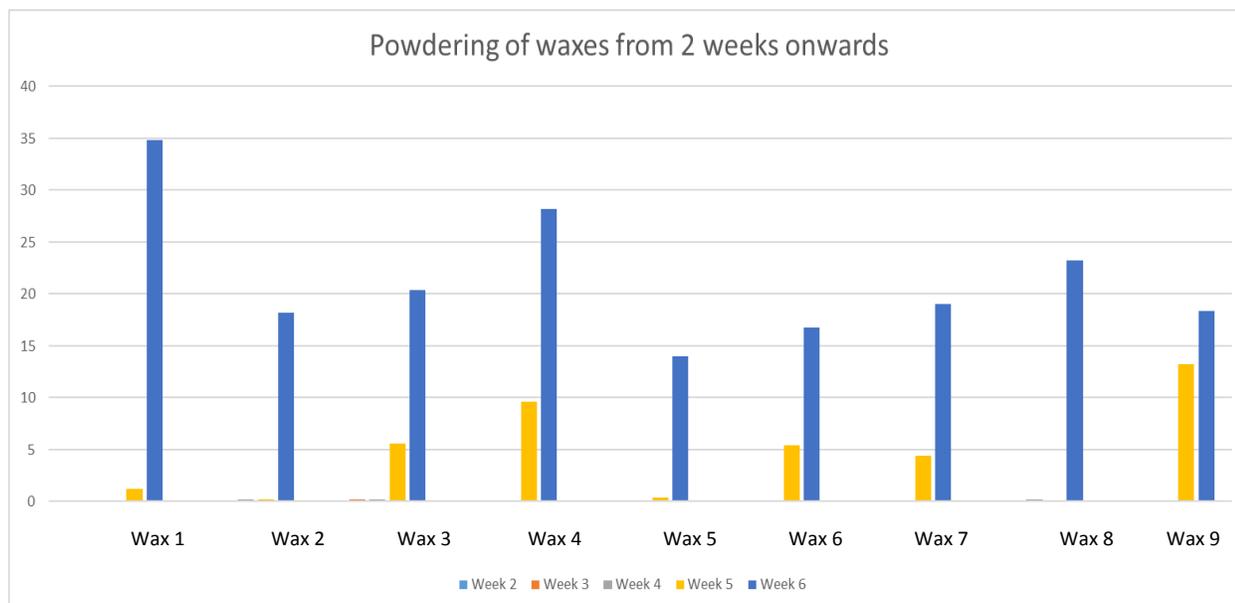


Figure 7. Summary of the week-by-week monitoring of increase in powdering by the different formulations kept at 8°C for 44 days.

Discussion

These two trials were very different in locality and fruit kind. No single wax can be highlighted as statistically more exceptional than another. The South African citrus industry is privileged to have access to a variety of quality products that can meet their individual requirements. The success that is achieved with a specific formulation depends on the diligent testing of this product in a packhouse, and on the citrus kinds being packed.

In the first trial an important point was that storage at ambient temperatures is not very useful. Although the fact that soft citrus was being used might have contributed to some waste being observed, the high storage temperature over 45 days still complicated the trial. Since there were only low numbers of rotten fruit, these were never removed, as the affected box would then have to be removed from the trial. Although this did have a minor effect on the mass of the affected boxes, the size of the trial did provide ample resilience to the observations. The difference between polyethylene waxes and carnauba based waxes are noticeable over the very long time of the trial (more than 6 weeks in both cases). While following applicable market requirements, where possible, this aspect should be considered when exporting to distant markets with protracted export periods. As an extension tool, this trial will benefit from a repeat in 2018. The support of the manufacturers will be most useful in order to expand on the formulation types across more citrus kinds in more production areas.

APPENDIX 3

Report of Pilot Trial: AGRIDISINFECT

On behalf of: Tony Schlebush
Researchers: Dr Wilma du Plooy
Fruit type: Navels
Origin: Nelspruit area
Date: 15 August 2017

Objectives

Agridisinfest is a proprietary blend of various inorganic persulfate salts, ascorbic acid, citric acid, malic acid, and bioflavonoids. Alternative postharvest remedies are of particular importance with increasing pressure on conventional chemical control measures currently available. Not only in terms of acceptability (perceived human toxicity, emotional connotations, phytotoxicity, resistance development) but also in terms of market pressures exploiting the factors that determine acceptability. Although natural products and alternative remedies are often not successful on their own, it is hoped that a synergistic relationship can alleviate some of the market pressure experienced by the fruit producers.

Protocol

Navels were collected from a reputable commercial packhouse. They were washed with ozone, with excess water removed using a roller bed of standard brushes whereafter they were air dried, and stored at ≈ 23 °C for one day before the trial commenced.

1. For each trial, four repeats with 12 fruit per repeat was used.
2. The fruit was inoculated with a strain of *Penicillium digitatum* (PD) previously isolated from navels.
3. Inoculations were done by piercing the rind using a probe with a 2 mm² flat point. The probe is dipped in a 10⁶ PD spore suspension for each wound made. Four equidistant wounds are made on the shoulder area of the stem end of each fruit, resulting in 4 small piercings per fruit.
4. Treatments were:
 - Treated control (Imazalil at 500ppm).
 - Treatment – Agridisinfest 1% solution (v/v)
 - Treatment – Agridisinfest 5% solution (v/v)
 - Treatment – Agridisinfest 10% solution (v/v)
 - Untreated control

Results

Agridisinfest is a locally developed blend of organic acids and bioflavonoid compounds. The fruit was inoculated with a strain of *Penicillium digitatum* (PD) previously isolated from navels.

- In the control group an average disease development of 96% was observed, whilst the IMZ control had only 2% of rot. This confirms that the techniques used were appropriate.
- None of the Agridisinfest solutions were able to exert any control over inoculated PD in any of the trialled fruit, with the best control at 35% only.

Discussion

In this trial Agridisinfect did not prove to be an effective stand-alone product. The failure rate makes it improbable that it will have a synergistic effect.

Conclusion

No further work in a postharvest application will be done with this product.

APPENDIX 4

TRIAL: Efficacy, phytotoxicity of Obstructo 250 SC as post-harvest dip for the control of green mould - 16ICA025

On behalf of: ICA International Chemicals

BY: Wilma du Plooy

DATE: 19 September 2017

Objectives

Testing azoxystrobin as a potential postharvest fungicide in a commercial product.

Crop: Citrus - type Valencia

Disease/pathogen: Green Mould - *Penicillium digitatum*

Materials and Methods

1. Navels were collected from a reputable commercial packhouse and stored at ≈ 23 °C for two days before the trial commenced. The fruit was removed from cold storage and allowed to reach ambient temperature before use in the trial.
2. The trial was set up according to the descriptions below.
3. Each treatment had five replicates with 10 fruit in each repeat, except where residue samples were taken, in which case the repeat had six extra prepared for residue determinations.
4. For all a sensitive strain of *Penicillium digitatum* (PD) were used.
5. A 10^6 spore suspension was prepared using the standard in-house laboratory technique.
6. Inoculation was done 6 hours prior to treatment.
7. Fruit was dipped in the remedies for 120 seconds, removed, dried in a forced air drying tunnel and stored at ambient temperature.
8. Lesions were evaluated 6 days after inoculation.

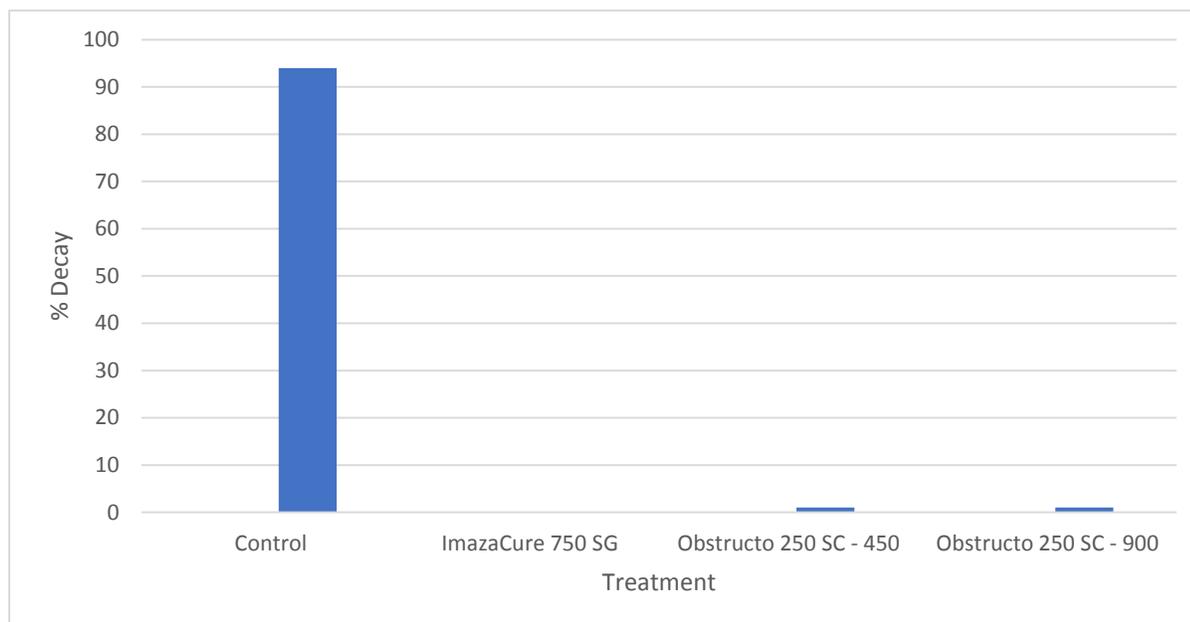
Table 1. Schedule of treatments used in the trial for OBSTRUCTO 250 SC.

Trmt no	Fungicide		Dosage (ml or g/100 ℓ water)
1	Control	-	-
2	ImazaCure 750 SG	Imazalil Sulphate	67
3	Obstructo 250 SC	Azoxystrobin	450
4	Obstructo 250 SC	Azoxystrobin	900

Results and Discussion

1. The control fruit had 100% rot on all the fruit in all five replicates, proving successful and viable inoculation.
2. Imazacure, as the industry standard, had resulted in 100% decay control.

3. Both applications of Obstructo 250 SC (i.e. at 450 g/100L and 900 g/100L) resulted in 1% decay or, conversely, 99% control. Neither of the applications had any phytotoxic effects on the fruit.



Graph 1. Decay control of Obstructo 250SC

MRL Data

Will be made available once processed by Hearshaw and Kinnes, Cape Town.

Conclusion

Obstructo 250 SC is an effective fungicide for the control of *P. digitatum* and could present an alternative treatment to Imazalil 750 SC. Being from FRAC group 11, this is an important consideration in resistance management of the pathogen.

DISCLAIMER

This report contains information and results from a confidential trial, but does not imply any product endorsement by the CRI.

3.5.5 PROGRESS REPORT: Epicuticular wax composition of CBS resistant and susceptible citrus cultivars

Project 1135 (2016/17-2017/19) by Wilma du Plooy (CRI), Wilma Augustyn (TUT), Charl Kotze (CRI), Paul Fourie (CRI), and Jan van Niekerk

Summary

Plants produce an extensive diversity of compounds known as phytochemicals, all of which are functional in the plant in some or other way. The role of phytochemicals in either eliciting or inhibiting the ability of *Phyllosticta citricarpa* (citrus black spot, CBS) to infect citrus fruit is being investigated. Cultivars with varying susceptibility to CBS are being investigated, looking at the apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycosides) fractions in the rind phytochemistry. Currently used cultivars include Bitter Seville, which is accepted to have low susceptibility to CBS infection, Valencia orange with medium susceptibility, and highly susceptible lemon. In addition, kumquats are included as a resistant type. The composition of the epicuticular wax layer of the different citrus cultivars over time (from fruit set until harvest) will be determined. Comparison over similar developmental periods will be attempted amongst all citrus cultivars studied. Bitter Seville is believed to

contain the favourable hard C₂₈ – C₃₃ chains from an earlier stage of fruit development, rendering it more resistant towards citrus black spot infections. Alternatively, the non-susceptible nature of kumquats may reside in the phytochemical composition of the peel of this unique citrus.

Opsomming

Plante produseer 'n baie uitgebreide diversiteit van verbindings bekend as fitochemikalieë, wat almal op een of ander wyse funksioneel is in die plant. Die rol wat fitochemie speel om die infeksie deur *Phyllosticta citricarpa* (sitrus swartvlek, SSV) òf aan te moedig, òf te inhibeer, word in hierdie studie ondersoek. Kultivars met verskillende vatbaarhede vir SSV word ondersoek deur bestudering van die apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloïede, glikosiede) in die vrugskil. Huidiglik word Bitter Seville as 'n lae vatbare tipe, medium vatbare Valencia en hoogsvatbare suurlemoen tipes gebruik, terwyl kumkwat as 'n nie-vatbare tipe bygevoeg is. Die samestelling van die epikutikulêre waslaag van die verskillende sitrustipes tydens die verskillende ontwikkelingsfasies (vrugset tot volwasenheid) sal ondersoek word. Daar sal gepoog word om die vergelykings oor die ontwikkelingstydperke te maak tydens die bestudering van die verskillende kultivars. Bitter Seville het skynbaar gunstige harde C₂₈ – C₃₃ wassettings tydens vroeë ontwikkelingsstadiums, wat dit meer bestand maak teen SSV infeksie. Alternatiewelik mag die nie-vatbare aard van kumkwatvrugte wees as gevolg van die fitochemiese samestelling van die skil van hierdie unieke sitrus.

3.5.6 PROGRESS REPORT: Studies on the management of sour rot and green mould with propiconazole
Project 1141 (2015/2016-2016/19): by Lindokuhle C. Mamba (SU), Charles Stevens (SU), Cheryl Lennox (SU), Julia Meitz-Hopkins (SU), Wilma du Plooy (CRI), Paul Fourie (CRI)

Summary

A new fungicide to control sour rot, caused by the ascomycete *Galactomyces citri-aurantii* is needed since guazatine, a guanidine fungicide effective against sour rot, is being phased-out for citrus fruit exported to the EU. Propiconazole is a postharvest fungicide that is available to the South African citrus industry. This demethylation-inhibitor (DMI) triazole fungicide has recently been registered for postharvest use on sour rot decay on citrus fruit in South Africa. Baseline sensitivity to propiconazole was determined for single spored South African isolates of *G. citri-aurantii* (sour rot) and *Penicillium digitatum* causing green mould, from one unexposed orchard population in the Eastern Cape and one population in the Western Cape. The mean effective concentration for 50% reduction of mycelial growth (EC₅₀ value) was 0.682 µg/ml (range 0.451-0.888 µg/ml) in the Eastern Cape *G. citri-aurantii* population and 0.313 µg/ml (range 0.004-1.087 µg/ml) in the Western Cape population. For *P. digitatum*, the mean EC₅₀ (EC) was 0.068 µg/ml (range 0.007-0.084 µg/ml), and the mean EC₅₀ (WC) was 0.149 µg/ml (range 0.025-0.405 µg/ml). Drench application of propiconazole was studied to determine the time frame (6, 14, 18, 24 h) in which the fungicide has to be applied from harvest until fruit arrives on the packline. Additionally, the exposure time (1, 2, 3 min) required to obtain adequate coverage of the fungicide applied to the fruit was determined for three cultivars ('Nules' clementines, 'Eureka' lemon, navel orange). Results from inoculations of lemons with a *G. citri-aurantii* isolate indicated that propiconazole can effectively control sour rot infection when treatment is applied at 600 mg/L with 1 min exposure time (flow rate approximately 500 L/min), if applied within 14 h of inoculation. In comparison, 'Nules' clementine treatments had to be applied within 6 hours after inoculation to reach full effectivity.

Opsomming

Sitrusuitvoere na die EU benodig 'n nuwe swamdoder teen suurvrot (veroorzaak deur die askomiseet *Galactomyces citri-aurantii*). Die gebruik van guasatien, 'n guanidienswamdoder effektief teen suurvrot, is uitgefasseer vir hierdie markte. Propikonasool, is 'n na oes-swamdoder wat beskikbaar in die Suid-Afrikaanse sitrusbedryf. Hierdie demetilasië-inhibeerder (DMI) triasool swamdoder is nou geregistreer vir na-oes gebruik teen suurvrot, veral op sitrusvrugte. Basislynsensitieweit vir propikonasool was getoets vir Suid-Afrikaanse isolate van *G. citri-aurantii* (suurvrot), en *Penicillium digitatum*, wat groenskimmel veroorsaak. Twee boorde wat nie vantevore

aan propikonasool blootgestel was nie, was gebruik: een elk in die Oos-Kaap en Wes-Kaap. Die gemiddelde effektiewe konsentrasies vir 50% vermindering van miseliumgroei (EC50-waarde) in the Oos-Kaap boord, vir *G. citri-aurantii*, was 0.682 µg/ml (bestek 0.451-0.888 µg/ml), en in die Wes-Kaap boord was 0,313 µg/ml (bestek 0,004-1,087 µg/ml). Die EC50-waarde teen *P. digitatum* in the Oos-Kaap boord was 0.068 µg/ml (bestek 0.007-0.084 µg/ml) en in die Wes-Kaap, 0,149 µg/ml (bestek 0,025-0,405 µg/ml). Stortvloedaanwending van propikonasool was bestudeer om die tyd (6, 14, 18, 24 h) waarbinne die swamdoder moet aangewend word, te bepaal: vanaf pluk tot die vrugte op die paklyn verwerk word. Hiervoor was 'Eureka' suurlemoene, navel lemoene en 'Nules' clementine gebruik. Verder was die blootstellingstyd (1 min, 2 min, 3 min) wat nodig is om goeie bedekking van die swamdoder op die vrugte te kry, getoets. Toetse op suurlemoene met 'n *G. citri-aurantii* isolaat, het aangedui dat propikonasool suurvrot infeksie effektief kan beheer, mits die behandeling aangewend word teen 600 mg/L vir 1 min binne 14 uur van inokulasie (vloeitempo ongeveer 500 L/min). Toetse met 'Nules' clementine het aangedui dat die behandeling binne 6 ure aangewend moet word.

3.5.7 PROGRESS REPORT: Fungal degradation of wood pallets used in export of citrus fruit
Project 1165 (2016/17-2017/19) by Wilma du Plooy (CRI), and Jan van Niekerk (CRI)

Summary

Recent fungal contamination on wooden pallet bases used for export highlighted a problem of which the source is apparently unknown. This degradation of pallet bases when the consignment reaches its market has raised questions on the quality of the wood used in the manufacturing of these bases. Further concerns were the contribution of packhouse storage methods to the fungal contamination of the bases, and the possible role of environmental factors (for instance moisture, UV degradation, and insect infestation). However, it is fungal decay that is currently the hazard with the highest priority. Heavily contaminated pallets are a point source of fungal spore dissemination that poses a deterioration risk to the boxes stacked on these pallets, will increase the spore load in cold rooms, and presents a phytosanitary concern. This study looks at the microbiome on contaminated pallet bases, manufacturing aspects of the bases, the role of storage at packhouses, options for wood treatment, as well as possible contribution from the shipping containers to the degradation of wooden pallet bases.

Opsomming

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

3.6 CRI DIAGNOSTIC CENTRE (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, and Jan van Niekerk)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematode:Roots	41	917	58	993
Nematode:Soil	10	35	113	825

<i>Phytophthora</i>	6934 ¹	1213	176	1127
Water spore trap	178	0	2	0
Black spot identification (PCR)	0	151	0	12
Black spot benzimidazole resistance	0	93	0	0
Citrus greening (PCR)	0	2	0	0
Post Harvest Resistance	0	73	5	53
Fruit & Foliar identification	0	38	15	34
Soil dilution plating	0	432	6	19
Internal Fruit Quality	0	0	0	0
SUB-TOTALS	7163	2954	375	3063

¹ Total samples received for citrus nurseries – includes quarterly samples, re-tests and non-certified nurseries

Citrus Certified Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 5841² nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 6.13% tested positive. In addition to soil and water samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans*. For the nematode root samples, 0% tested positive and for the nematode soil samples 0% tested positive.

Commercial samples

Samples were received from the following citrus growing areas: Eastern Cape, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, Swaziland, Western Cape and Zimbabwe. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Twenty three percent of the 917 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Fifty nine percent of the 1213 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of Avo, Grape, Macadamia, Pecan and Tobacco. Nematodes found present on these crops included: *Criconema*, *Scutellonema*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Xiphinema*, *Paratrichodorus*, *Longidorus*, *Hemicriconemoides*, *Paratylenchus*, *Hoplolaimus*, *Helicotylenchulus*, *Hemicycliophora* and *Tylenchulus semipenetrans*. *Phytophthora* and *Pythium* analyses were done on Avo, Blueberries, Grape, Macadamia and Soybean. The diagnostic centre analysed 35 soil samples from macadamia nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 2945 samples from experimental trials. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols.

Footnote:

² Sample number and the percentage positive are only for certified nurseries and only for the quarterly samples received.

4 PORTFOLIO: CITRICULTURE

4.1 PORTFOLIO SUMMARY

By Paul Cronjé (CRI-SU)

In the Citriculture portfolio the aim is to ensure the sustainable and profitable production and export of citrus fruit via research in cultivar and rootstock evaluation, preharvest production practise as well as aspects that can impact on fruit quality during the postharvest handling and cold chain. During the season, the cultivar team continued to evaluate promising cultivars per production area in order to serve as support for growers' decisions on orchard establishment or replacement. Comprehensive details are supplied in this report in addition to updating of the cultivar fact sheets. The research project on alternative bearing came to an end. It contributed not only significantly to understanding this physiological phenomenon, but also to leading new recommendations to manage this problem. The shade netting projects are near completion and 2018 will be the last season, however, clear fundamental information as well as practical recommendations have already stemmed from this project. Research into irrigation in collaboration with the Water Research Commission (WRC) will draw to a close and a full conclusive report will be available in 2019. New products and techniques have been tested in the portfolio including pruning strategies, a new chemical thinning agent as well as a growth retardant. The cold chain of the SA citrus industry is complex and the reduced shipping regimes to the EU have highlighted various factors, i.e. effective precooling as well as ambient loading. The diversity and complexity of subjects addressed in this portfolio are vast but the development of new incremental growth in technology to facilitate quality and quantity of citrus fruit produced and exported remains critical for the citrus industry.

PORTEFEULJE OPSOMMING

In die portefeulje Sitrusproduksie is die doel om deur middel van cultivar- en onderstam-evaluering, navorsing in voor-oes produksiepraktyk die volhoubare en winsgewende produksie en uitvoer van sitrusvrugte te verseker, verder word aspekte wat die vrugkwaliteit kan beïnvloed tydens die na-oes hantering en koue ketting ook ondersoek. Gedurende die seisoen het die cultivar-evalueerders weereens alle belowende kultivars per produksiegebied geëvalueer ten einde steun te verleen aan produsente tydens besluitneming oor die vestiging of vervanging van boorde. In hierdie verslag word volledige besonderhede verskaf wat gebruik word in die opdatering van die cultivar feitegids. Die navorsingsprojek oor alternatiewe drag het tot 'n end gekom en beduidende bydrae was gemaak tot die begrip van hierdie fisiologiese verskynsels en het direk aanleiding gegee tot nuwe aanbevelings om hierdie probleem te bestuur. Die skadunet projekte kom tot 'n einde in 2018 maar alreeds is fundamentele inligting ingewin asook praktiese aanbevelings ontwikkel. Navorsing oor besproeiing in samewerking met die Waternavorsingskommissie (WVK) sal tot 'n einde kom en 'n volledige verslag sal in 2019 beskikbaar wees. Nuwe produkte en tegnieke is in verskeie projekte in die portefeulje getoets wat insluit; snoei-strategieë, 'n nuwe chemiese uitdunningsmiddel sowel as 'n nuwe vegetatiewe groeimanipuleer middel. Die koueketting van die SA sitrusbedryf is kompleks en die verlaging in verskeppingsregime temperatuur na die EU het verskeie faktore beklemtoon wat impak maak op vrugkwaliteit, nl. effektiewe verkoeling asook die warm laai van vrugte in verkoelde houers. Die diversiteit en kompleksiteit van die onderskeie spesialisgebiede wat in hierdie portefeulje aangespreek word, is aansienlik, maar die ontwikkeling van nuwe tegnologie deur inkrementele vordering in hierdie portefeulje is belangrik om sitrusvrugte van kwaliteit en kwaliteit te geproduseer en uit te voer.

4.2 PROGRAMME: RIND CONDITION

Programme coordinator: Paul Cronjé (CRI-SU)

4.2.1 PROGRESS REPORT: Chilling injury of lemon fruit

Project 1169 (2017/8-2019/20) by Paul Cronje, Jade North (CRI) and Nicola Kirsten (SU)

Summary

Export of lemons could take place in new regimes that include pulp temperature options of 2 or 4°C. However, at these temperatures, lemon fruit are still sensitive to chilling injury, especially under long durations such as 24 days. In addition to the duration of a protocol, the logistical implications in the cold chain of cooling fruit, as well as

loading and off-loading, often results in an additional 3 to 6 days' exposure to low temperatures. During 2017 a set of experiments was conducted to firstly determine factors influencing CI susceptibility in lemon fruit. Secondly, postharvest actions were tested to reduce the impact of CI in lemon fruit. No variation occurred between canopy positions, but fruit maturity and cultivar do contribute to higher susceptibility. Postharvest wax application containing higher solid contents of 18-20%, showed a significant reduction in CI incidence. These initial results will be followed up in 2018 to determine if consistent factors could be identified.

Opsomming

Die uitvoer van suurlemoene teen pulptemperatuuropsies van 2 of 4°C kan moontlik ingesluit word in nuwe regimes. Teen hierdie temperature is suurlemoenvrugte egter steeds sensitief vir koueskade, veral onder lang verskeping tydperke soos 24 dae. Benewens die verlangde tyd van koue-protokol, lei die logistieke implikasies in die koelketting tot addisionele blootstelling dae aan lae temperature wat kan strek van 3 tot 6. Daarom is dit belangrik om dus informasie beskikbaar te stel oor die voorkoming van koueskade in suurlemoene. Gedurende 2017 is 'n stel eksperimente uitgevoer om eerstens faktore te bepaal wat CI vatbaarheid beïnvloed in suurlemoensvrugte. Tweedens is na-oes-aksies getoets om die impak van CI in suurlemoensvrugte te verminder. Geen variasie het plaasgevind tussen posisie in die blaardak nie, maar vrug volwassenheid en kultivar dra egter by tot groter vatbaarheid. Na-oes waks toediening met hoër vastestof inhoud (18-20%) het 'n beduidende vermindering in CI-voorkoms getoon. Hierdie aanvanklike resultaat sal opgevolg word in die 2018, om te bepaal of hierdie faktore geïdentifiseer in 2017 konsekwente bydrae tot koueskade in suurlemoene.

Objectives

1. Quantify the influence of pre-harvest factors on CI susceptibility, i.e., production areas, canopy position, fruit maturity and cultivar selection.
2. Develop postharvest treatments to reduce the incidence of CI, i.e., chemical treatments (Auxin and TBZ), wax type and content and *add hoc* product evaluations.

Scope and techniques

Cold temperature protocols used in the various experiments depended on the research question asked and are summarized below. Fruit will be evaluated for CI incidence as well as severity upon completion of cold duration as well as after 7 d shelf life

1. -0.6°C for 12, 22 and 32 days
2. 2°C for 12, 22 and 32 days
3. 7°C for 12, 22 and 32 days

The first cold regime, i.e., USDA type sterilisation protocol were used, even though it is not a commercial used regime, to test the efficacy of various factors and treatments as it is the most stressful cold treatment. In addition to evaluation CI, rind colour, rind thickness as well as pigment and carbohydrate content was measured and will be included in the 2019 report as part of a completed MSc thesis.

Results and discussion

The underlying first theme in this research project is to identify factors which contribute significantly to the variability in CI susceptibility of lemons fruit. In order to gain this insight, various experiments were done where fruit were sampled and or subjected to a treatment prior to a similar cold storage treatment. For these experiments, no wax or TBZ was applied in order to test the inherent susceptibility of the fruit without alterations.

Canopy position is known to affect citrus fruit internal (TSS) external (colour) quality as well as the susceptibility to physiological disorders such as rind breakdown a chilling injury in grapefruit. However, no data exist for lemons. After harvesting fruit from the inside and outside of lemon trees in three production areas, no significant pattern was visible in CI susceptibility (Fig.1). This results will be followed up in the 2018 season. However, do to the growth habit and tree architecture of lemons trees being more open compared the grapefruit or clementine it could be reasoned that there are not such a large difference between these positions in general.

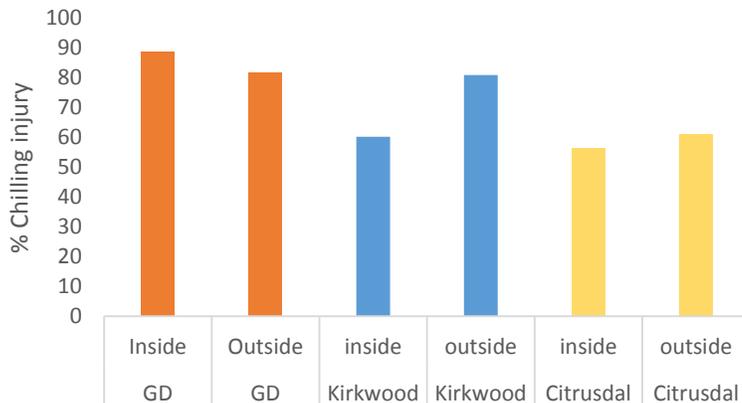


Fig. 1. Impact of canopy position, i.e. inside vs. outside on CI susceptibility. Fruit were harvested from one orchard per area and 10 trees per orchard (n=10) and stored for 32 days at -0.6°C and evaluated after 7 days shelf life.

In lemons fruit maturity always pose an interesting question as the juice % are the primary factor to determine harvest date since the fruit will be degreened. In the occurrence of rind disorders, rind maturity are known to affect susceptibility, and immature and over mature fruit have a higher possibly to develop a disorder. To sheds, some light on this aspect in lemons fruit were sample (and cold stored at -0.6°C for 32 days) from one orchards over 6 weeks in May - June 2017. The results indicate a higher susceptibility in the initial weeks of the planned commercial harvesting window (Fig. 2).

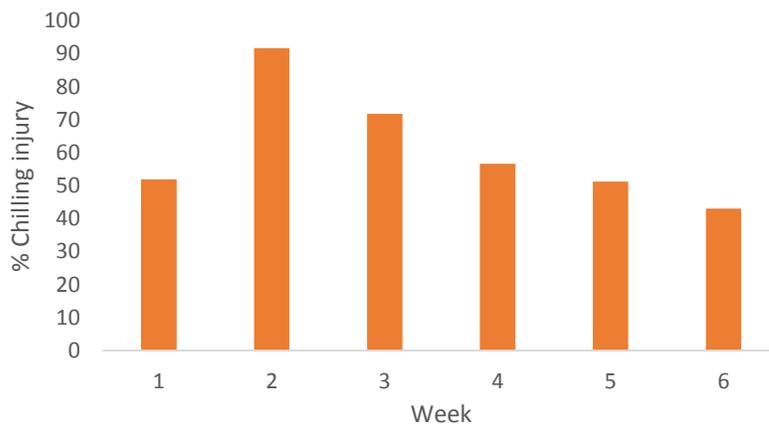


Fig. 2. Variation and change in CI susceptibility over a 6-week period during commercial harvest from a lemons orchard in Stellenbosch Western Cape. Fruit were harvested from 10 trees in one orchard (n=10) and stored for 32 days at -0.6°C and evaluated after 7 days shelf life.

Limited cultivars are available for lemons and Eureka dominate the production for fresh use in South Africa. In order to determine if there are any difference in CI susceptibility fruit form a cultivar evaluation block in Citrusdal were harvested on the same day and put into cold storage. In 2018 seasons the new seedless cultivar are being developed will also be evaluated. In the 2017 season, Lisbon were the culitvar with the least amount of CI compared to Eureka and Genoa (Fig. 3).

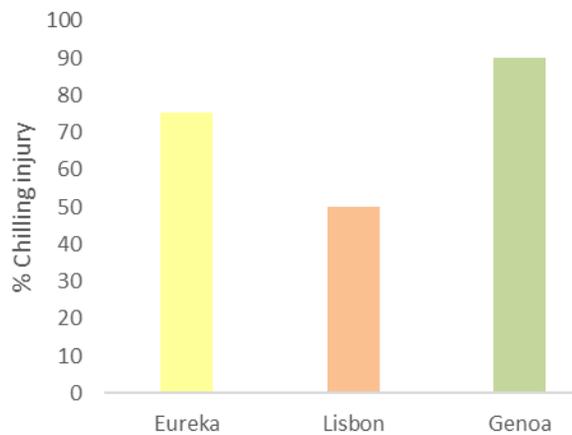


Fig.3. Influence of cultivar on CI susceptibility of fruit harvested in Citrusdal. Fruit were harvested from 10 trees in one orchard (n=10) and stored for 32 days at -0.6°C and evaluated after 7 days shelf life.

The postharvest actions can be influential in determine the degree to which a fruit develop a physiological disorder such as chilling injury. The second aim of this project is, therefore, to create postharvest handling strategies to minimise the impact of CI on lemons fruit. In the first experiment, a time temperature duration study was done to determine how long lemon fruit can be exposed to specified temperatures. For this experiments, no wax or TBZ was applied in order to test the inherent susceptibility of the fruit without alterations. The incidence of CI followed the know pattern of increasing at all temperatures as the duration escalates (Fig. 4). In addition, the influence of temperature was also evident with a higher incidence early on at -0.6°C compared to 2°C.

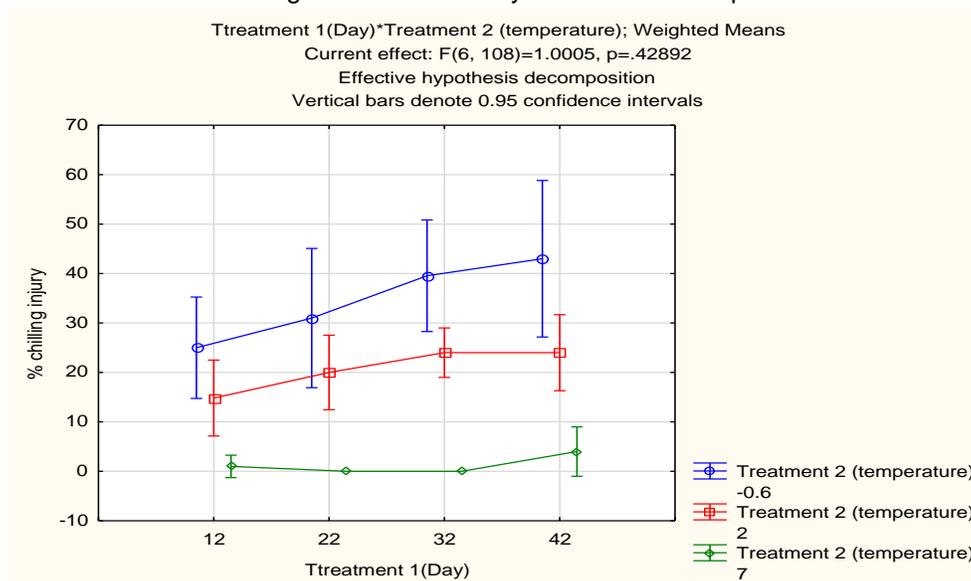


Fig 4. The Interaction of time x temperature on CI susceptibility of lemon fruit harvested at commercial maturity in Robertson, Western Cape in 2017 in June (n=10).

The application of wax as a protective layer has been used in horticulture to reduce the symptom development of CI in various crops. In citrus, this is also known to be true, however, for lemons no data have shown how CI development wax of different solid content and chemical composition influence.

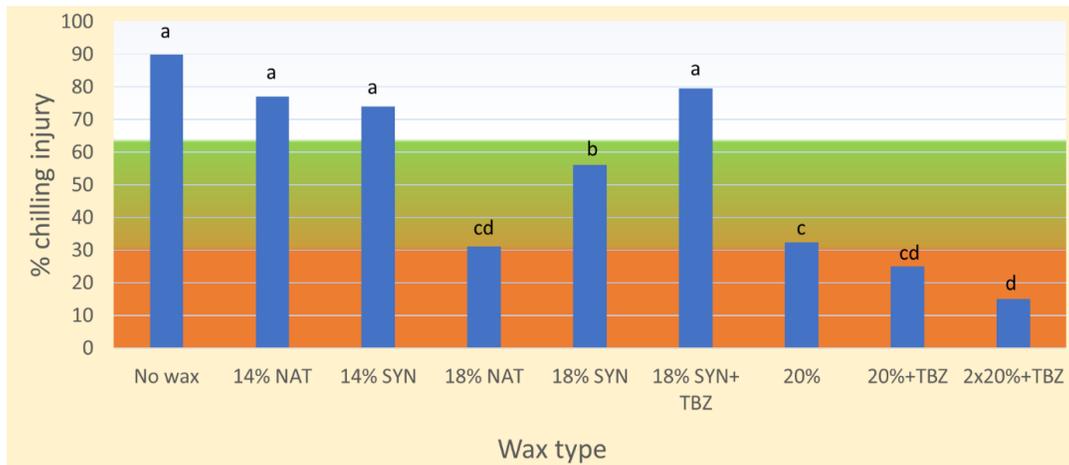
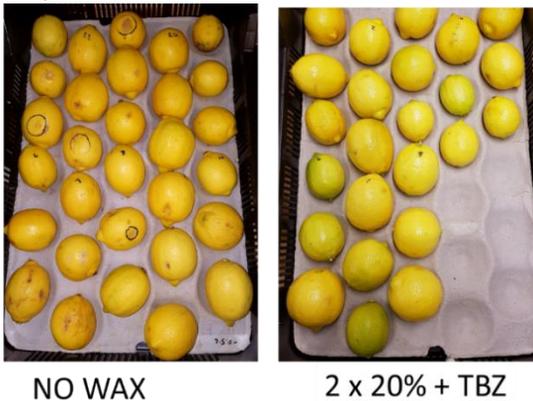


Fig 5. The use of various wax formulations, i.e. natural vs. synthetic and solid % on CI of lemons stored at -0.6°C for 32 days (n=10).

Photo 1. An example of the impact of the wax treatment compared to the control. Higher rind collapse due to the temperature stress could be seen in the control fruit.



Preliminary conclusion

During the first seasons of this project new information on the factors which could influence susceptibility of lemons fruit was tested. Maturity seems to be an important aspect to consider in more depth. However in the subsequent seasons, more experiments will be conducted to determine area influence as well a localised variation with in orchards.

The use of high solid content wax was shown to be effective in reducing the incidence of CI significantly and the preliminary recommendation of using 18-20% solid content wax on lemon fruit exported under low temperature, i.e. $<4^{\circ}\text{C}$ could be made.

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4.3 **PROGRAMME: FLOWERING AND FRUIT SET**

Programme coordinator: Jakkie Stander (CRI)

4.3.1 **Programme summary**

Research within this programme aims to (1) optimise flowering potential and (2) optimise fruit set. In alternate bearing 'Nadorcott' mandarin trees, flowering and fruit load, root growth during spring and summer, and summer vegetative shoot development were identified as the major predictors of intensity of return bloom flowering and yield potential. Results showed that flowers and fruit are the major carbohydrate sinks, and disturb the balance between root growth and vegetative shoot development. The lack of development of summer vegetative shoots and flowers following in "on" trees was not related to leaf carbohydrate or macro-nutrient concentrations. An inhibition of summer vegetative shoots is related to high concentrations of the hormones 1 *H*-indole-3-acetic acid (IAA) and dihydrophaseic acid and the abscisic acid (ABA) glucose ester in leaves, and high gibberellin (GA) concentration in leaves in May and June contributes to limited flowering following an "on" year. A new model for alternate bearing in mandarins is presented (4.3.4). A pyramid pruning treatment in young citrus trees stimulated new vegetative growth and resulted in a 30% increase in yield response compared to an open vase pruning treatment (4.3.2). However, an early shaping or training of newly established trees stimulated vigorous vegetative growth and is not recommended. In mature trees, light selective pruning treatments resulted in highest yields for 2 consecutive seasons, and severe mechanical pruning, the lowest. Strategic removal of medium sized branches that restrict light or cross with other branches appeared to be the best approach of pruning 'Nadorcott' mandarin to maintain productivity and obtain adequate fruit size. In flower induction experiments, foliar GA₃ treatments of 40 ppm during May to June resulted in an inhibition of flowering on vegetative shoots (4.3.3). Treatments aimed at increasing return bloom flowering on summer vegetative shoots should therefore be timed during May to June. For example, a 2-fold elevating of leaf carbohydrate content by girdling and de-fruiting during this period significantly increased return bloom flowering, and soil and foliar treatments of "on" trees with 1000 mg·L⁻¹ paclobutrazol or uniconazole, gibberellin biosynthesis inhibitors during this period increased flowering and resulted in fruit development from buds of "on" shoots. Two research projects on the effects of permanent shade netting found that 20% white shade nets reduced air temperatures, leaf vapour pressure deficit (VPD), solar radiation and wind speed, and improved tree water usage efficiency (4.3.5). The shade net treatment increased fruit yield (no. of fruit per tree), larger fruit size counts, and significantly reduced superficial rind blemishes. Shade netting reduced fruit rind strength at time of commercial harvest, as well as rind carbohydrate contents. A novel chemical thinning agent used in pome fruit, Nevis®, with the active ingredient metamitron is being evaluated in citrus. A treatment of 300 ppm metamitron at fruitlet diameter of 8 or 15 mm significantly reduced tree total fruit yield and enhanced the fruit size distribution and did not negatively influence fruit quality. Outputs of research within this programme provide novel insights into complex production problems, but at the same time practical, readily-applicable and cost-effective solutions.

Programopsomming

Navorsing binne hierdie program het ten doel om (1) blompotensiaal te optimaliseer en (2) vrugset te optimaliseer. In alternerende drag in 'Nadorcott' mandaryne is blom- en vruglading, wortelgroei gedurende lente en somer, en die somer-vegetatiewe groei geïdentifiseer as die belangrikste voorspellers van die intensiteit van opvolgblom- en opvolg opbrengspotensiaal. Resultate het getoon dat blomme en vrugte die belangrikste koolhidraatverbruikers is en die balans tussen wortelgroei en vegetatiewe loot-ontwikkeling versteur. Die gebrek aan ontwikkeling van somer vegetatiewe lote en blomme in "aan" bome hou nie verband met blaarkoolhidraat- of makro-nutriëntkonsentrasies nie. 'n Inhibisie van somer vegetatiewe lootgroei is nou gekoppel met hoë konsentrasies van die hormone 1 H-indool-3-asynsuur (IAA) en dihydrofaas suur en die ABA-glukose ester in blare. Hoë gibberellien (GA) konsentrasie in blare in Mei en Junie dra by tot 'n beperkte blom na 'n "aan" jaar. 'n Nuwe model vir alternerende drag dra in mandariene is voorgestel (4.3.4). 'n Piramied snoei-behandeling in jong sitrusbome stimuleer nuwe vegetatiewe groei en het gelei tot 'n 30% toename in opbrengs vergeleke met 'n oop-kelk snoei behandeling (4.3.2). 'n Vroeë vorming of snoei van nuut gevestigde bome het egter sterk vegetatiewe groei gestimuleer en word nie aanbeveel nie. In volwasse bome het ligte selektiewe snoei behandelings die hoogste opbrengs vir 2 agtereenvolgende seisoene tot gevolg gehad, en strawwe en meganiese snoei, die laagste. Strategiese verwydering van mediumgrootte takke wat kruis met ander takke beperk, was die beste benadering vir snoei van volwasse 'Nadorcott' mandaryn bome om produktiwiteit te handhaaf en voldoende vruggrootte te verkry. In blominduksie eksperimente het blaar GA₃ behandelings van 40 dpm gedurende Mei tot Junie gelei tot 'n inhibisie van blomontwikkeling op vegetatiewe lote (4.3.3). Behandelings wat daarop gemik is om goeie blom op die somer vegetatiewe lote te stimuleer moet dus gedurende Mei tot Junie toegedien word. Byvoorbeeld, 'n 2-voudige vermeerdering van blaarkoolhidraatinhoud deur ringelering en ontvrugting gedurende hierdie tydperk het 'n aansienlike toename in opvolgblom tot gevolg gehad, en grond- en blaarbehandelings van "aan" bome met 1000 mg·L⁻¹ paclobutrasool of unikonazool, gibberellienbiosintese-inhibeerders gedurende hierdie tydperk, het blom en oeslading in die volgende seisoen vermeerder. Twee navorsingsprojekte op die effekte van permanente skadunette het bevind dat 20% wit skadunette die lugtemperatuur, blaardampdruk tekort (VPD), sonstraling en windsnelheid verminder het, en die doeltreffendheid van boom waterverbruik verbeter het (4.3.5). Die skadunet behandeling het vrugopbrengs (aantal vrugte per boom) verhoog, groter vruggrootte tellings, en aansienlik minder vrugletsels tot gevolg gehad. Skadunette het vrugskil sterkte verlaag, asook die inhoud van skilskoolhidrate. 'n Nuwe chemiese uitdunningsmiddel in kernvrugte, Nevis®, met die aktiewe bestanddeel metamitron word tans vir effektiwiteit in sitrus geëvalueer. 'n Behandeling van 300 ppm metamitron by vrugdeursnee 8 of 15 mm het die totale oes opbrengs aansienlik verminder en die vruggrootte verspreiding verbeter, sonder om vrugkwaliteit negatief te beïnvloed. Uitsette van navorsing in hierdie program bied nuwe insigte in komplekse produksieprobleme, maar terselfdertyd praktiese, toepaslike en koste-effektiewe oplossings.

4.3.2 FINAL REPORT: Effect of pruning on fruit production of Nadorcott mandarin

Project number 522019 (2014/04 – 2018/03) (New Number: 000190) by R.B. Cronje, C.F. Human and I.M. Ratlapane (ARC-TSC)

Summary

A project on pruning strategies for 'Nadorcott' mandarin for newly established, young and old trees was initiated in 2014. The aim of the project was to determine the effect of various pruning methods on flowering, yield, fruit quality, starch reserves and alternate bearing pattern as well as to develop a practical and economical pruning practice for 'Nadorcott' mandarin.

The trial on newly established and young trees (1 and 2.5-year old at trial start, respectively), included three treatments, namely two selective hand-pruning treatments (pyramid and open vase shape) and a control (untreated until trees touched each other). The trial on old trees (8-year old at trial start) consisted of six treatments including selective pruning by hand (light and severe after harvest or just after fruit drop), mechanical pruning after

harvest, a combination of hand and mechanical pruning in alternate years and a control (farm practice). Pruning was carried out in July/August, after fruit drop (one treatment only) and January (water shoot control). Data collection included total amount of removed plant material, tree height before and after pruning, stem circumference at harvest, leaf starch content (at harvest (Jun/Jul), fruit set (Sep), after fruit drop (Nov), flower initiation (Apr)), return bloom, yield and fruit size.

Timing and severity of pruning influenced return bloom, yield, fruit size and leaf starch content. High crop load reduced fruit size and leaf starch content during fruit development and vice versa. Early shaping or training of newly established trees stimulated vigorous vegetative growth and is not recommended. Pruning of young 'Nadorcott' trees when reaching their allotted space in the row (around year 3) allowed easy shaping and removal of branches that were restricting light into the canopy. After three years of pruning, the pyramid shape appears to be beneficial for 'Nadorcott' mandarin, despite the higher amount of branches removed. Pruning according to a near pyramid shape removes strong shoulder branches, which otherwise only cause overhangs once full of fruit. Fruit on such overhanging branches coloured up less evenly compared with fruit on upright branches. Pruning of old, overgrown 'Nadorcott' trees appeared to be challenging. Severe pruning of such trees caused vigorous vegetative growth in the following season with a decrease in yield, but an increase in fruit size. Moderate pruning after harvest or after fruit drop by removing smaller branches (Control and Selective after fruit drop treatment) showed the lowest impact on yield, fruit size, return bloom and leaf starch content. Mechanical pruning alone or combined with selective hand pruning in alternate years did not show any benefits. On the contrary, used alone it created a dense canopy with short and weak bearing shoots. It is recommended that pruning of 'Nadorcott' mandarins should start once they fill their space in the row. Strategic removal of medium sized branches that restrict light or cross with other branches appeared to be the best approach of pruning 'Nadorcott' mandarin to maintain productivity, and adequate fruit size.

Opsomming

'n Projek oor snoeistrategieë vir 'Nadorcott' mandaryne vir nuut gevestigde, jong en ou bome is in 2014 begin. Die doel van die projek was om die effek van verskillende snoeimetodes op blom-, opbrengs-, vrugkwaliteit, styselreserwes en alternatiewe drag te bepaal sowel as om 'n praktiese en ekonomiese snoei praktyk vir 'Nadorcott' mandaryne te ontwikkel. Die proef op nuut gevestigde en jong bome (onderskeidelik 1 en 2.5 jaar oud met die aanvang van die proef) het drie behandelings ingesluit, naamlik twee selektiewe handsnoeibehandelings (piramide en oop kelk vorm) en 'n kontrole (onbehandel tot bome aan mekaar raak). Die proef op ou bome (8 jaar oud met die aanvang van die proef) het bestaan uit ses behandelings, insluitend selektiewe snoei met die hand (lig en swaar na oes of net na vrugval), meganiese snoei na oes, 'n kombinasie van hand- en meganiese snoei in alternatiewe jare en 'n kontrole (plaaspraktyk). Snoei is uitgevoer in Julie/Augustus, na vrugval (slegs een behandeling) en Januarie (waterlootbeheer). Dataversameling het ingesluit die totale hoeveelheid verwyderde plantmateriaal, boomhoogte voor en na snoei, stamomtrek tydens oes, blaarsystelinhoud (oes (Jun/Jul), vrugset (Sept), na vrugval (Nov), blominisiasie (Apr)), herblom, opbrengs en vruggrootheid ingesluit. Tydsberekening en hardheid van snoei het herblom, opbrengs, vruggrootheid en blaarsystelinhoud beïnvloed. Hoë oestheid het vruggrootheid en blaarsystelinhoud tydens vrug ontwikkeling verminder en omgekeerd. Vroeë vorming of opleiding van nuutgevestigde bome het sterk vegetatiewe groei gestimuleer en word nie aanbeveel nie. Snoei van jong 'Nadorcott' bome sodra hulle hul spasie in die ry bereik het (ongeveer 3 jaar) het maklike vorming en verwydering van takke, wat lig in die boom beperk, moontlik gemaak. Na drie jaar se snoei blyk die piramide vorm voordelig te wees vir jong 'Nadorcott' mandaryne, ten spyte van die groter hoeveelheid takke wat verwyder word. Snoei volgens 'n amper piramide-vorm verwyder sterk skouertakke wat andersins sou veroorsaak dat daardie takke wanneer hulle vol vrugte is oorhang. Vrugte op sulke oorhangende takke het minder eweredig verkleur in vergelyking met vrugte op regop takke. Snoei van ou, oorgroeide 'Nadorcott' bome was uitdagend. Harde snoei van sulke bome het in die volgende seisoen sterk vegetatiewe groei veroorsaak, met 'n afname in opbrengs, hoewel die vruggrootheid toegeneem het. Matige snoei na oes of na vrugval waar kleiner takke verwyder is (Kontrole en Selektiewe na vrugval behandelings) het die laagste impak op opbrengs, vruggrootheid, herblom en blaarsystelinhoud gehad. Meganiese snoei alleen of gekombineerd met selektiewe hand snoei in alternatiewe jare

het geen voordele getoon nie. Inteendeel, meganiiese snoei alleen, het 'n digte blaardak met kort en swak dralote geskep. Dit word aanbeveel om met snoei van 'Nadorcott' mandaryne te begin sodra hulle hul spasie in die ry vul. Strategiese verwydering van mediumgrootte takke, wat lig binne die boom beperk, of kruis met ander takke, was die beste manier om te snoei.

Introduction

'Nadorcott' is a popular late mandarin cultivar, not only because of its good eating quality but also its excellent bearing capacity. Despite its good bearing and fruit quality characteristics, 'Nadorcott' is different from many other mandarins. It is characterized by vigorous growth, an upright tree structure, alternate bearing and a late and long harvesting period. Because of these characteristics managing 'Nadorcott', especially its alternate bearing and tree size, becomes a challenge. Pruning as a way to manipulate both tree growth and bearing patterns is an important orchard management tool, but pruning of 'Nadorcott' after harvest is difficult because of the short period between harvest and flowering. It is therefore important to develop pruning strategies specifically for 'Nadorcott'. Yield and fruit size are very important in 'Nadorcott' production. One of the most limiting factors in tree productivity is the lack of optimum light. Therefore, the main aim of pruning is to improve light penetration through the tree canopy in order to produce strong bearing branches outside and inside the tree that can bear fruit of good size and quality. Research has shown that tree shape and light conditions within the tree affect the microclimate inside the tree (Suzuki et al., 1973) which in return influences emerging time and strength of flushes and flowers and fruit quality parameters such as colour, size, TSS, TA (Suzuki et al., 1973; Davenport, 1990; Tucker et al., 1994; Verreynne et al., 2004; Khurshid and Krajewski, 2010). The type of bearing branch (strong or weak) will determine the type of inflorescence it will bear as well as the fruit set potential. Strong bearing branches are thick and produce mainly leafy inflorescences, which have a high fruit set rate and produce a good fruit size, whereas weak bearing branches have the opposite effect (Davenport, 1990; Krajewski and Rabe, 1995; Krajewski and Pittaway, 2000; Khurshid and Krajewski, 2010). This applies especially to mandarins and oranges (Davenport, 1990; Krajewski and Rabe, 1995). Furthermore, there is an interaction between crop load, carbohydrate levels and alternate bearing (Garcia-Luis et al., 1988; Goldschmidt, 1999; Iglesias et al., 2003; Verreynne and Lovatt, 2009) which can be influenced positively by correct pruning.

Due to the complexity of the tree structure but also because of the above-mentioned interactions between light, type of bearing branch, fruit quality, crop load, carbohydrate levels and alternate bearing it is important to study tree phenology of 'Nadorcott'. Understanding its growth pattern and reaction to certain pruning times and techniques will enable growers to prune strategically for sustainable yields with low or no alternate bearing, economical fruit size and quality, whilst maintaining tree size for better orchard management and harvesting. Therefore, the aim for the project was to develop practical as well as economical pruning practices for 'Nadorcott'.

Stated Objectives

- To determine the effect of timing of various pruning techniques on flowering, fruit set, yield and fruit quality (size) of 'Nadorcott' mandarin
- To determine the effect of various pruning techniques on carbohydrate reserves and alternate bearing pattern
- To determine the effect of pruning time and type of cuts on re-growth

Materials and methods

Experimental site and trial design

The study was conducted on a commercial farm (Indigo Fruit Farming) near Nelspruit, in Mpumalanga Province between 2014 and 2017 (3 seasons). 'Nadorcott' trees of various ages (1, 2.5 and 8 years at trial start) were pruned after harvest of each year.

Pruning of newly established and young trees

Young trees were trained to determine the best structure for a strong framework that can produce many bearing branches.

Treatments for young trees (1 and 2.5 years at trial start) were trained/pruned immediately after harvest as follows:

1. **Control:** Trees remained unpruned until they touched each other in the row. Thereafter light standard pruning was carried out by removing medium sized branches that crossed other branches or were too closely spaced. Follow-up shoot control was carried out in summer (January), where necessary.
2. **Open Vase:** Trees were selectively hand pruned after harvest by removing medium to big branches to form an open tree structure, i.e similar to open vase structure (Fig. 1, middle row). Long shoots were shortened by one-third. Follow-up shoot control was carried out in summer (January), where necessary.
3. **Pyramid:** Trees were selectively hand pruned by removing medium to big branches to form a pyramid shaped structure with central leader(s) (Fig. 1, bottom row). Long shoots were shortened by one-third. Follow-up shoot control was carried out in summer (January), where necessary.

The trial was laid out in a randomized complete block design with 10 replicates per treatment and three trees per replication, of which the middle tree was used for data collection.

Pruning of older trees

Older trees were pruned as proposed below to maintain tree size, increase light within the canopy and establish a productive canopy with many bearing branches.

Treatments for older trees (8 years at trial start) were carried out as follows:

1. **Severe selective hand pruning after harvest:** Strong water shoots and big excess branches were removed to open up the canopy. Long shoots were shortened by one-third. Follow-up shoot control was carried out in summer (January) by removing water shoots.
2. **Light selective hand pruning after harvest:** Only strong water shoots and few out-of-place branches were removed to open up the canopy. Follow-up shoot control was carried out in summer (January) by removing water shoots.
3. **Selective hand pruning after fruit drop:** Pruning was similar to the light selective pruning treatments but carried out after fruit drop in November only. This treatment also served as fruit thinning at the same time. Follow-up shoot control was carried out in summer (January) by removing water shoots.
4. **Light mechanical pruning after harvest:** Pruning was done with a hedge pruner. Cuts were no thicker than 10 mm diameter. Trees were shaped according to a pyramid form. Where trees were very dense, especially after the first season, hand pruning was used (after trees were pruned mechanically) to open up the canopy and create windows. Follow-up shoot control was carried out in summer (January) by removing water shoots.
5. **Selective hand pruning (year 1) and mechanical pruning (year 2) after harvest:** Selective hand pruning was carried out for sufficient light for new growth inside the tree in the first year by removing medium to big branches. In the following year, mechanical pruning was carried out with a hedge pruner to uniformly cut back/remove long branches and shape the trees into a pyramid form. Follow-up shoot control was carried out in summer (January) by removing water shoots.
6. **Control:** Standard selective hand pruning was carried out immediately after harvest by pruning contractors used by the farm by removing medium sized branches. Follow-up shoot control was carried out in summer (January) by removing water shoots.

The trial was laid out in a randomized complete block design with 5 replicates per treatment and four trees per replication, of which the two middle trees were used for data collection.

Data collection and statistical analysis

All removed plant material was weighed and recorded for each pruning time (Jul/Aug, Nov (where applicable), Jan). Tree response after pruning was monitored by observing 10 shoots per tree at flowering and fruit set. Each

shoot consisted of 8 internodes on average. Percentage white blossom, green blossom, vegetative flush, dormant internodes and fruit set was calculated from the observed shoots.

Thirty leaves per tree were collected at harvest (Jul), full bloom (Sept), fruit set (Oct), after fruit drop (Nov/Dec), after water shoot thinning in summer (Jan) and at flower induction (Apr) to determine starch reserves of leaves. For analysis of starch reserves, leaves were dried at 60°C after sampling. The samples were then analyzed for starch content using the Iodine Colorimetric Method by Xu et al. (1998). At harvest, yield (per tree) and fruit size (mm diameter of 50-100 fruits/tree) were determined.

Data for yield and percentage flowering were subjected to a combined analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc., Cary, USA). The Shapiro-Wilk test showed normal distributions for all data. Fisher's least significant difference (LSD) was calculated at the 5% level to compare treatment means. Pearson's product-moment correlation was performed on all data using PROC CORR of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Principal Component Analysis was performed with XLSTAT version 2015.

Results and discussion

'Nadorcott' tree structure

It was observed that as 'Nadorcott' shoots grow they branch into several second order shoots of which one is bigger than the rest. This stronger branch then extends and branches again into third order shoots, and again one of these is stronger and thicker than the rest. When this stronger branch bears fruit, it pulls the entire branch down. As these kind of branches are usually on the outside of the canopy and shoulder area of the tree, they create an overhang. Fruit on such overhanging branches colour-up less evenly compared with fruit on upright branches and are therefore less desirable. Removing these bigger shoots/branches was often enough to achieve the desired tree shape, especially for younger trees. Trees with overhanging branches cause shading on themselves as well as opposite trees. Removing these branches will improve light penetration considerably in 'Nadorcott' mandarin.

Pruning of newly established trees (1-year-old at trial start; 2015-2017)

Removal of plant material

Newly established trees were trained for the first time in July 2015, one year after planting, according to pyramid or open vase shape or remained unpruned. Figure 1 displays the effect of various pruning treatments on tree shape and size in 2015, 2016 and 2017. To establish a pyramid shape, big off-centered branches were completely removed and very small branches, which only die off later and damage fruit, on the inside of the trees were removed. For the open vase treatment, branches that closed up the center, were removed and water shoots were shortened. No records for removed plant material were taken in 2015 due to the low amount removed. Tree height of Pyramid and Open Vase trees were reduced on average by 20 cm in 2015 compared with the Control trees (data not shown). In 2016 and 2017, tree height was adjusted in all data trees to 2.0 and 2.2 m, respectively. This was on average a height reduction of 5, 5 and 7.5% for the Control, Pyramid and Open Vase treatments, respectively (data not shown). Most plant material was removed from the Open Vase trees, followed by the Selective and Control treatments (Fig. 2). This influenced the first crop of the trees significantly (Fig. 3). There were no statistical differences in stem circumference between treatments at harvest in 2015 and 2016 (data not shown). However, stem circumference increased in all treatments as trees grew each year, which was to be expected.

Yield and fruit size

The more plant material was removed through pruning/water shoot thinning the lower was the yield. Therefore, the Control had the highest yield while the Open Vase treatment had the lowest yield (Fig. 3). However, there was no difference in fruit size and most fruit were in the size categories 2 to 1XX (Fig. 4).

Return bloom

Flowering was only monitored in 2017. Although not significant, percentage leafless and leafy inflorescences (white and green blossom) were lower and percentage dormant internodes higher in the Control compared with the other treatments (Fig. 5). Percentage internodes with vegetative growth (flush) were significant higher in the Control trees (Fig. 5). Lower percentage inflorescences and higher percentage vegetative growth in the Control can be attributed to the high yield in July 2017, which affected flower induction and bearing potential for the following season, which is in accordance to other studies (Mazhar et al., 2007; Verreyne and Lovatt, 2009; Martinez-Fuentes et al., 2010; Shalom et al., 2012). Unfortunately, no fruit set or other data could be collected after September 2017 as a hail storm caused severe damage to the trees in October 2017. The trial was therefore stopped.

Starch reserves

Leaf samples for starch analysis were taken in April, July and September 2017 as this was the first season for bearing. Significant differences between treatments could only be observed in April, where the Pyramid treatment had a significantly higher starch content compared to the other treatments (Fig. 6). However, this did not have a positive effect on the same year's yield or fruit size of this treatment.

Further data is required to draw sound conclusions on the effect of tree training/pruning on long-term bearing potential of very young trees. However, significant (although not strong) correlations between yield and height reduction, leaf starch content in April and flushing during flowering (Table 1) give an indication that removal of large amounts of plant material through tree training and pruning at this young tree age have a negative impact on the trees and can possibly cause alternate bearing from a very early age. From the limited observations made over the two seasons on these very young trees, it appears more beneficial to allow the trees to fill their allocated space in the row and only start pruning and shaping them once they encroach each other. Due to their vigorous growth, 'Nadorcott' trees tend to replace removed branches with strong water shoots that are not productive. Allowing the tree establish itself for the first two to three years and then removing branches that interfere with tree shape and canopy density will be less disruptive to tree phenology or reduce early onset of alternate bearing. This could be shown in the second pruning trial on 2.5-year old trees (at trial start) and is discussed below.

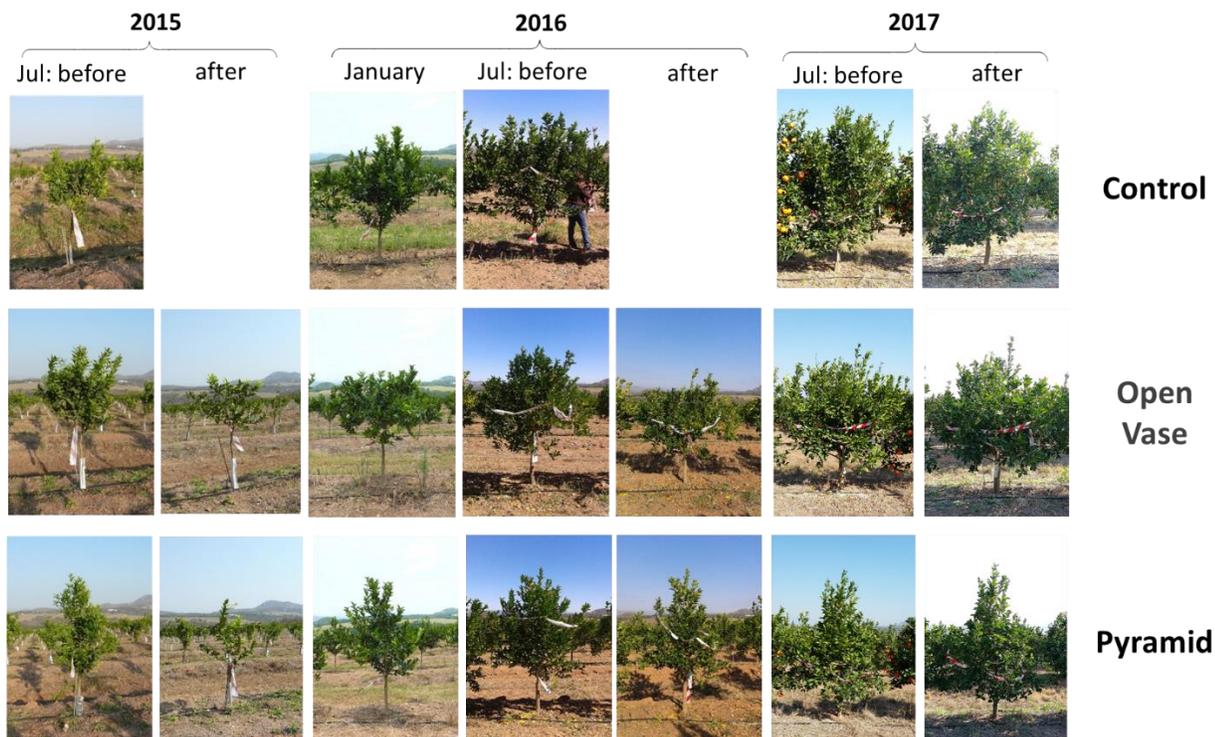


Figure 1. Tree shape of the Control (unpruned; top), Open Vase (middle) and Pyramid treatment of young 'Nadorcott' trees between July 2015 and July 2017 (before and after pruning).

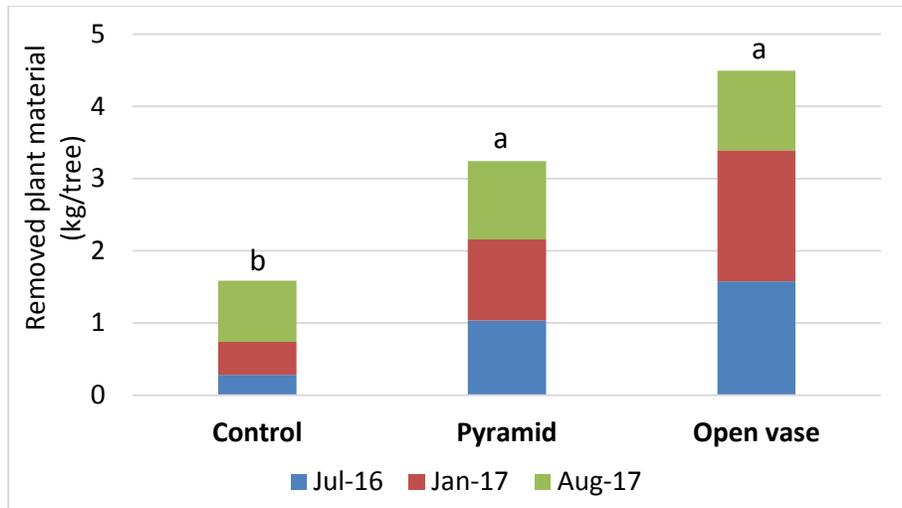


Figure 2. Removed plant material through pruning and water shoot control in newly established 'Nadorcott' trees between July 2016 and August 2017. Different letters above columns denote significant differences between treatment means (P < 0.05).

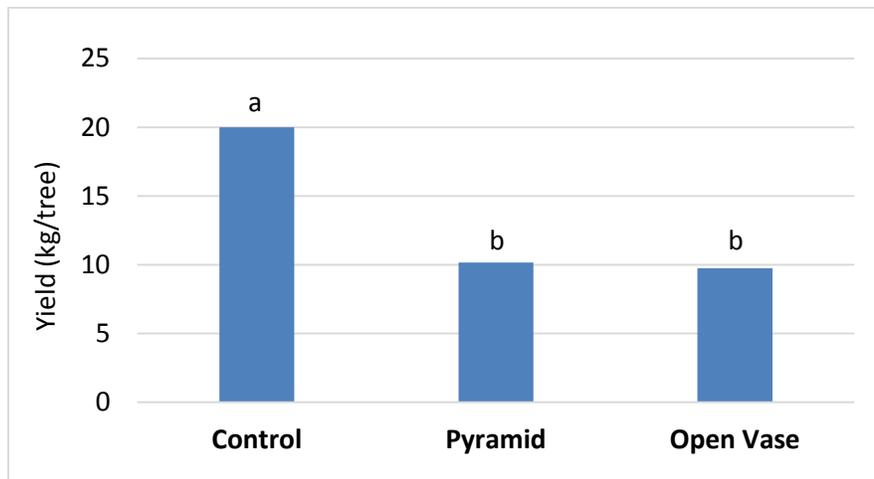


Figure 3. Effect of various pruning treatments on yield (kg/tree) of 3-year old 'Nadorcott' trees in 2017. Different letters above columns denote significant differences between treatment means (P < 0.05).

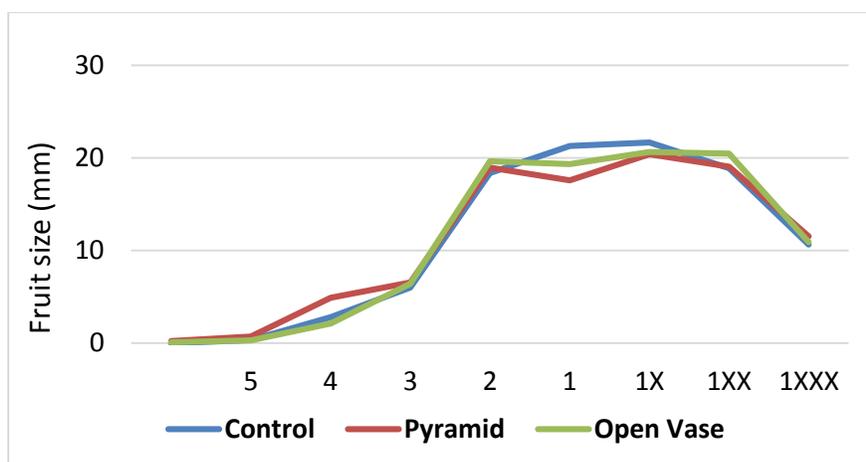


Figure 4. Effect of various pruning treatments on fruit size distribution of 3-year old 'Nadorcott' trees in July 2017.

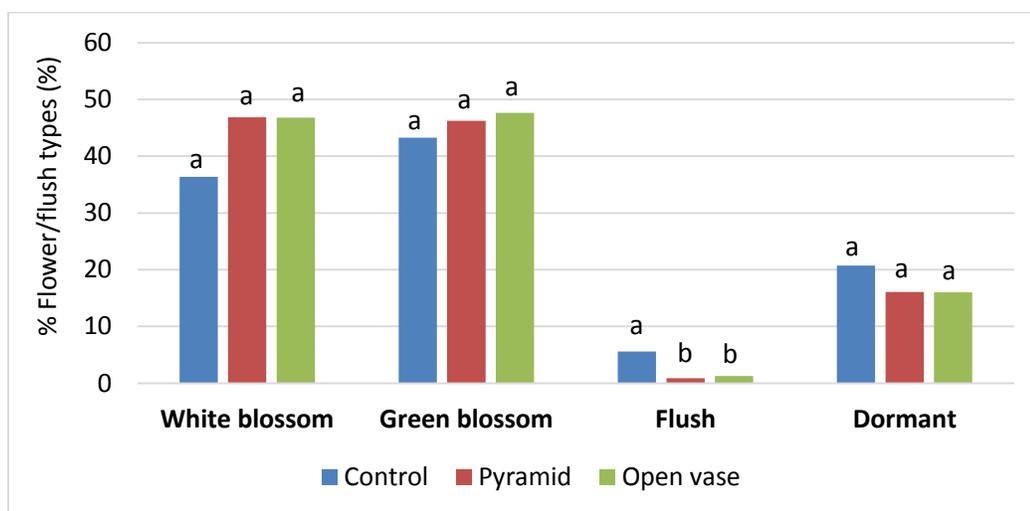


Figure 5. Effect of different pruning treatments on percentage flower/shoot type of 3-year old 'Nadorcott' trees in September 2017. Different letters above columns denote significant differences between treatment means ($P < 0.05$).

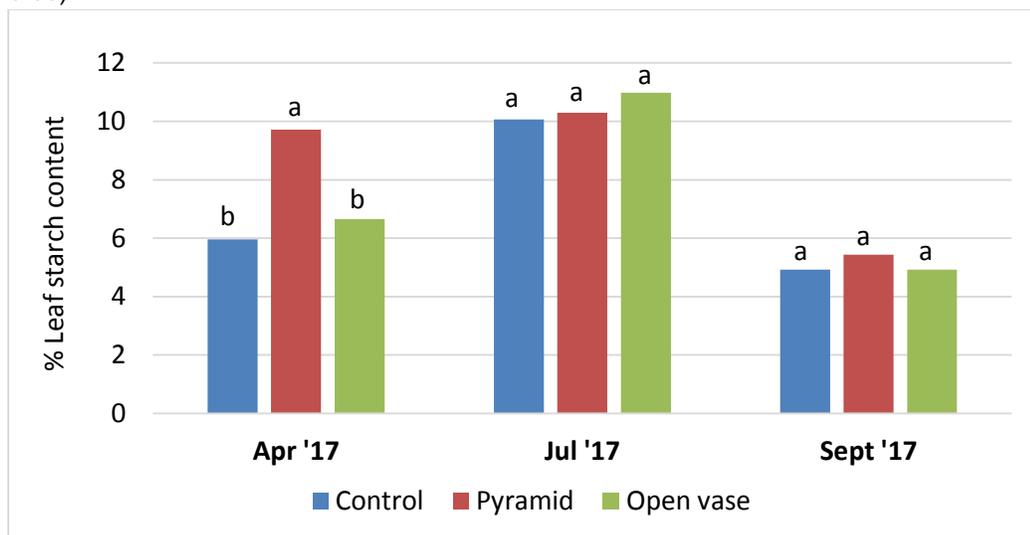


Figure 6. Leaf starch content of 3-year old Nadorcott trees in 2017. Different letters above columns denote significant differences between treatment means ($P < 0.05$).

Table 1. Pearson's correlation coefficients between various tree parameters in 2017.

Parameter	Yield 2017
Height reduction 2016	-0.50871 P=0.0041
% Flush Sep 2017	0.58167 P=0.0007

Pruning of young trees (2.5-year old at trial start; 2014-2017)

Removal of plant material

In the second trial, 2.5-year old 'Nadorcott' trees were pruned according to pyramid, open vase shape or left uncontrolled until intervention was needed. Figure 7 displays removed plant material after harvest (July) and during shoot control in January for the 2014 to 2017 seasons. The highest amount of plant material was removed in the Pyramid treatment, followed by the Open Vase and Control treatment (Table 2). As trees grew bigger more material had to be removed year on year (Table 2). No branches were removed in the Control during 2014/15, but some pruning had to be done during 2015 and more pruning during 2016. The more severe pruning was after harvest, the more vigorous was re-growth, i.e. water shoots in January (Fig. 7) confirming similar results in other studies (Tucker et al., 1994; Krajewski and Pittaway, 2000). Height reduction was on average 0.27 m for 2015 and 2016 (11.5%) and there were no statistical differences between treatments (data not shown). There were no statistical differences in stem circumference between treatments within a year, but a significant increase year on year independent of treatment as trees grew bigger, similar to the very young trees (data not shown).

Yield

Removal of plant material after harvest and strong re-growth had an effect on the next season's flowering and fruiting. The Control treatment, where no pruning was done in 2014, had a significantly higher yield with 45 kg/tree compared to around 30 kg/tree for the Pyramid and Open Vase treatments (Fig. 8). In 2016, however, yield in the Control was lower compared with the other treatments, although not significantly, followed by the highest yield in 2017 (Fig. 8). Despite the significantly higher yield in the Control (45.6 kg/tree) over all three years compared with the Open Vase (35.9 kg/tree) and higher yield compared with the Pyramid treatment (39.3 kg/tree), an alternate bearing pattern was observed in the Control trees but not in the other two treatments. On the contrary, yield in the Pyramid and Open Vase treatments increased year on year (Fig. 8). This might indicate that moderate pruning of young trees to form a productive canopy with strong bearing branches may reduce alternate bearing in this cultivar (Tucker et al., 1994).

Fruit size

Crop load influenced fruit size and fruit size distribution (Fig. 9-11; Table 3). The higher the yield, the lower was fruit size, a common and known phenomenon in many fruit crops (Guardiola, and Garcia-Luis, 2000; Krajewski and Pittaway, 2000; Khurshid and Krajewski, 2013). This was also shown with the negative correlation between yield in 2017 and fruit size in 2015 and mean yield (2015-17) and mean fruit size (2015-17), which were highly significant ($r=-0.66512$; $P<0.0001$ and $r=-0.6730$; $P<0.0001$, respectively). Where yield between treatments was similar, such as in 2016, no statistical differences in fruit size were evident. Mean plant removal (2014-16) and mean fruit size (2015-17) were also significantly correlated ($r=0.61626$; $P=0.0003$) revealing that heavy pruning influences crop load and thus fruit size either through removing too much bearing wood or due to high vegetative re-growth. Over all the three seasons, Control fruit were significantly smaller than fruit of the other two treatments (Table 3).

Return bloom and fruit set

After the first harvest in 2015, flowering pattern were monitored. Table 4 displays the various phenological stages during the flowering period for 2015-2016. In 2015, after a high yield, the Control trees has significantly lower percentage white blossom and significantly higher percentage dormant internodes per shoot, and less green

blossoms compared to the other treatments. Correlations performed on the 2015 data also confirmed the interaction between crop load and return bloom with highly significant results (Table 6). Similar results were found by other researchers (Mazhar et al., 2007; Verreynne and Lovatt, 2009; Martinez-Fuentes et al., 2010; Shalom et al., 2012). It is also interesting to note that in both flowering seasons, percentage flushing and dormant internodes were highest, and green blossoms lowest in the Control trees compared to the other treatments (Table 4). Despite difference in flowering/flushing intensities, there were no significant differences in fruit set between treatments, although the Pyramid treatment showed slightly higher fruit set in both years (Table 5). Overall fruit set, irrespective of treatment, was significantly higher in 2015 than in 2016.

Starch reserves

Crop load, fruit size and flowering pattern influenced starch reserves of the trees. Figure 12 shows percentage leaf starch content throughout the trial period. High crop load reduced starch reserves during fruit development, which can especially be seen in the Pyramid treatment during 2016 and the Control treatment during 2017 (Fig. 12). There was a slight recovery in starch reserves towards harvest for both on- and off-years. It appears that younger trees, which still have a moderate yield, start to recover their starch reserves already around harvest. However, leaf starch reserves overall were relatively low in both years. When comparing years, it can also be seen that years with higher yields, e.g. 2017, had on average lower starch reserves compared to years with lower yields, e.g. 2016, indicating the high use of reserves for the crop (Fig. 12; horizontal lines). In 2015, highly significant correlations between leaf starch in June and yield and fruit size could be found (Table 6). In 2017, significant negative correlations between yield and leaf starch in January ($r=-0.46734$; $P=0.0106$), April ($r=-0.5293$; $P=0.0031$) and July (-0.62295 ; $P=0.0003$) were observed, illustrating the high demand for reserves by the developing crop. This is confirmed in other studies (Tucker et al., 1994; Goldschmidt, 1999; Iglesias et al., 2003; Khurshid and Krajewski, 2010; Van der Merwe, 2012). Furthermore, significant positive correlations between leaf starch in June and return bloom, flush and dormant internodes in 2015 demonstrate the importance of starch reserves at harvest for the new flowering season (Table 6). Managing the effects of pruning on crop load and starch reserves will be the key to sustainable production with little alternate bearing.

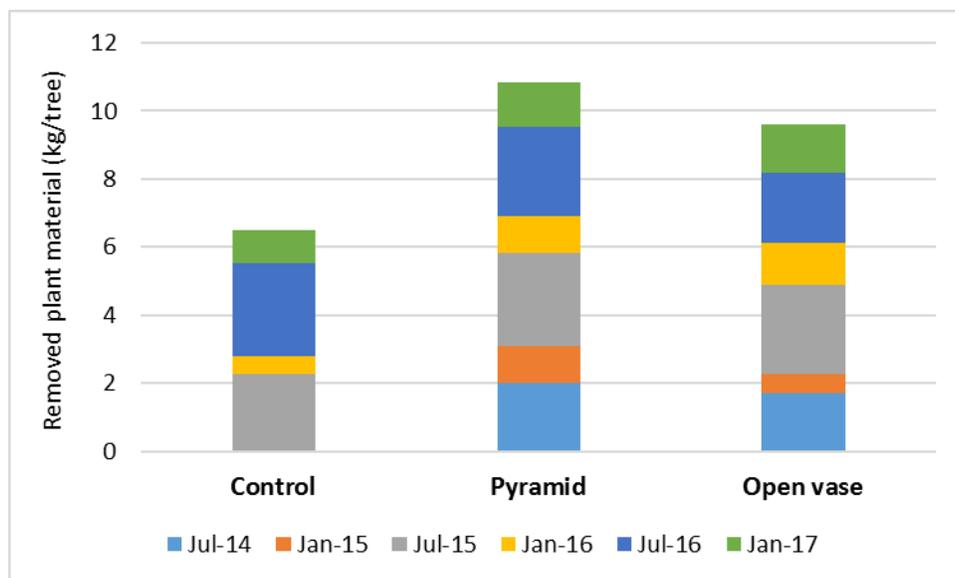


Figure 7. Removed plant material in various pruning treatments and times of young ‘Nadorcott’ trees during the 2014/15, 2015/16 and 2016/17 seasons.

Table 2. Plant material removed per growing season (post-harvest pruning to harvest) for young ‘Nadorcott’ trees.

Treatment	Mean removed plant material (kg/tree)			Mean per treatment 2015-17 (kg)
	2014/15	2015/16	2016/17	
Control	2.2	2.8	2.8	2.6
Pyramid	2.0	2.8	2.8	2.5
Open vase	1.8	2.8	2.8	2.5

Control	0.00 b	2.79 a	3.70 a	2.16 b
Pyramid	3.10 a	3.82 a	3.92 a	3.61 a
Open Vase	2.26 a	3.88 a	3.47 a	3.20 ab
Overall mean per year (kg)	1.78 b	3.49 a	3.70 a	

Means per year and over three years followed by different letters differ significantly at $P < 0.05$.

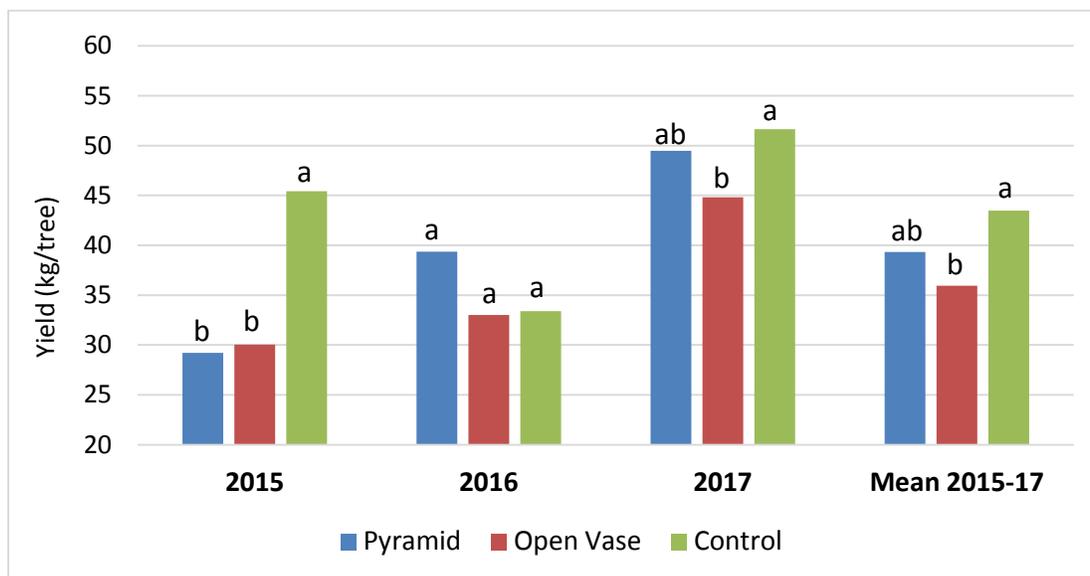


Figure 8. Effect of various pruning treatments on yield (kg/tree) of young 'Nadorcott' trees at harvest time in July 2015, 2016 and 2017. Different letters above columns denote significant differences between treatment means per year and mean over years ($P < 0.05$).

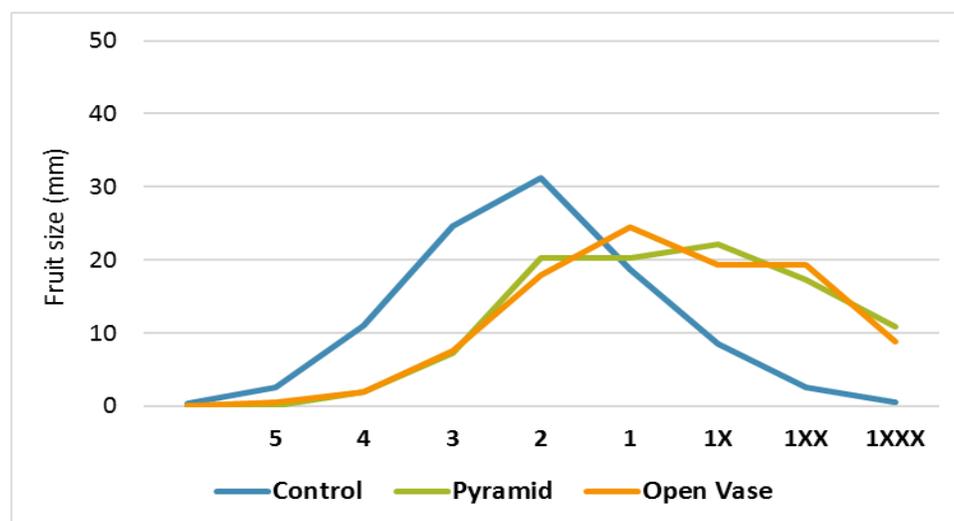


Figure 9. Effect of various pruning treatments on fruit size distribution of young 'Nadorcott' trees in July 2015.

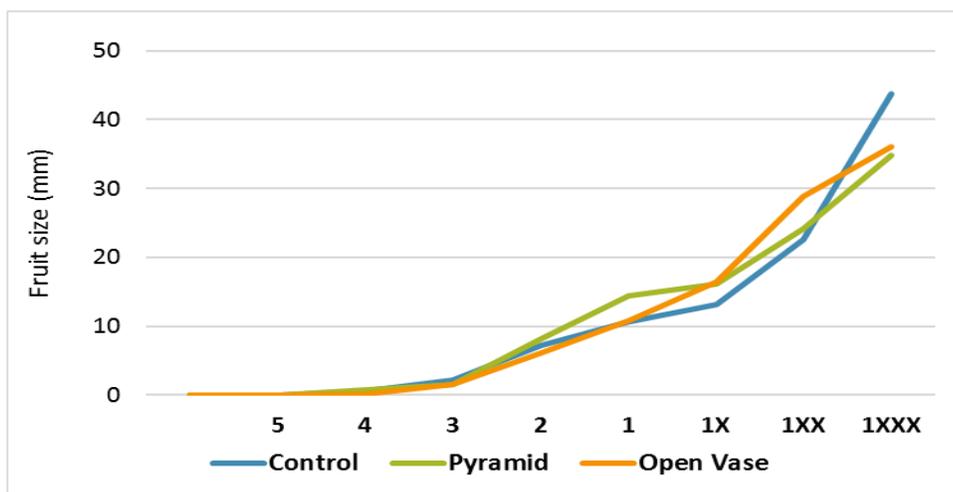


Figure 10. Effect of various pruning treatments on fruit size distribution of young 'Nadorcott' trees in July 2016.

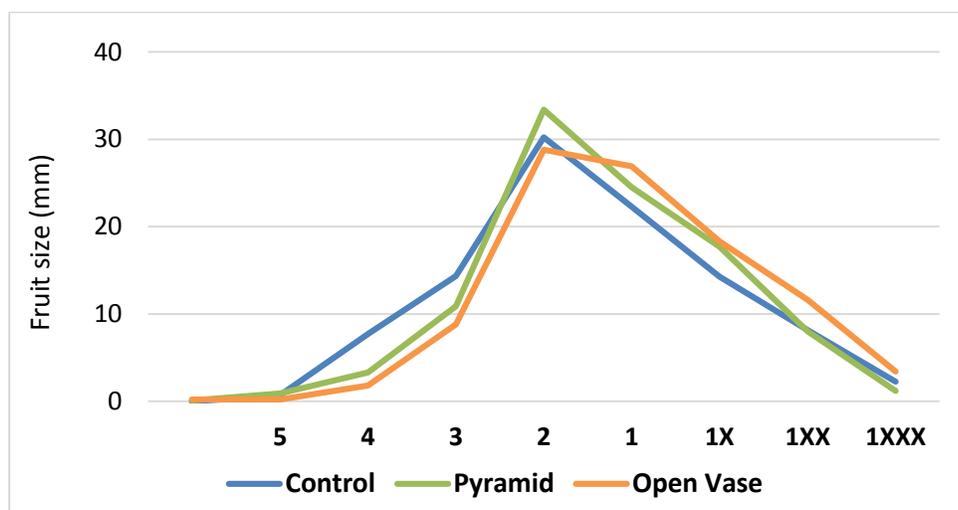


Figure 11. Effect of various pruning treatments on fruit size distribution of young 'Nadorcott' trees in July 2017.

Table 3. Effect of various pruning treatments on fruit size of young 'Nadorcott' trees in 2015, 2016 and 2017.

Treatment	2015	2016	2017	Mean 2015-17 (mm)
Control	61.07 b	76.79 a	63.44 b	66.54 b
Pyramid	68.53 a	74.86 a	64.49 ab	69.29 a
Open Vase	68.80 a	75.60 a	65.84 a	70.08 a

Means per year and over three years followed by different letters differ significantly at $P < 0.05$.

Table 4. Effect of various pruning treatments on return bloom of young 'Nadorcott' trees.

Treatment		% White blossom	% Green blossom	% Flush	% Dormant
Control	2015	14.53 b	39.43 a	17.58 a	37.92 a
	2016	74.89 a	10.63 b	2.33 a	12.15 a
	Mean 2015-16	44.71 a	25.03 b	9.96 a	25.04 a
Pyramid	2015	31.78 a	44.67 a	12.19 a	27.53 ab

	2016	67.37 a	19.97 ab	0.59 a	12.05 a
	Mean 2015-16	49.57 a	32.32 ab	6.39 a	19.79 a
Open Vase	2015	26.96 ab	51.97 a	7.03 a	25.46 b
	2016	65.92 a	21.73 a	0.41 a	11.93 a
	Mean 2015-16	46.44 a	36.85 a	3.72 a	18.70 a

Means per year and over two years followed by different letters differ significantly at $P < 0.05$.

Table 5. Effect of various pruning treatments on percentage fruit set of young 'Nadorcott' trees.

Treatment	% Fruit set		Mean over years 2015-16
	2015	2016	
Control	58.98 a	18.72 a	38.85 a
Pyramid	60.41 a	23.55 a	41.98 a
Open Vase	50.22 a	21.31 a	35.76 a
Mean per year	56.54 a	21.19 b	

Means per year and over two years followed by different letters differ significantly at $P < 0.05$.

Table 6. Pearson's correlation coefficients and significance levels of various tree parameters in 2015.

Parameter	Yield 2015	%White blossom 2015	%Green Blossom 2015	%Flush 2015	%Dormant 2015	Fruit size 2015
Leaf starch Jun 2015	-0.84012 P<0.0001	0.72787 P<0.0001	0.52367 P=0.0036	-0.58183 P=0.0009	-0.65363 P=0.0001	0.65188 P=0.0001
Yield 2015		-0.75999 P<0.0001	-0.41115 P=0.0240	0.45738 P=0.0110	0.64463 P=0.0001	-0.66519 P<0.0001

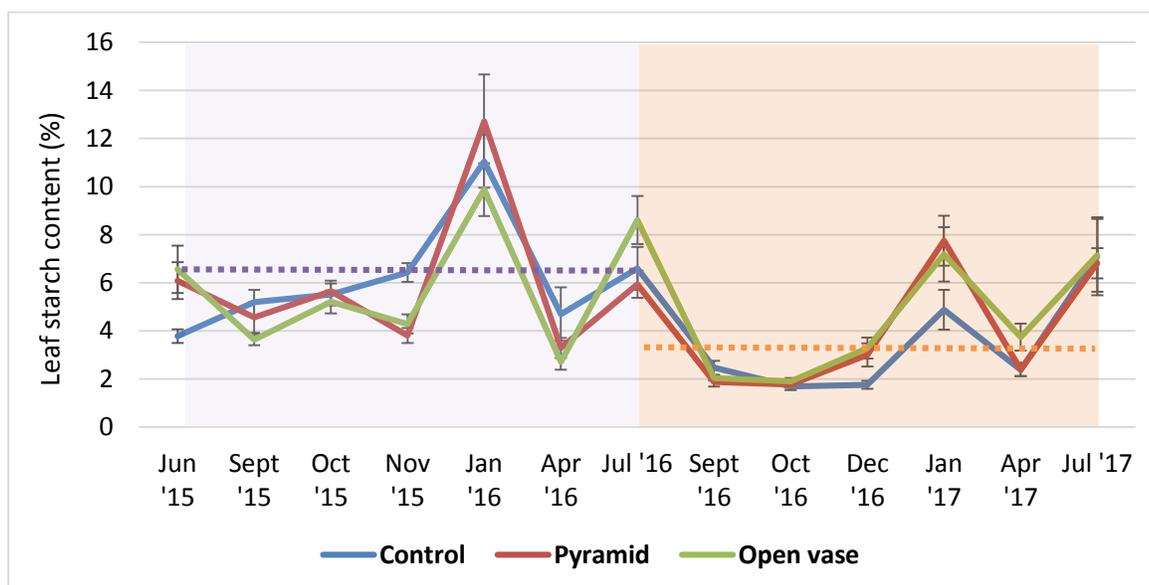


Figure 12. Effect of various pruning treatments on leaf starch content of young 'Nadorcott' trees between June 2015 and July 2017, indicating off- (blue field) and on-years (orange field) and overall mean leaf starch content per season (.....; 2015/16 season: 6.1%; 2016/17 season: 3.8%). Bars indicate the value means ($n = 10$) \pm standard error (SE).

Pruning of old trees (8-year old at trial start; 2014-17)

Removal of plant material

Due to the overgrown stage of the trees, pruning was very challenging. More branches had to be removed in August 2014 than initially planned. Most plant material was removed by the two 'Selective after harvest' treatments (Fig. 13; Table 7). In the following years, less material was removed because of the high thinning effect of the previous season's pruning. Overall, more water shoots had to be removed for the two 'Selective after harvest' treatments due to the severe pruning after harvest.

The planned pruning of the 'Selective after fruit drop' treatment could not be done in November 2015 as the pruning contractors pruned these trees in August although they were clearly marked. Therefore, the removed amount of plant material in this treatment does not reflect the true amount removed. The lowest amount of plant material over all three years was removed in the 'Light selective after harvest' and the 'Mechanical after pruning' treatment.

Height reduction was between 14 and 33.5% in 2014, between 0.6 and 12.1% in 2015, and between 8.8 and 14.7% in 2016 (data not shown). There was no significant influence of pruning on stem circumferences between treatments (data not shown).

Yield

Figure 14 displays yield data for 2014 (before trial start) to 2017. Yield was recorded for 2014 to determine an alternate bearing pattern. The 'Light selective' treatment was only included from 2015 onwards, hence no data is available for 2014 for this treatment.

After the first year of pruning, the highest yield was seen in the 'Selective after fruit drop' treatment, followed by the Control. The other treatments had similar yields around 15 kg/tree. These low yields were not only a result of pruning but also as a result of alternate bearing (high yield in 2014) since both light and heavy pruned trees showed low yields. However, as seen with the younger trees, lighter pruning had less effect on flowering and crop load compared with more severe pruning. Despite yield difference between treatments in 2014 and 2015, they were not different. In 2016, yields varied between 79 and 102 kg/tree, with the Control and 'Mechanical after harvest' treatments having the highest yield and the 'Selective after harvest' treatments having significantly lower yields. In 2017, yields decreased compared to 2016, clearly showing alternate bearing (Fig. 14). The Control and 'Selective after fruit drop' treatments showed the highest overall mean yield (2015-17). Although these treatments had more plant material removed than some of the other treatments, the type of cuts (medium branches) made were clearly less invasive for sustainable yields. 'The Light selective' pruning for instance where very few but bigger branches were removed, could not achieve sustainable high yields despite the low amount of plant material removed and low percentage re-growth. Higher canopy density in this treatment could have been a reason for its poor performance. This confirms that moderate pruning of old, overgrown 'Nadorcott' trees does not create enough light inside the tree canopy for good flowering and cropping. Therefore, a good balance in the removal of bigger and smaller branches, all of which inhibit light to enter the canopy, is necessary to re-gain or maintain good cropping (Tucker et al., 1994; Wheaton et al., 1995). Over the three seasons, none of the treatments were able to reduce alternate bearing. Longer trial periods are necessary to determine which pruning strategy has the potential to reduce alternate bearing with time. However, not only pruning but also other management practices, such as irrigation and fertilization, have to be adjusted to reduce and minimize alternate bearing, especially for old trees that already show high alternate bearing.

Fruit size

As for the young trees, crop load also affected fruit size. Although fruit size distribution was similar for all treatments in 2015, slightly more fruit could be found in the three biggest size classes for the treatments with the more severe pruning, i.e. 'Severe' and 'Light selective after harvest' and 'Mechanical after harvest' (Fig. 15). In 2016, however, differences in fruit size were more prominent. The 'Selective after harvest' treatment, which had the lowest yield, had bigger fruit compared with the other treatments, with 25% of the fruit being in the biggest size class (Fig. 16). This once again confirmed other research reports on crop load affecting fruit size (Guardiola, and Garcia-Luis, 2000; Krajewski and Pittaway, 2000; Van der Merwe, 2012; Khurshid and Krajewski, 2013). There were no differences in fruit size distribution among the other treatments. Fruit size distribution in 2017 was similar to 2016,

except for the Light selective treatment having the highest percentage fruit in the bigger fruit size classes (Fig. 17). Table 8 displays mean fruit size per year and overall mean.

Return bloom and fruit set

Flowering data was only taken in 2015 and 2016 and is displayed in Table 9. In 2015, an off-year, few difference in return bloom between treatments were observed. Percentage white blossom was considerably higher compared with percentage green blossom in all treatments. The 'Mechanical after harvest' treatment showed highest percentage white blossoms and lowest percentage flushing and dormant internodes. The 'Selective after harvest' treatment showed highest percentage green blossom. In 2016, an on-year, differences in return bloom were more pronounced. Treatments with high yields in the previous season, such as Control, 'Selective after fruit drop' and 'Light selective after harvest', had higher flushing and dormant internodes compared with lower yielding treatments, such as 'Selective after harvest' and 'Selective year 1, Mechanical year 2'. The lower yielding treatments also had higher percentage white and green blossom compared to higher yielding treatments. This confirms data obtained in the young trees and demonstrates the inhibitory effect crop load has on flowering (Verreyne and Lovatt, 2009; Martinez-Fuentes et al., 2010; Muños-Fambuena et al., 2011; Shalom et al., 2012). There were no significant differences in fruit set in 2015 (Table 9). In 2016, fruit set was highest for the 'Selective after harvest' treatment, which also had the highest percentage white and green blossom, while the Control had the lowest fruit set (Table 10). There is not enough data available to draw sound conclusions on the effect of flower type on fruit set. Besides, climatic effects also influence fruit set.

Starch reserves

Leaf starch reserves in old 'Nadorcott' trees followed similar trends to young trees. In off-years (2014/15 and 2016/17) starch reserves in leaves were higher compared with on-years (2015/16) (Fig. 18). High crop load reduced starch reserves during fruit development and at harvest irrespective of the alternate bearing cycle. For example, in 2015, the 'Selective after fruit drop' treatment had the highest yield but the lowest leaf starch reserves. Likewise, the 'Selective after harvest' and Control treatments had the highest yields in 2017, but lowest starch reserves during fruit development. Within a season, there was a build-up of starch reserves after harvest, followed by a slight decrease just during and after the fruit drop period, another increase during early fruit development and another decrease during rapid fruit growth. This was true for all three seasons. Unlike with young trees, there was no or hardly any recovery in starch reserves towards harvest. However, overall starch reserves were higher in old trees compared to young trees (Fig. 12). As mentioned before, pruning influences re-growth and cropping potential, and therefore starch reserves.

Due to the high variation in the data, Pearson's product-moment correlation only yielded weak correlations and data is therefore not shown.

In order to evaluate the potential of the treatments as practical and economical pruning practices a principle component analysis (PCA) was performed with the parameters yield, fruit size, leaf starch at harvest and plant removal amount and three axes. The PCA explains 81% of the differences between treatments. The Control treatment lies separate from the other treatment and is clearly associated with yield in all three years (Fig. 19). The treatments with the lowest plant removal rates ('Selective after fruit drop', 'Light selective' and 'Mechanical after harvest') were closely associated with each other and negatively correlated with removal rates for all years. On the other hand, the 'Selective after harvest' and 'Selective year 1, Mechanical year 2' treatments, which had the highest removal rates, were closely associated with removal rates and fruit size in all years in the biplot. These treatments were also negatively correlated to yield. To summarize, high removal of branches after harvest caused reduction in yield, increase in fruit size and affected return bloom and alternate bearing.

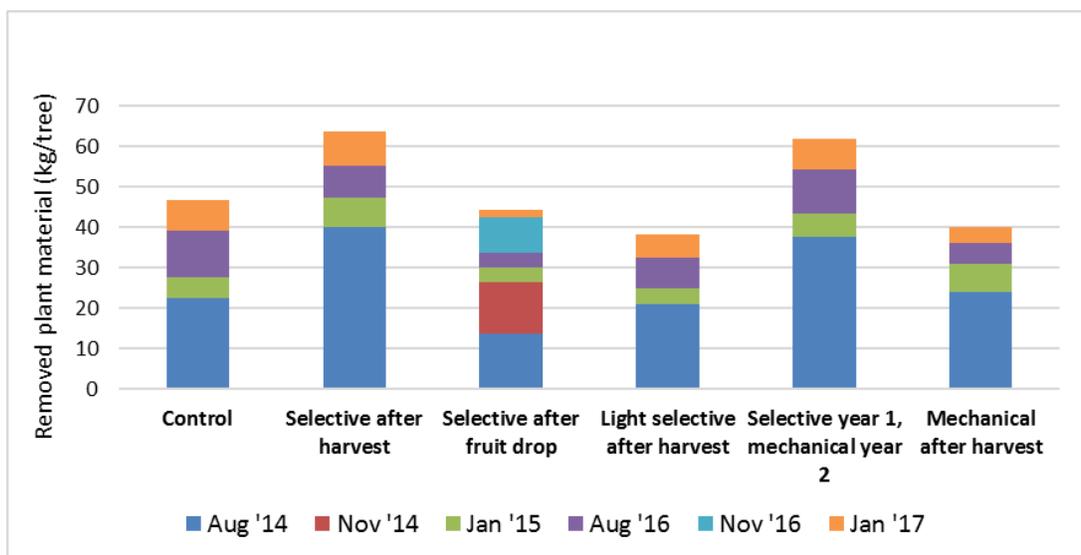


Figure 13. Removed plant material of three pruning season of old 'Nadorcott' trees between August 2014 and January 2017.

Table 7. Plant material removed per growing season (post-harvest pruning to harvest) for old 'Nadorcott' trees.

Treatment	Mean removed plant material (kg/tree)			Mean per treatment 2015-17 (kg)
	2014/15	2015/16	2016/17	
Control	27.61 b	9.81 a	19.16 a	21.52 bc
Selective after harvest	47.44 a	12.08 a	16.28 a	28.65 a
Selective after fruit drop	30.17 b	0.6 c *	16.12 ab	19.21 bcd
Light selective after harvest	24.95 b	6.44 abc	13.33 ab	16.95 cd
Selective year 1, mechanical year 2	43.49 a	9.08 ab	18.32 a	23.51 b
Mechanical after harvest	30.95 b	3.31 bc	9.09 b	15.73 d
Overall mean per year (kg)	34.10 a	12.66 b	15.66 b	

Means per year and over three years followed by different letters differ significantly at $P < 0.05$.

*November data missing due to pruning contractors pruning data trees without permission

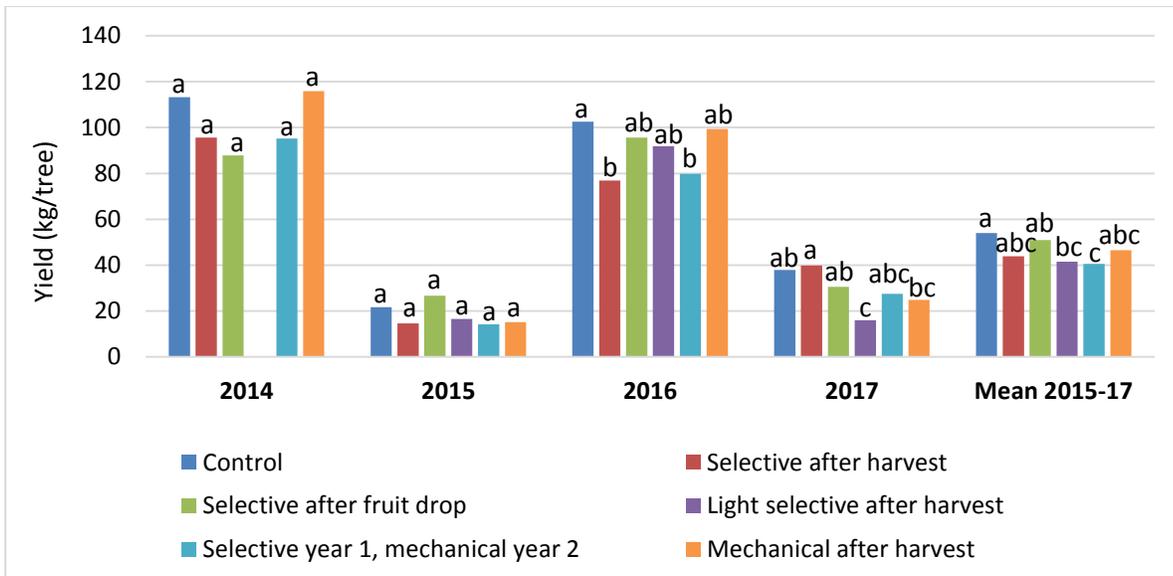


Figure 14. Effect of various pruning treatments on yield (kg/tree) of old 'Nadorcott' trees from 2014 (before trial start) to 2017. Different letters above columns denote significant differences between treatment means per year and mean over years ($P < 0.05$).

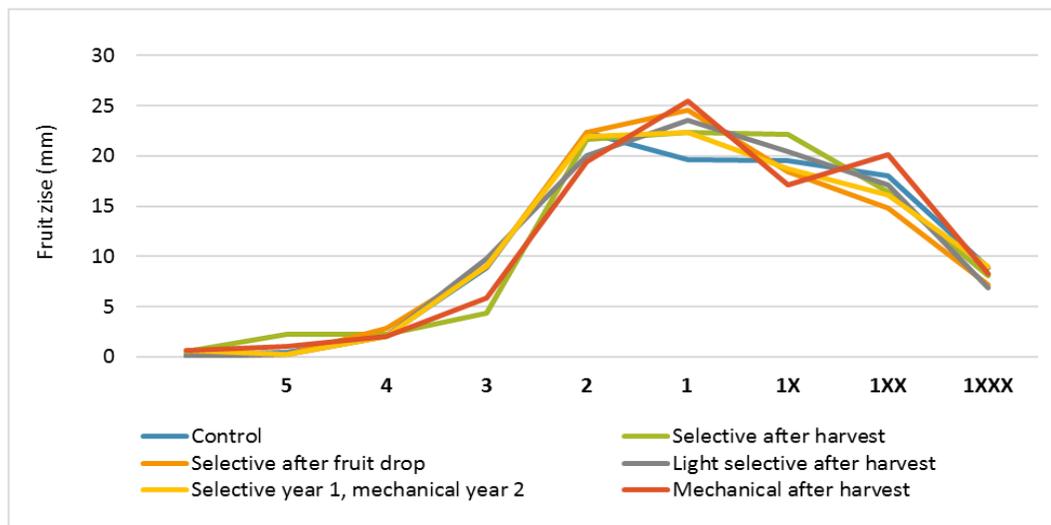


Figure 15. Effect of various pruning treatments on fruit size distribution of old 'Nadorcott' trees in July 2015.

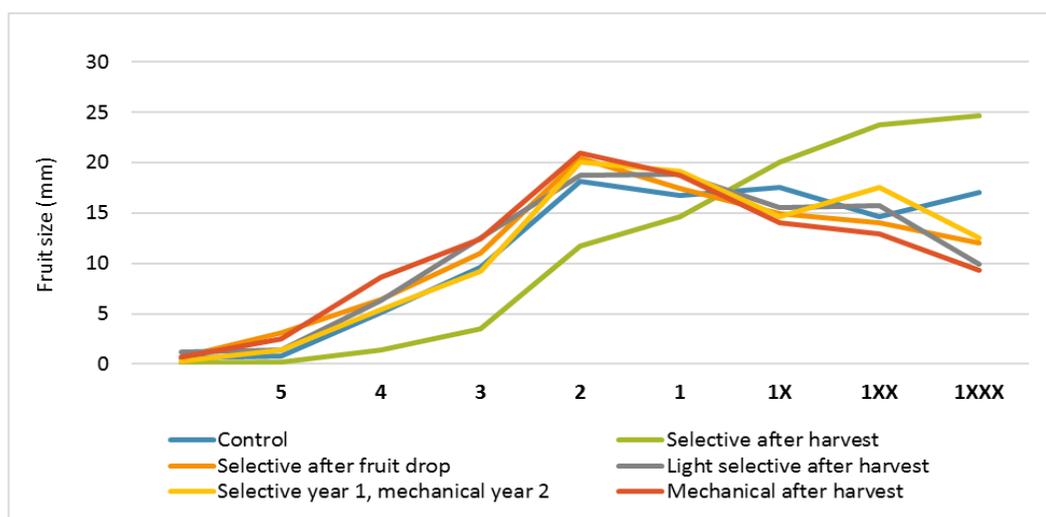


Figure 16. Effect of various pruning treatments on fruit size distribution of old 'Nadorcott' trees in July 2016.

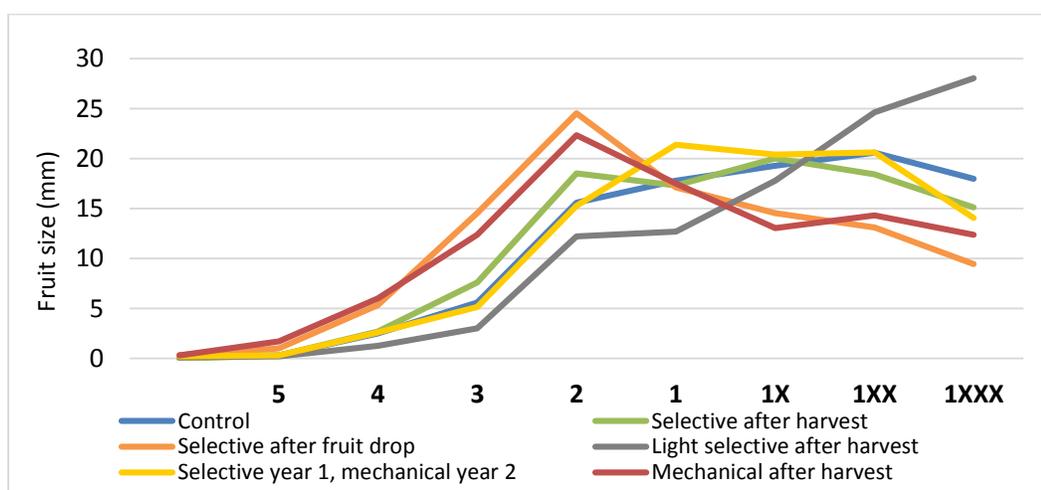


Figure 17. Effect of various pruning treatments on fruit size distribution of old 'Nadorcott' trees in July 2017.

Table 8. Effect of various pruning treatments on fruit size of old 'Nadorcott' trees in 2015, 2016 and 2017.

Treatment	2015	2016	2017	Mean 2015-17 (mm)
Control	67.65 a	68.69 ab	70.06 ab	68.79 ab
Selective after harvest	67.42 a	72.12 a	69.12 ab	69.61 a
Selective after fruit drop	66.82 a	66.47 b	65.99 b	66.48 b
Light selective after harvest	67.21 a	66.43 b	72.71 a	68.98 ab
Selective year 1, mechanical year 2	67.35 a	67.74 ab	69.39 ab	68.16 ab
Mechanical after harvest	67.77 a	65.55 b	66.82 b	66.71 b

Means per year and over three years followed by different letters differ significantly at $P < 0.05$.

Table 9. Effect of various pruning treatments on return bloom of old 'Nadorcott' trees in 2015 and 2016.

Treatment		% White blossom	% Green blossom	% Flush	% Dormant
Control	2015	76.66 ab	16.5 b	1.38 a	12.55 a
	2016	11.30 b	10.73 bc	39.58 a	33.08 a

	<i>Mean 2015-16</i>	<i>57.99 b</i>	<i>15.03 b</i>	<i>12.29 ab</i>	<i>18.41 a</i>
Selective after harvest	2015	70.83 b	28.29 a	2.06 a	12.57 a
	2016	33.25 a	22.38 ab	19.6 b	25.73 a
	<i>Mean 2015-16</i>	<i>60.09 b</i>	<i>26.6 a</i>	<i>7.07 bc</i>	<i>16.33 a</i>
Selective after fruit drop	2015	78.02 ab	19.38 ab	0.97 a	9.77 a
	2016	26.28 ab	7.8 c	28.35 ab	16.85 a
	<i>Mean 2015-16</i>	<i>63.24 b</i>	<i>16.07 b</i>	<i>8.79 abc</i>	<i>14.65 a</i>
Light selective after harvest	2015	79.14 ab	19.08 ab	2.01 a	10.13 a
	2016	11.75 b	14.78 ab	39.85 a	34.08 a
	<i>Mean 2015-16</i>	<i>59.89 b</i>	<i>17.85 b</i>	<i>12.82 a</i>	<i>16.97 a</i>
Selective year 1, mechanical year 2	2015	76.59 ab	19.05 ab	1.04 a	11.52 a
	2016	42.15 a	24.63 a	18.25 b	16.33 a
	<i>Mean 2015-16</i>	<i>66.75 b</i>	<i>20.64 ab</i>	<i>5.96 cd</i>	<i>12.89 ab</i>
Mechanical after harvest	2015	81.71 a	18.35 b	0.99 a	8.27 a
	2016	*	*	*	*
	<i>Mean 2015-16</i>	<i>81.71 a</i>	<i>18.35 b</i>	<i>0.99 d</i>	<i>8.27 b</i>
Overall Mean 2015-16	2015	24.95 b	20.15 a	1.41 b	10.80 b
	2016	77.16 a	16.06 a	29.13 b	27.21 a

Means per year and over two years followed by different letters differ significantly at $P < 0.05$.

*Missing data

Table 10. Effect of various pruning treatments on percentage fruit set of old 'Nadorcott' trees in 2015 and 2016.

Treatment	% Fruit set		Mean over years 2015-16
	2015	2016	
Control	45.82 a	28.54 bc	37.18 bc
Selective after harvest	42.48 a	56.45 a	49.49 ab
Selective after fruit drop	45.13 a	46.99 ab	46.06 ab
Light selective after harvest	45.36 a	62.5 a	52.98 a
Selective year 1, mechanical year 2	43.88 a	37.50 ab	40.69 abc
Mechanical after harvest	50.76 a	9.38 c	32.37 c
Mean per year	45.57 a	40.53 a	

Means per year and over two years followed by different letters differ significantly at $P < 0.05$.

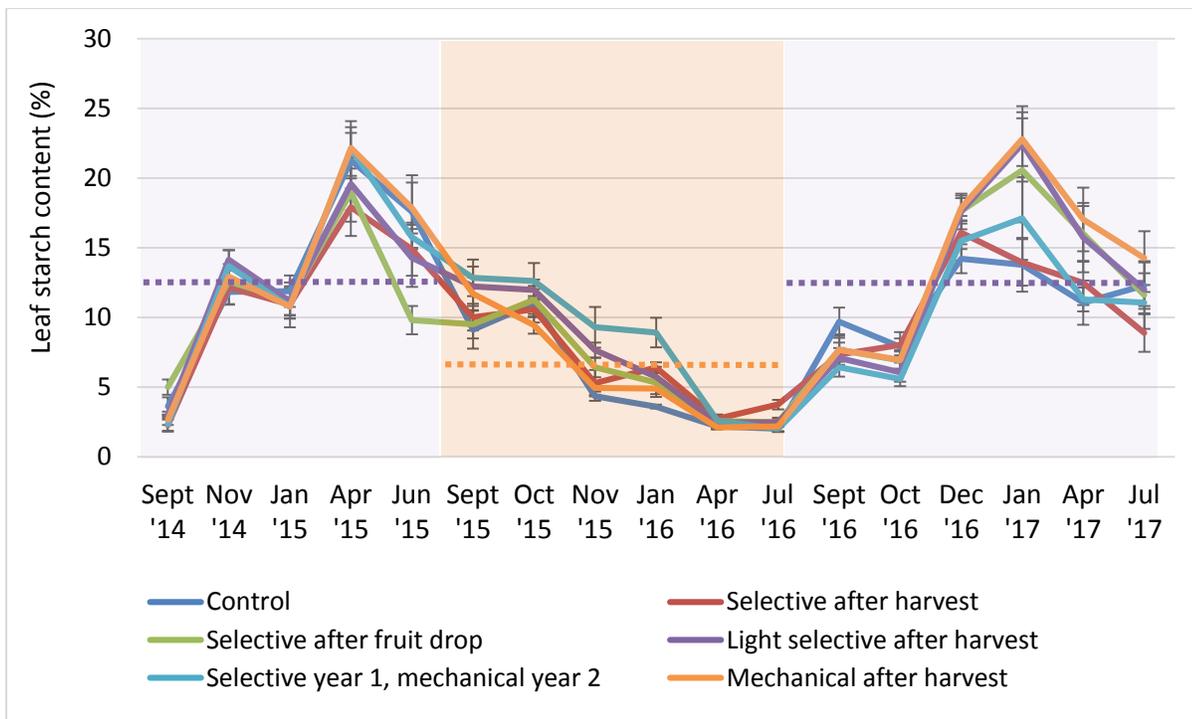


Figure 18. Leaf starch content of various pruning treatments on young Nadorcott trees between June 2015 and July 2017, indicating off- (blue fields) and on -years (orange field) and overall mean leaf starch content per season (; 2014/15 season: 12.5%; 2015/16 season: 6.5%; 2016/17 season: 12.5%). Bars indicate the value means (n = 10) ± standard error (SE).

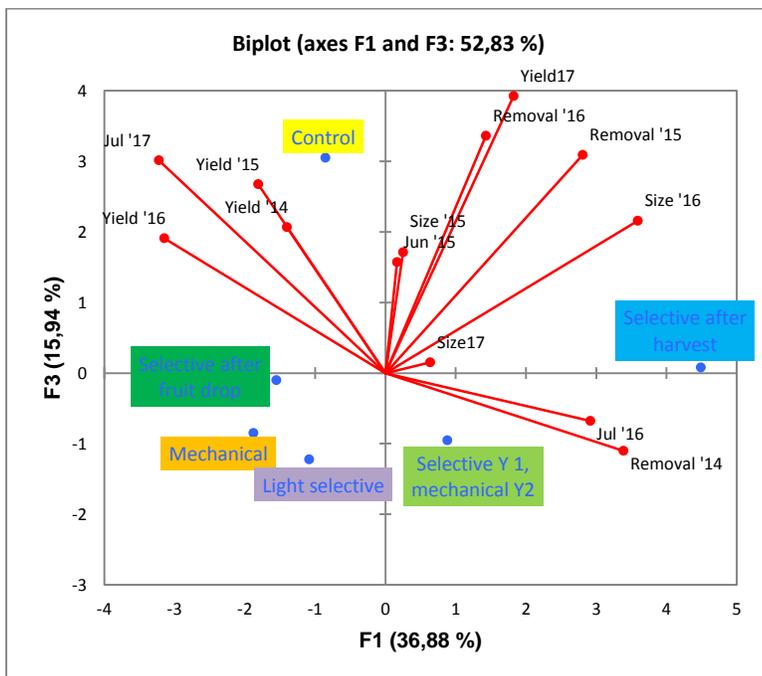


Figure 19. Principle Component Analysis between growth parameters (yield, leaf starch at harvest, fruit size and plant removal amount) and treatments for the entire trial period.

Conclusion

Various pruning strategies for 'Nadorcott' trees revealed that early shaping of newly established, non-bearing trees showed little carry-over effect due to the vigorous growth of this cultivar. The Pyramid treatment maintained the best tree shape. Until trees have filled their allocated space in the row, it appears more beneficial to remove only branches that cross each other and strong water shoots.

For young and older trees, thinning out of big branches was essential to improve light penetration and fruit quality. The Pyramid treatment appeared to be most beneficial for 'Nadorcott' as it reduced overhangs that produced shade and reduced fruit colouring. Less pruning produced higher yields and smaller fruit size and vice versa. The higher the crop load, the lower were starch reserves at harvest, which affected return bloom. Alternate bearing patterns as results of the previous year's pruning were also clearly visible and pruning should therefore start during an expected on-year to minimize alternate bearing and improve fruit quality.

Considering all available results, it can be concluded that selective hand pruning is preferred above mechanical pruning, although it should not be severe. Mechanical pruning creates a dense canopy (reduces light) and is therefore not recommended as pruning method after harvest. However, it can be considered to reduce tree height after fruit set.

Moderate removal of mainly medium and some big branches, like in the Control, appeared to be most beneficial especially for older, overgrown trees in the long-term without a significant impact on yield and fruit size. However, none of the treatments on old trees were able to reduce alternate bearing during the trial period and a long-term study is necessary to evaluate the potential of pruning strategies on reduction of alternate bearing.

Future research

'Nadorcott' is a vigorous growing cultivar and is susceptible to alternate bearing, although this behaviour can differ depending on the climatic area it has been planted. In order to fully understand and draw clear conclusions on the effect of various pruning strategies on alternate bearing, carbohydrate reserves and sustainable cropping potential of the cultivar further studies under different climatic conditions (warmer and cooler areas) are recommended. Additional pruning techniques and times currently used by growers showing potential, should be included in such future work. A more detailed study and evaluation on pruned branch sizes could also shed some light on the percentage of certain branches that may be removed in order to maintain production, achieve adequate and high percentage of marketable fruit size and reduce alternate bearing.

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Technology transfer

Papers presented at symposia:

Cronje, R.B., Human, C.F. and Ratlapane, I.M. 2016. Effect of various pruning strategies on fruit production of Nadorcott mandarin. CRI Research Symposium, Drakensberg, 22-24 August 2016.

Human, C.F., Cronje, R.B. and Ratlapane, I.M. 2017. Effect of different pruning methods on fruit production of Nadorcott mandarin. SASAT Conference, Cape St. Francis, 5-8 September 2017

Publications are planned in journals (e.g. South African Fruit Journal) and presentations at study groups/workshops (as required by the industry).

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4.3.3 **FINAL REPORT: Determining the time and duration of flower induction in early vs late mandarin cultivars and evaluating the effect of hand thinning, pruning and girdling on leaf and root carbohydrate levels, fruit size, vegetative regrowth and alternate bearing in 'Nadorcott' mandarin.**

Project 1106 (Apr 2014-Mar 2018) by Jakkie Stander and Paul Cronje (CRI at SU)

Summary

The objective of this project was to pinpoint and compare the time and duration of flower induction in mandarins; by measuring the flowering inhibition response to GA₃ applications at different times throughout the expected flower induction period. The project also evaluated manipulations of vegetative and reproductive growth to change carbohydrate allocation and/or restore carbohydrate levels and reduce the effect of endogenous gibberellins on flower induction. 'Nadorcott' mandarin trees were used in experiments to establish whether there are significant treatment effects on carbohydrate availability that correlate with the following season's fruit load and quality. The treatment effects on problems such as small fruit size and vigorous vegetative regrowth were quantified throughout.

Opsomming

Die doel van hierdie projek was om die tyd en durasie van blominduksie in mandaryne te bepaal en te vergelyk deur die blomreaksie op verskillende GA₃ toedienings tydens die verwagde blominduksie periode te meet. Daarna is verskillende manipulasies van vegetatiewe, sowel as reprodktiewe groei vanaf Januarie tot April ge-evalueer met die doel om koolhidraat allokasie tussen sinkorgane te manipuleer en/of om koolhidraatvlakke te herstel en die inhiberende effek van interne gibereliene op blominduksie vanaf Mei tot Augustus te verminder. 'Nadorcott' mandaryn bome is in eksperimente gebruik om vas te stel of daar enige betekenisvolle effek van behandelings op blaar- en wortel koolhidraat-vlakke is en dit is gekorreleer met vruglading en kwaliteit. Behandelingseffekte is ook addisioneel evalueer op probleme soos klein vrugte en aggresiewe vegetatiewe groei.

Introduction

Alternate bearing is a phenomenon occurring in a variety of horticultural crops (Monselise and Goldschmidt, 1982), in citrus specifically Mandarin hybrids (Monselise et al., 1981). Alternate bearing is characterized by a year of high fruit load ("on" year), followed by a year of low fruit load ("off" year). In studies on alternate bearing in citrus the emphasis has been on carbohydrate availability during flowering, fruit set and fruit growth, as well as manipulations to ensure sufficient supply to developing sink organs during this critical period, stretching from October to January in the Southern Hemisphere ("nutritional hypothesis"). However, by measuring leaf carbohydrate levels, Van der Merwe (2012) was able to compare the carbohydrate status of "on" and "off" 'Nadorcott' mandarin trees, a cultivar particularly prone to alternate bearing, throughout two seasons. He reported that at onset of flowering, both "on" and "off" trees had similar leaf carbohydrate levels and that the only significant difference between the carbohydrate levels of "on" and "off" trees occurs in April prior to flower induction.

Flower induction in perennial fruit trees precedes flower initiation and is initiated by exogenous environmental cues i.e. low temperatures and/or water stress (Davenport, 1990) stimulating certain metabolic and regulatory pathways that leads to the expression of key flowering genes within citrus leaves and buds (Goldberg-Moeller et al., 2013; Muñoz-Fambuena et al., 2012). Citrus is relatively day-length neutral and under subtropical conditions, the predominant promotive stimulus for flower induction is of an exogenous nature and that of sufficient low temperatures (<15°C) (Bangerth, 2006). However, the lack of flowering response in citrus buds on fruit-bearing shoots in the presence of these conditions (Valiente and Albrigo, 2004), suggests an endogenous signal transmitted within a shoot ("hormonal hypothesis"), inhibiting floral gene expression on a shoot and reducing the inflorescence in the following spring, leading to the manifestation of alternate bearing (Shalom et al., 2012).

Fruit rinds and seeds are major sources of endogenous gibberellins (GAs) (Ben-Cheikh et al., 1997) and the suggestion of their inhibiting effect on flowering is substantiated by Koshita et al., (1999), who related the lack of flower bud formation to three-fold higher GA content in the leaves of fruit-bearing shoots during time of flower induction. The inhibiting effect of GAs on flower induction has furthermore substantially been proven by the lack of inflorescence obtained by exogenous applications of gibberellic acid (GA₃) during flower induction when environmental conditions for flower induction were optimal (Davenport, 1990). Only recently has significant insight into the metabolic method of inhibition of externally applied GA during flower induction been obtained, when Muñoz-Fambuena et al., (2012) demonstrated a reduced expression of a key flowering gene, the citrus flowering

locus T (CiFT) in the leaves and buds in response to exogenously applied GA₃. A similar response was obtained by Goldberg-Moeller et al., (2013).

This project aimed at pinpointing and comparing the time and duration of flower induction in mandarins by means of GA₃ applications at two-week intervals from May to August (Objective A). Logging of soil and atmospheric temperatures throughout the expected flower induction period and correlating it with the flowering inhibition response to GA₃ applications at different times throughout the season may prove to be an excellent indicator of exactly when and under which environmental conditions flower induction occurs.

Thereafter (Objective B-E), the project evaluated manipulations of vegetative growth and reproductive growth from January (summer) to April (autumn) to change carbohydrate allocation and/or restore carbohydrate levels and to reduce the effect of endogenous gibberellins on flower induction in May to August.

With this in mind, 'Nadorcott' mandarin trees were used in experiments and the following treatments were applied alone or in combination, during the period October-April: (1) summer and (2) autumn hand thinning treatments; (3) summer and autumn girdling treatments (4) crop load management by chemical thinning agents; and (5) management of vegetative growth and improving fruit quality with different pruning strategies. Measurements of tree carbohydrates were conducted to establish significant treatment effects on carbohydrate availability and possibly correlating it with the following season's fruit load and quality.

Objectives

Preliminary study: Evaluate the effects of two hand-thinning treatments in January (summer) 2014, as well as hand thinning in summer and April (autumn) 2015 respectively, on fruit size, yield (kg and no. fruit per tree), and return bloom of 'Nadorcott' mandarin.

Objective A: Determining the time and duration of flower induction of 'Nadorcott' mandarin by means of GA₃ applications at two-week intervals from May to August.

Objective B: Evaluate the effect of different treatments (girdling and novel chemical thinning practices) on leaf carbohydrate content of 'Nadorcott' mandarin.

Objective C: Evaluate the effect of different treatments (girdling and novel chemical thinning practices) on fruit growth rate and final fruit size.

Objective D: Evaluate the effect of different treatments (girdling and novel chemical thinning practices) on vegetative regrowth.

Objective E: Evaluate the effect of different treatments (girdling and novel chemical thinning practices) on return bloom, fruit set percentage and fruit load of 'Nadorcott' mandarin, over a period of three production seasons.

Background

A preliminary study was conducted to evaluate the potential of different intensities of summer hand thinning treatments on fruit quality and return crop in 'Nadorcott' mandarin. The study was completed in 2015. Thereafter, flower inhibition response to different timings of fall-winter foliar GA₃-treatments was evaluated, to determine time and intensity of flower induction in 'Nadorcott' mandarin. Chemical fruit thinning experiments were subsequently conducted.

Materials and methods

Preliminary study

In a preliminary study, an experiment was conducted on Nadorcott mandarin trees at Tienrivieren, Citrusdal (Mouton Citrus) to evaluate the effect of two hand-thinning treatments in January 2014 on fruit size. The trial consisted of one control and two treatments, with 6 single-tree replications for each treatment in a

randomized complete block design (n=18). Only healthy, uniform trees were selected. Treatments consisted of the following:

Treatment nr	Treatment
1	Control
2	Hand removal of all fruit ≤ 20 mm
3	Hand removal of all fruit ≤ 25 mm

Ten fruit were tagged on each replicate on the day directly after the first thinning treatment occurred in January 2014 to avoid tagging of incorrect (too small) fruit that were to be thinned. Fruit diameter was measured monthly from January and the yield (kg.tree⁻¹) for each tree determined at time of commercial harvest (July), as well as the fruit size distribution by measuring the diameter of 150 fruit randomly distributed within the tree canopy of each replicate.

Objective A:

Experimental design: For the experiment determining time and duration of flower induction, six treatments of 8 single-tree replications were used on three different mandarin cultivars, in a randomised complete block design (n=8). Treatments consisted of the following:

Treatment nr.	Treatment
1	Control
2	40 mg.L ⁻¹ GA ₃ + 5 ml/100L Breakthru; 02 May + 16 May 2014
3	40 mg.L ⁻¹ GA ₃ + 5 ml/100L Breakthru 16 May + 27 May
4	40 mg.L ⁻¹ GA ₃ + 5 ml/100L Breakthru 27 May + 14 June 2014
5	40 mg.L ⁻¹ GA ₃ + 5 ml/100L Breakthru 14 June + 01 July 2014
6	40 mg.L ⁻¹ GA ₃ + 5 ml/100L Breakthru 01 July + 15 July 2014

Plant material and treatment application method: The experiment was conducted in De Doorns, South Africa (33°51'S 19°51'E), on three-year old 'Nadorcott' mandarin trees (*Citrus reticulata* Blanco) budded on Carrizo citrange rootstock [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.]. A commercial orchard with non-bearing trees was selected, to eliminate the inhibiting effect of fruit load on flower induction (as suggested in correspondence with Proff. E Goldschmidt). Experimental trees were selected on the basis of uniform size and appearance. GA₃ (ProGibb®, Valent BioSciences®, 40 g/kg active compound (40%)) was applied at a rate of 40 mg.L⁻¹ and a non-ionic wetting agent (Break-Thru®, Villa Crop Protection) with the active ingredient polyether-polymethylsiloxanecopolymer (1000 g.L⁻¹) added to the spray solution at a rate of 5 ml per 100 L of spray solution. Treatments were applied in two-week intervals, using a high-pressure handgun-sprayer until the point of run-off, with buffer trees as well as buffer rows to avoid drift from one treatment affecting another.

Data collection and evaluations: During the winter, five representative, complex shoots younger than 12 months, with a diameter of approximately 10-15 mm was selected and the total number of nodes (± 600) counted. During bud sprouting period in September the number of *flowers and vegetative* shoots that developed from the nodes on the tagged shoots in response to GA₃ treatments were determined and classed according to the type of inflorescence i.e. generative (leafless) mixed (leafy) and vegetative (only leaves). The flowering response of each treatment was then expressed as number of flowers per 100 nodes.

Objective B-E:

Girdling experiment: Two girdling experiments were conducted in summer and autumn 2015 and 2016, with the following treatments applied to branch replicates (n=8) in a randomised complete block design (tree=block) in January, and autumn, respectively:

1. Defruited and girdled
2. Fruiting and girdled
3. Defruited
4. Fruiting

Treatment effects on leaf colour, leaf total chlorophyll, and leaf carbohydrates were evaluated at two-week intervals, for a period of six weeks after treatments. Vegetative response and spring flowering (return bloom) were determined for various treatments.

Chemical thinning agents: Chemical thinning agents [Corasil P, Maxim and Brevis (Metamitron)] were evaluated for efficacy in 'Nadorcott' mandarin, as well as the potential of a novel chemical thinning agent. Treatments (n=8) of Corasil P, Maxim, and two timings of 300 ppm Metamitron (Brevis) were applied in November and December 2015 and were evaluated for effects on leaf carbohydrates, fruit growth, yield, fruit size and fruit quality. Two different treatments of Metamitron (150 ppm and 300 ppm) were applied in November 2016 and the effects on fruit size, yield and fruit quality were determined.

Results and discussion

Preliminary study: January fruit removal of <20 mm and <25 mm significantly increased the fruit growth rate (mm.day⁻¹) of remaining fruit, resulting in larger fruit at time of commercial harvest (Fig. 1) without significantly reducing yield (kg.tree⁻¹) (Table 1). Both thinning treatments resulted in a better and more even fruit size distribution by reducing the percentage of calibre 3 fruit and smaller (<59 mm) and increasing the percentage of calibre 1 fruit and larger (>64 mm) (Fig. 2). Although there were no differences in the total labour time for thinning and harvest between the control and the treatments, hand thinning reduced the duration of harvest by up to 8 minutes per tree (Fig. 3). In seasons of excessive fruit set or reduced effectiveness of chemical thinning agents, hand thinning of 'Nadorcott' mandarin in January thus has the potential to increase the growth rate (mm.day⁻¹) of remaining fruit as well as reduce duration of harvest, without significantly reducing yield (kg.tree⁻¹) or increasing labour. Although no statistical evidence can be provided, a delay in rind colour development was visually evident in fruit from the <25 mm treatment (Fig. 4). 'Nadorcott' fruit do not react sufficiently to the commercial practice of degreening, whereby fruit are submitted to ethylene gas and a predetermined threshold temperature to encourage postharvest fruit development. In the case of the <25 mm treatment, expected harvest time would therefore need to be delayed until sufficient fruit rind colour development has taken place, which in turn could encourage alternate bearing (Monselise and Goldschmidt, 1982). If this delay negates the benefit of the size improvement from an economic perspective, thinning of fruit <20 mm diameter would be the preferred option, as no delay in rind colour development was observed, compared to the control fruit. The effect of the <20 mm treatment on return bloom and yield should be evaluated in a follow-up study, as the removal of fruit has the potential of reducing the inhibitory effect of fruit on flower formation and negate alternate bearing (Shalom et al., 2014).

Objective A: Flower inhibition response to different timings of fall-winter foliar GA₃-treatments were evaluated in the spring (Fig. 5). Treatments during late fall-early winter (May-June) showed an inhibiting effect on reproductive inflorescence type as well as the total number of flowers per shoot. Later treatments (July-August) showed little or no flower inhibition response.

During flower induction, the expression of the flowering genes CiFT1, CiFT2 and CiFT3 are triggered by the accumulation of sufficient low temperatures, and the FT proteins transported via the phloem to susceptible buds. During flower initiation and differentiation, morphological development of the flowering parts leads to budbreak and sprouting of different inflorescence types during the spring, as the ambient temperature increases.

The expression of CiFT in leaves is inhibited by endogenous gibberellins, of which fruit is a major source. However, with no or little endogenous gibberellins within the tree (non-bearing trees and no active root growth), this result suggests that exogenous GA₃ treatments during May-June possibly inhibited expression of CiFT genes and as a result, inhibited flower induction. The flowering response obtained provides an indication of the time and duration of flower induction under prevailing environmental conditions.

Objectives B-E, girdling experiment: Summer girdling resulted in a rapid build-up of leaf carbohydrates (Figs. 6 and 75). When branches were both girdled and fruit removed, the effect was more pronounced and as a result, leaf chlorosis developed (Figs. 6 and 7). Whenever fruit were not removed or branches not girdled, symptoms of leaf chlorosis were less severe or absent. The summer vegetative response to the de-fruiting and girdling and the de-fruiting treatments was significantly higher compared to the fruiting and girdling, and the fruiting treatments (Table 2). Analysis of yellow leaves revealed a high starch:sugar ratio, compared to green, healthy leaves, which exhibited a high sugar:starch ratio. It seems that whenever sinks are removed during summer (girdle: removal of root sink), or defruited (removal of fruit sink), photo-assimilates are rapidly converted to storage carbohydrates in leaves. Compartmentalisation of complex starch granules in the leaf leads to rupturing and disintegration of the leaf chloroplasts, which eventually culminates in leaf chlorosis.

A similar trend was observed as a result of autumn girdling treatments (Figs. 6 and 7), however, leaf chlorosis and starch accumulation in leaves were only prevalent when both fruit were removed and girdling was applied. Whenever fruit prevailed, starch did not accumulate and no symptoms of leaf chlorosis developed (Figs. 6 and 7). The flowering response to the de-fruiting and girdling treatment was significantly higher compared with the rest of the treatments, in which no significant differences occurred between any of the treatments (Table 2).

These results indicate that both fruit and roots are major carbohydrate sinks during summer. Starch accumulates whenever removal of both roots (by girdling) and fruit removal (by de-fruiting) were applied. During winter, however, it appeared that fruit were the only major sink for photo-assimilates, and starch only accumulated whenever fruit are removed. During summer and in the presence of fruit, new vegetative shoots failed to sprout when leaf starch content of fruiting branches was significantly increased by girdling (Figs. 6 and 7). In contrast, when fruit were absent, new vegetative shoots sprouted regularly and irrespective of the leaf starch content. In addition, elevated leaf carbohydrate content in girdled and de-fruited branches failed to significantly increase the number of new vegetative shoots compared to non-girdled and de-fruited branches in which leaf carbohydrate content was significantly lower. In contrast, a 2-fold elevating of leaf carbohydrate content of de-fruited branches by girdling during flower induction in winter significantly increased return bloom flowering compared to non-girdled and de-fruited branches in which fruit were also absent, but leaf carbohydrate content was significantly lower (Figs.6 and 7).

Chemical thinning agents: In season 1, only the Maxim chemical thinning treatment significantly increased summer leaf carbohydrates (Fig. 8). Both Corasil P and Maxim treatments reduced the number of fruit per tree, but not the tree total fruit yield (kg per tree) (Table 2). Foliar application of Maxim resulted in severe fruit thinning ($\pm 25\%$ reduction in fruit numbers), increased fruit growth rate (Fig. 9) and shifted fruit size distribution by up to 2 commercial fruit size calibres (Fig. 10). Corasil P treatment increased fruit growth rate and only reduced the number of fruit in the smallest fruit size calibre (SC <5). Both Metamitron treatments resulted in a $\pm 25\%$ reduction in fruit numbers and significantly reduced tree total fruit yield (Table 2).

Foliar treatments of 150 ml/100L Corasil P applied at 8 mm fruitlet diameter, and 10g/100L Maxim applied at 15 mm fruitlet diameter, respectively, had no negative impacts on tree total fruit yield or fruit quality of 'Nadorcott' mandarin (Table 2), but increased potential grower returns by increasing fruit size and fruit size distribution per tree. Foliar treatments of 300 ppm Metamitron applied at a fruitlet diameter of 8 or 15 mm did not significantly influence fruit quality, but significantly reduced tree total fruit yield and influenced fruit size distribution (Fig. 10).

No symptoms of phytotoxicity were reported for Metamitron treatments and there appear to be significant potential for the use of Metamitron as a chemical thinning agent in 'Nadorcott' mandarin.

Technology transfer

A poster of results of the hand thinning experiment was presented at the CRI citrus research symposium in 2014, as well as in an article in the SAFJ of Dec/Jan 2014/15. Results from hand thinning and GA₃ experiments were presented at the annual CRI workshops in 2015, 2016 and 2017, as well as at the 2016 CRI citrus research symposium. The following peer-reviewed research articles on the results were published throughout the course of this study:

Stander, O.P.J. and Cronjé, P.J.R. 2016. Reviewing the commercial potential of hand thinning in citrus with a cost-benefit analysis of summer hand thinning of 'Nadorcott' Mandarin. HortTechnology. 26(2): 206–212.

Stander, O.P.J., G.H. Barry, and P.J.R. Cronjé. 2017. Fruit-load-induced starch accumulation causes leaf chlorosis in 'Nadorcott' mandarin. Scientia Hort. 222: 62–68.

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Tables and figures

Table 1. The effect of hand thinning treatments on 24/01/2014 on yield (kg.tree⁻¹) of 'Nadorcott' mandarin in Citrusdal (32°51'S 19°51'E), in the Western Cape, South Africa.

Hand thinning treatment	Mean no. fruit removed per tree	Trunk circumference (cm)	Yield (kg.tree ⁻¹)
Control	0	37.18 ^{ns}	78.64 ^{ns}
<20 mm	72	37.13	67.90
<25 mm	161	35.50	68.58
<i>P-value</i>		0.6548	0.2847

^{ns} No significant differences at the 5% level (LSD)

Table 2. The effects of different chemical thinning agents on fruit yield and important parameters of commercial fruit quality of 'Nadorcott' mandarin during the 2015/16 season.

Treatments (n=8)	Fruit yield		Fruit size			Rind colour				Internal quality										
	(kg per tree)		(g)		(mm)	lightness	chroma	hue	Juice %	Brix:TA ratio		Brix		Titratable acidity						
Control	32.4	ns	101.6	c	66.7	c	56.8	ns	55.2	ns	69.8	ns	49.1	a	9.2	b	9.4	ns	1.03	ns
Corasil P	36.4		116.3	ab	70.1	ab	55.3		51.9		72.6		49.6	a	9.1	b	9.0		1.00	
Maxim	32.6		128.4	a	73.2	a	55.5		52.6		70.9		45.4	b	10.6	a	9.2		0.88	
Metamitron Nov.	27.3		111.9	bc	69.0	bc	53.7		48.4		74.9		49.2	a	8.8	b	8.8		1.01	
Metamitron Dec.	27.4		104.5	bc	68.6	bc	55.8		53.1		70.2		51.5	a	9.5	b	9.2		0.98	
P-value	0.4542		0.0034		0.0051		0.1090		0.0690		0.1088		0.0098		0.0068		0.2730		0.0660	

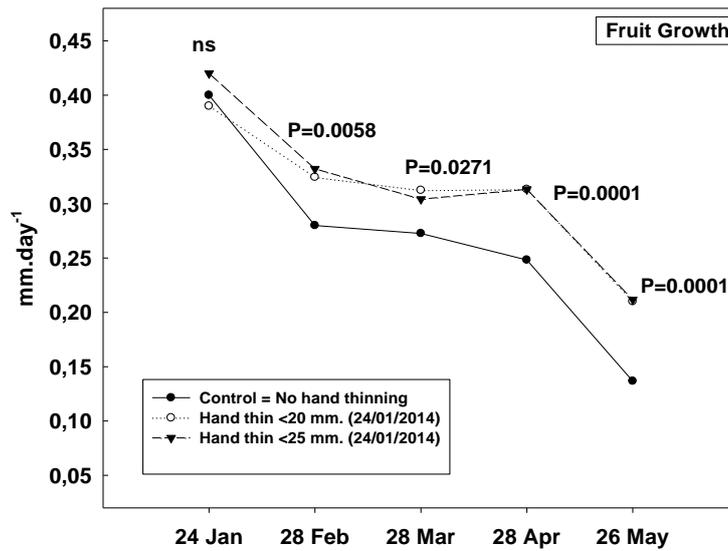


Fig. 1. The effect of two January hand thinning treatments on fruit growth rate (mm.day⁻¹) of 'Nadorcott' mandarin.

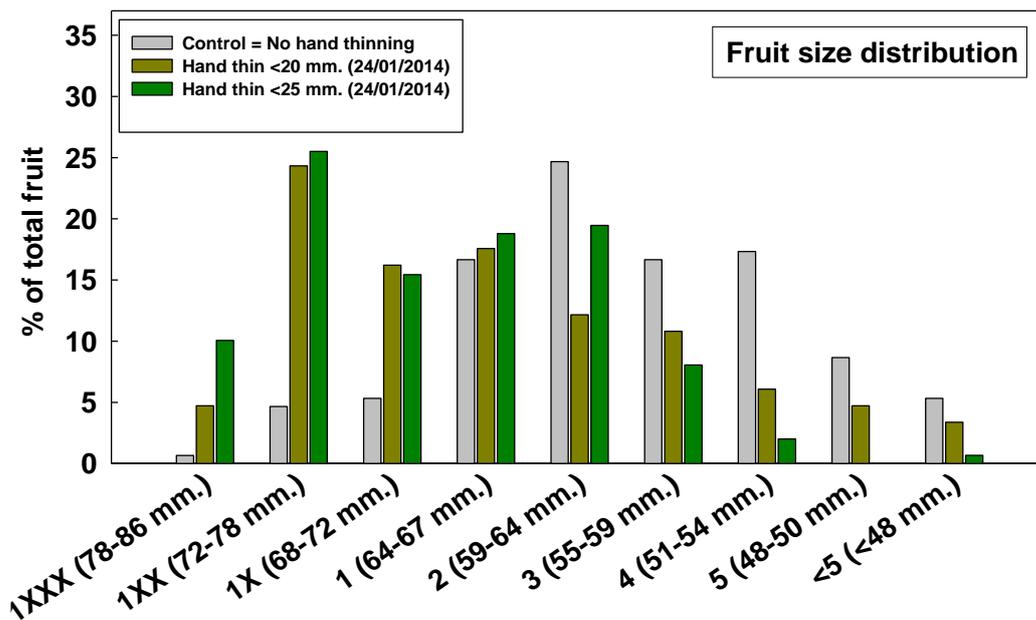


Fig. 2. The effect of two hand thinning treatments on fruit size distribution of 'Nadorcott' mandarin at time of commercial harvest (14/07/2014).

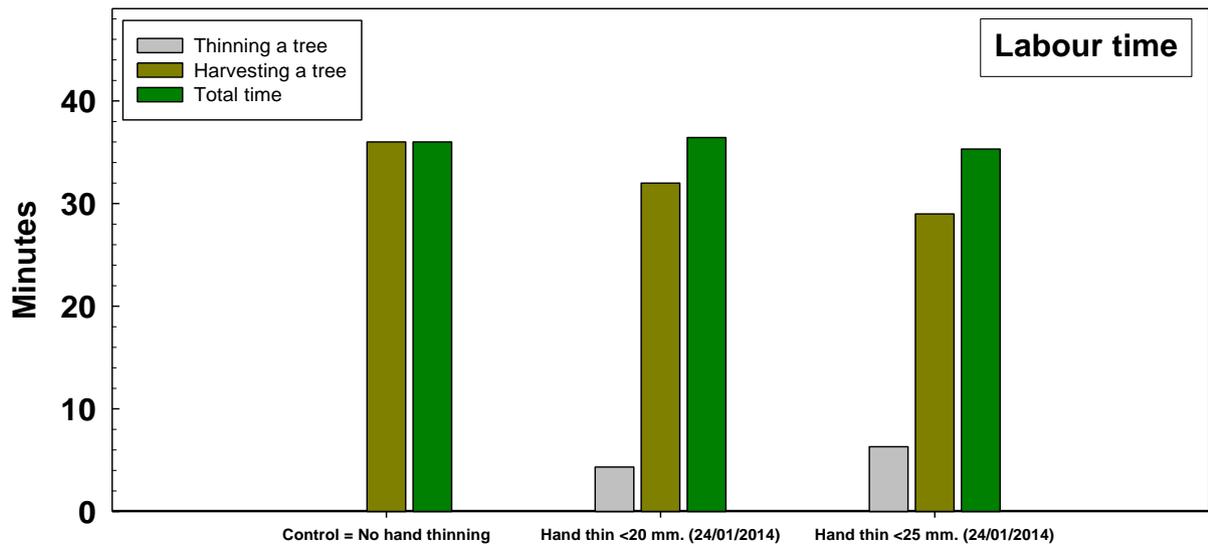


Fig. 3. Labour time required to thin and harvest 'Nadorcott' mandarin, compared to harvest of control trees.



Fig. 4. Although control trees had higher yield (kg.tree⁻¹) (1a vs 2a&3a), hand thinning resulted in significantly larger fruit (1b vs 2b&3b).

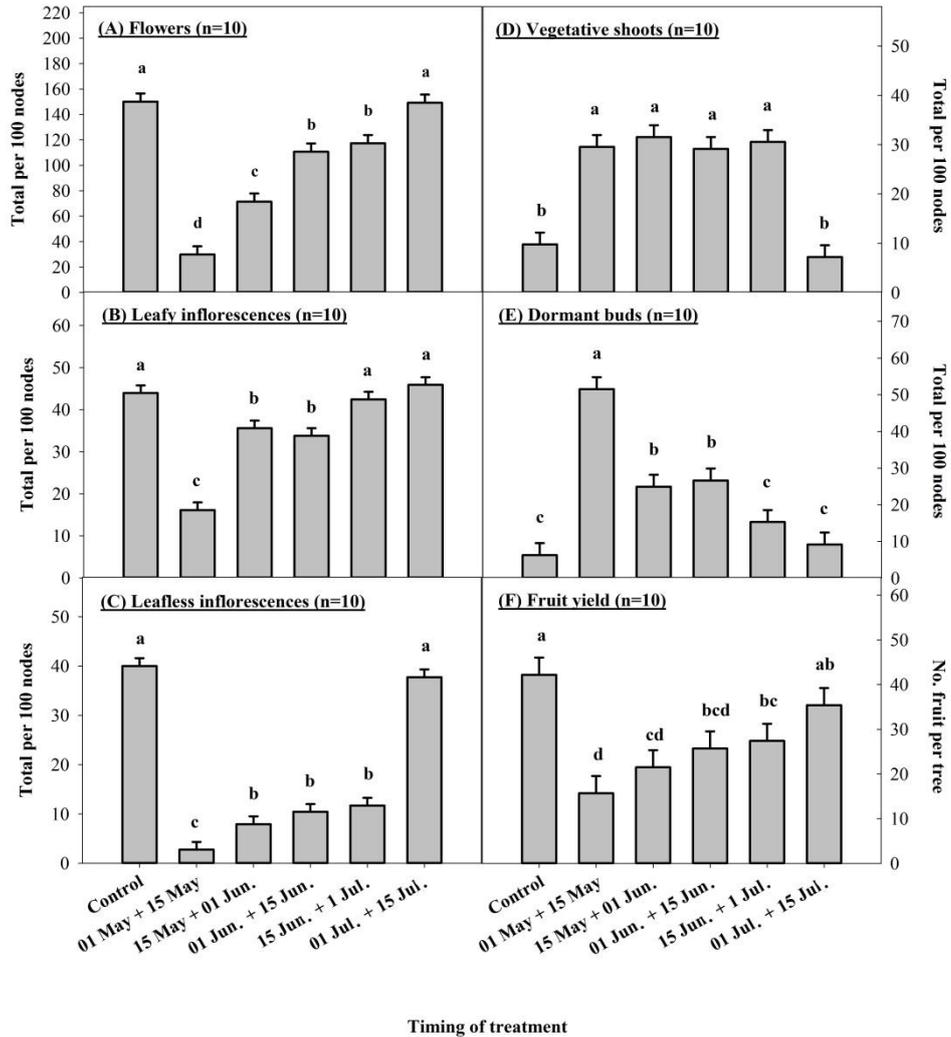


Fig. 5. The effects of different timings of foliar gibberellic acid (GA_3) treatments during winter in 2014, on flowering characteristics and fruit yield during return bloom and time of harvest in three-year-old, non-bearing 'Nadorcott' mandarin (*C. reticulata*) trees.

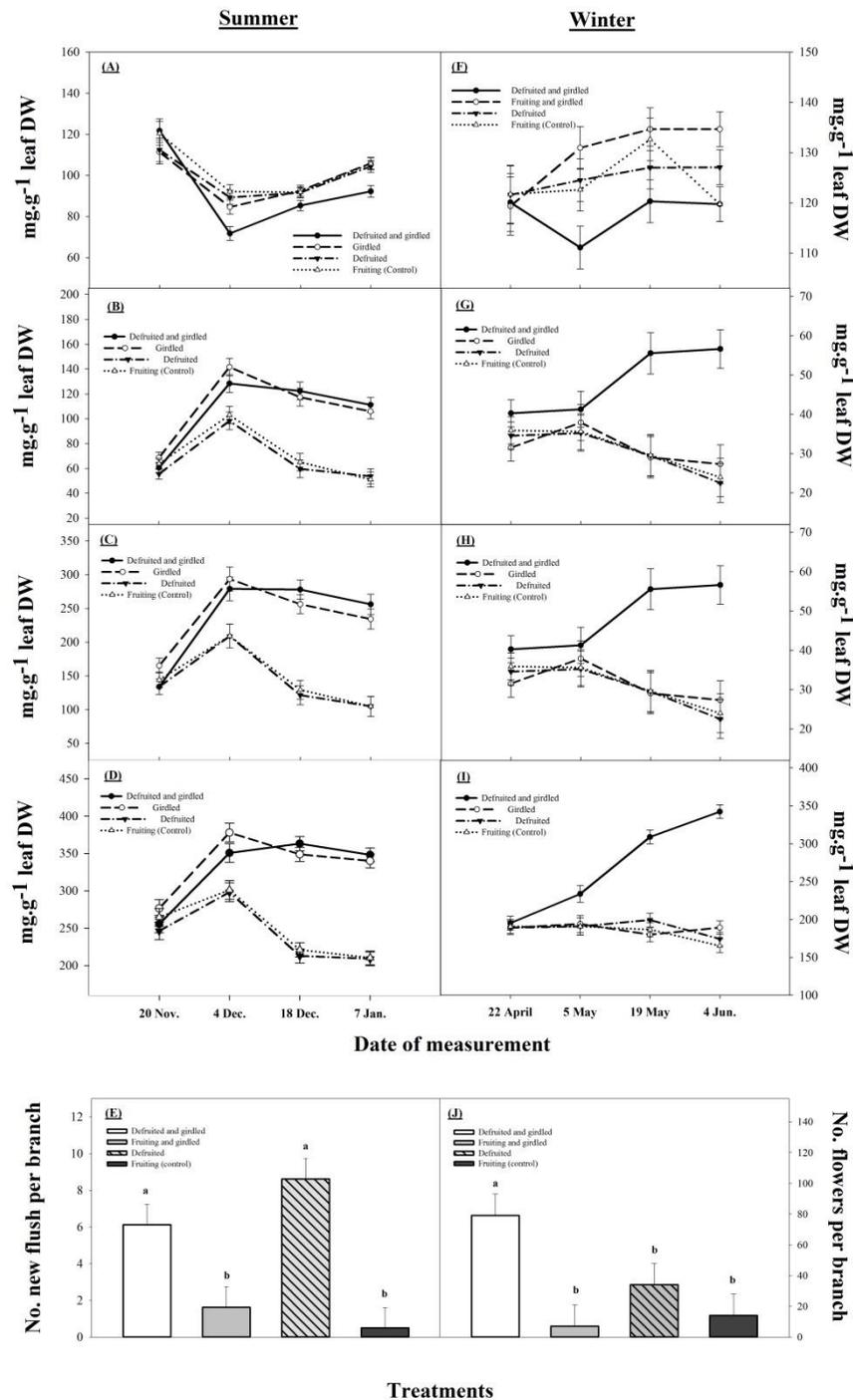


Fig. 6. The effects of branch source/sink alterations in moderate bearing ‘Nadorcott’ mandarin trees during summer, at the end of Nov. 2014, and winter, at the end of Apr. 2015, on the concentrations of: A and F) leaf sugars, B and G) leaf polysaccharides, C and H) leaf starch, and D and I) leaf total carbohydrates. The subsequent vegetative (E) and reproductive (J) responses to treatments were evaluated during autumn, and spring for each experiment, respectively. Data are expressed as means of eight replicates (n=8). DW = dry weight.

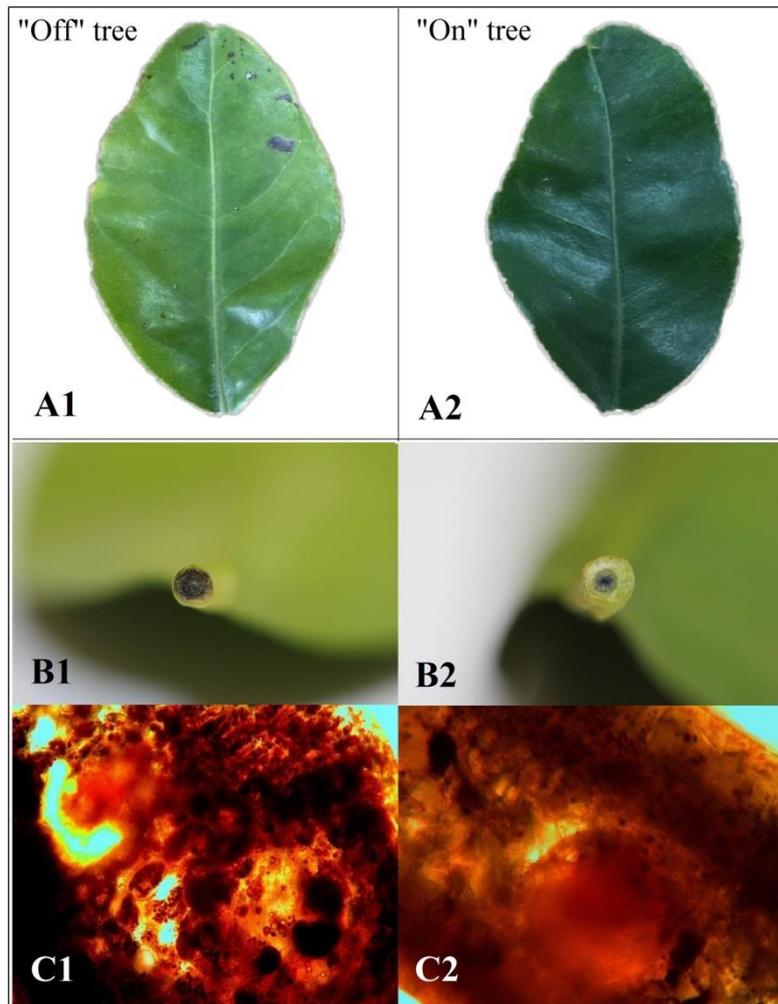


Fig. 7. The effects of fruit load (“off” = low fruiting; “on” = heavy fruiting) on leaf colour (**A1** and **A2**), and the distribution of starch granules in the leaf petiole (**B1** and **B2**) and the leaf blade (**C1** and **C2**) of ‘Nadorcott’ mandarin trees during winter in 2015. Leaves were dissected, stained with a 2% iodine solution, and examined using microscopic (Carl Zeiss ERc5s; Göttingen, Germany) photographic comparisons.

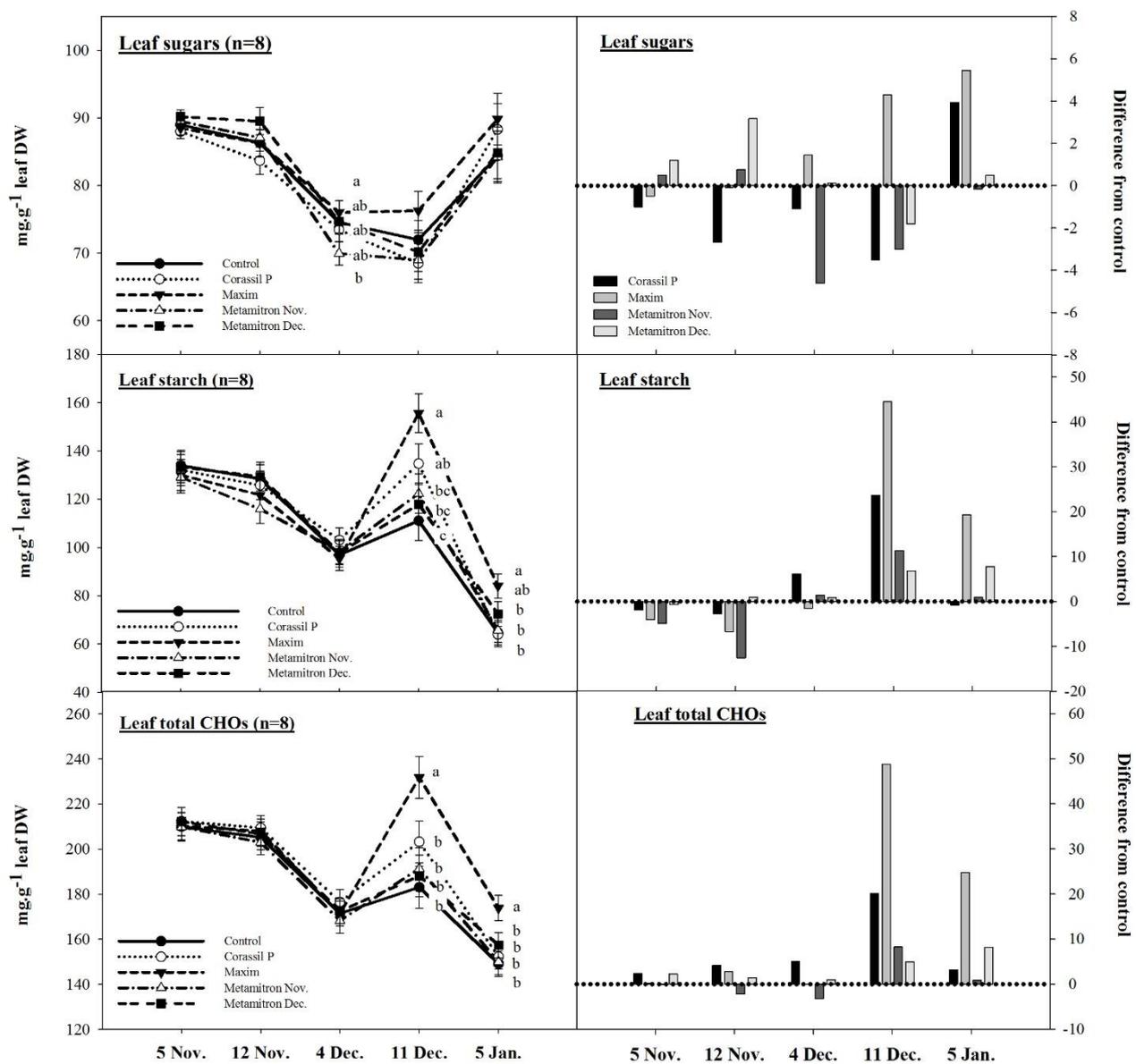


Fig. 8. The effects of different chemical thinning agents on leaf sugars, leaf starch and leaf total carbohydrates, compared with control, untreated 'Nadorcott' mandarin trees during the 2015/16 season.

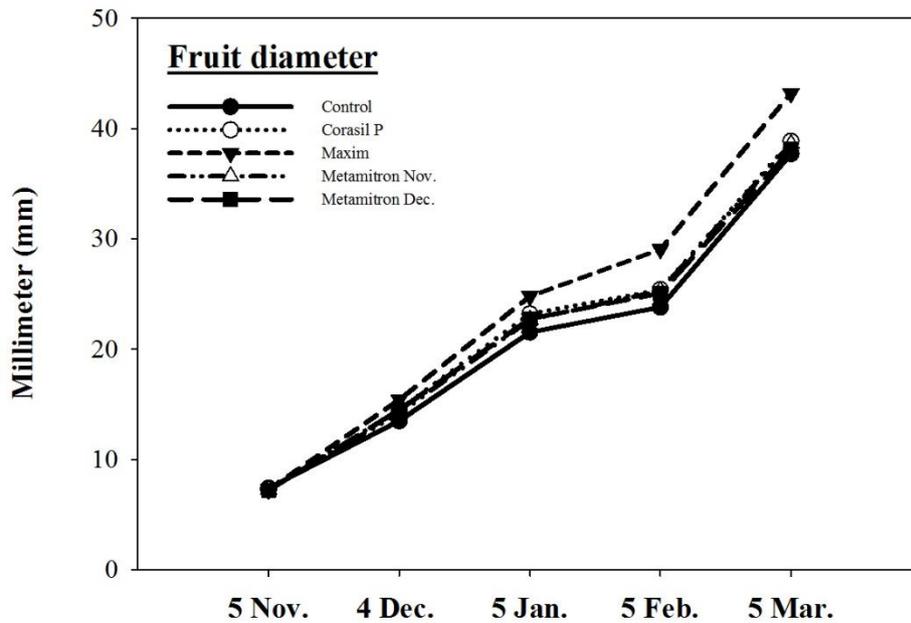


Fig. 9. The effects of different chemical thinning agents on fruit growth of 'Nadorcott' mandarin during the 2015/16 season.

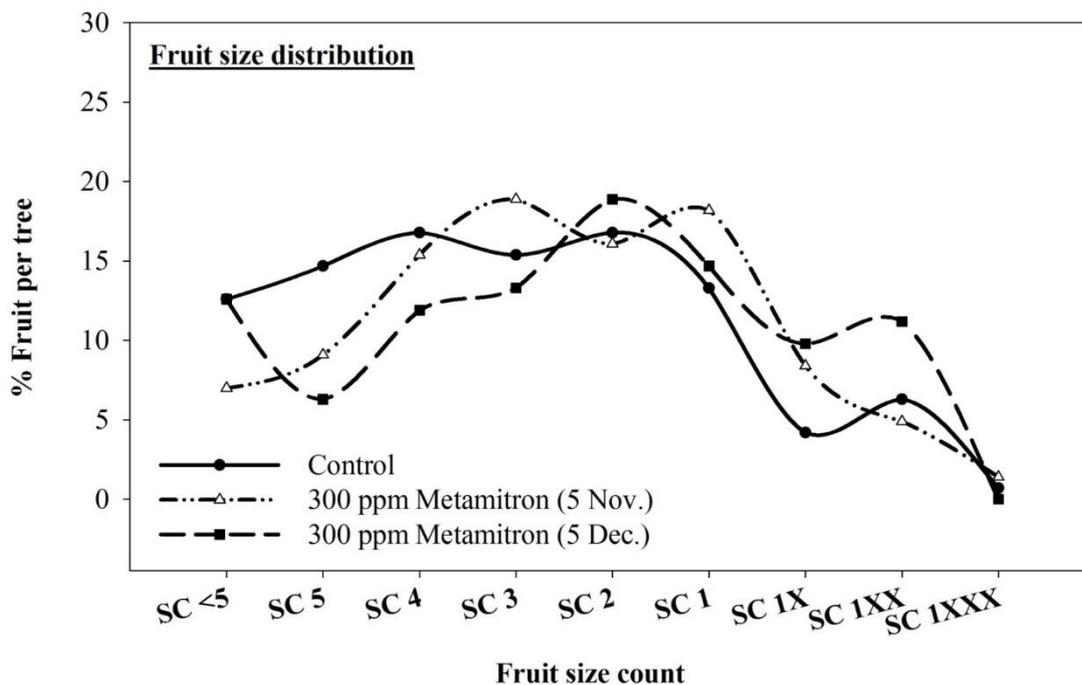


Fig. 10. The effects of different Metamitron chemical thinning treatments on fruit size distribution at time of commercial harvest (end of July) of 'Nadorcott' mandarin during the 2015/16 season.

4.3.4 FINAL REPORT: Studies on the reproductive development of 'Nadorcott' mandarin (*C. reticulata* Blanco)

Project 1131 (Apr 2015-Mar 2018) by Jakkie Stander and Dr. P.J.R. Cronje (CRI), and Dr G.H. Barry (XLnT Citrus)

Summary

The significance of carbohydrates, mineral nutrients and phyto-hormones was investigated in relation to their possible roles in selected phenological events in alternate bearing 'Nadorcott' mandarin (*C. reticulata* Blanco) trees. Crop load in 'Nadorcott' mandarin trees was influenced by flowering intensity. The most important determinants of flowering intensity were the amount of new vegetative shoot growth and resulting number of new potential floral buds that developed during summer, and the influence of fruit on floral bud development during winter. The lack of development of summer vegetative shoots in "on" trees was not related to leaf carbohydrate concentration. In "off" trees, root sugar concentration peaked during full bloom and high root growth activity was observed prior to the vegetative shoot flush in summer. In "on" trees, fruit were the major carbohydrate sinks and probably disturbed the balance between vegetative shoot development and root growth. Sugar concentration in roots in "on" trees was ≈ 3 -fold lower, root growth was absent, and shoot growth was halved. The concentration of mineral nutrients in leaves was a response to fruit load and not related to parameters of flowering or vegetative shoot growth. Measurements of phyto-hormones in leaves and roots confirmed that the inhibition of summer vegetative shoots was related to a high concentration of 1 *H*-indole-3-acetic acid (IAA) in leaves. High concentrations of dihydrophaseic acid and the abscisic acid (ABA) glucose ester suggested that IAA might have acted synergistically with ABA to create a growth inhibition in fruiting shoots. As a result, cytokinins did not contribute to the development of new summer vegetative shoots. High gibberellin concentration in leaves in May and June contributed to limited flowering in "on" trees. Consistent with this interpretation, treatment of "off" trees with 40 mg·L⁻¹ gibberellic acid inhibited flowering, whereas soil and foliar treatments of "on" trees with 1000 mg·L⁻¹ paclobutrazol or uniconazole, gibberellin biosynthesis inhibitors, increased flowering and resulted in fruit development from buds of "on" shoots.

Opsomming

Die verband tussen die konsentrasies van koolhidrate, minerale nutriente en fito-hormone, en belangrike fenologiese gebeure is ondersoek in 'Nadorcott' mandaryn (*C. reticulata* Blanco) bome met 'n alternerende drag patroon. Vruglading was beïnvloed deur blomintensiteit. Intensiteit van opvolgblom is bepaal deur die aantal beskikbare blomposisies wat gedurende die voorafgaande seisoen se somer ontwikkel het, asook deur die invloed van vrugte op blomontwikkeling gedurende winter. Die gebrek aan somer vegetatiewe lootgroei in "aan"-bome was nie verwant aan die konsentrasie van blaarkoolhidrate nie. Die suikerkonsentrasie in wortels was die hoogste in "af"-bome en tydens volblom, en wortelgroei is waargeneem voor die vegetatiewe lootgroei-stuwing in die somer. Vrugte was die sterkste koolhidraat sink in "aan"-bome en het waarskynlik die balans tussen loot- en wortelgroei versteur. Die suikerkonsentrasie in wortels van "aan"-bome was laer, wortelgroei was afwesig en lootgroei gehalveer. Die inhoud van makro-elemente in blare was 'n reaksie op vruglading en nie verwant aan vegetatiewe lootgroei of blom nie. Bepaling van fito-hormoon vlakke in blare en wortels het bevestig dat indool-3-asynsuur (IAA) primêr verantwoordelik was vir die inhibisie van somer vegetatiewe lootgroei. Hoë konsentrasies van dihidrofaasuur en die absisiensuur (ABA) glukose-ester in blare kon moontlik sinergisties met IAA opgetree het om te lei tot die lootgroei-inhibisie in "aan"-bome. Gevolglik het sitokinien toedienings nie somer vegetatiewe lootgroei gestimuleer nie. Hoë gibberellien inhoud in blare gedurende die vroeë winter het bygedra tot die ontwikkeling van min of geen blomme in "aan"-bome. Behandeling van "af"-bome en lote met 40 mg·L⁻¹ gibberelliensuur gedurende winter het opvolgblom inhibeer, terwyl behandelings met 1000 mg·L⁻¹ paclobutrazol of unikonasool op dieselfde tyd gelei het tot blomvorming en vrugontwikkeling vanaf knoppe op "aan" lote.

Introduction

The production of late mandarins has become an integral part of the South African citrus industry. These cultivars can produce exceptional yields and fruit attain high prices in the export market. However, due to the late harvesting of these cultivars it could pose various horticultural problems such as irregular flowering, poor fruit set and strong vegetative growth. All these aspects are thought to influence the vegetative:reproductive balance in a tree and can contribute to seasonal fluctuation in fruit load, an occurrence generally referred to as alternate bearing.

Alternate bearing is a phenomenon occurring in a variety of horticultural crops (Monselise and Goldschmidt, 1982), and in citrus specifically so in mandarin hybrids (Monselise et al., 1981). Alternate bearing is

characterized by a year of heavy fruit load (“on” year) which inhibits flowering in the following spring which results in an “off”-year (Monselise and Goldschmidt, 1982). In “on”-years, fruit are small, the rinds thin and more susceptible to various physiological disorders and higher acidity. In “off”-years, fruit are few and large, with rough and thick rinds and accompanied increased vegetative growth. Alternate bearing can occur in an orchard, in a tree, or on individual limbs and shoots (Verreyne and Lovatt, 2009). It is a complex horticultural problem that requires an understanding of the phenological stages during both reproductive and vegetative development, as well as the interaction with prevailing environmental conditions.

The objective of this PhD research project was to study the reproductive development of “on” and “off” ‘Nadorcott’ mandarin (*C. reticulata* Blanco) trees throughout fruit development. During the first year of the project, data was collected throughout the production seasons to determine possible correlations between various horticultural responses such as root growth, flowering, fruit set, vegetative growth, fruit load and fruit quality, to measurements of physiological parameters such as leaf and root carbohydrate levels, leaf endogenous hormone content, leaf nutrient content and photosynthesis.

The overall aim of this multi-season research project was to identify, measure and integrate the various aspects that could influence yield, i.e. carbohydrates, phytohormones, and photosynthesis capacity into a crop model. Such a model would potentially be used to identify opportune timing of a horticulture manipulation technique during the various tree phenological stages to obtain a consistent yield.

Objectives

Objective A: Monthly leaf and root carbohydrate analysis of “on” and “off” ‘Nadorcott’ mandarin trees.

Objective B: Determining leaf endogenous hormonal content of “on” and “off” ‘Nadorcott’ mandarin trees in September, December, March and June.

Objective C: Leaf nutrient analysis of “on” and “off” ‘Nadorcott’ mandarin trees in September, December, March and June.

Objective D: Monthly measurements of leaf photosynthesis of “on” and “off” ‘Nadorcott’ mandarin trees.

Objective E: Measurement of horticultural aspects relating to reproductive development of ‘Nadorcott’ mandarin.

Materials and methods

Plant material and experimental site:

Ten year-old ‘Nadorcott’ mandarin trees grown under field conditions and budded on ‘Carrizo’ citrange [*C. sinensis* × *Poncirus trifoliata* (L.) Raf.] rootstock were selected from orchards with a history of alternate bearing in De Doorns (lat. 33°51’S, long. 19°52’E) and Citrusdal (lat. 32°81’S, long. 19°01’E) in the Western Cape Province of South Africa. Trees were spaced at 5 × 2 m in a sandy soil with pH_(KCl) 4.4. The Western Cape Province of South Africa experiences Mediterranean-type climatic conditions; summer typically occurs from December to February; autumn from March to May; winter from June to August and spring from September to November. The region receives an annual rainfall of between 400 and 600 mm, with the majority occurring from May to August. The orchards were cultivated, pruned, and sprayed according to good agricultural practices: trees were watered using a drip irrigation system with four emitters per tree, and the amount of water applied to each tree amounted to ≈4000 L per annum. The fertilizer rate [kg per hectare (ha)] was based on annual leaf mineral nutrient analysis and potential yield (kg fruit per ha). Nitrogen (N) was annually supplied at a rate of 240 kg N per ha, with 25% applied foliar, 20% as a soil application, and 55% dissolved in the irrigation solution (fertilization) and split uniformly into applications from September to April.

Treatments and experimental design

The experiments were set up as a two-factorial completely randomised design using whole trees (factor 1) and shoots (factor 2) as experimental units (n=10). Heavy- (“on”) and low-fruited (“off”) trees were selected based on their contrasting fruit loads. To ensure that trees were uniformly selected, trunk circumferences of individual trees were measured and canopy volumes determined at the beginning of the experiment by measuring tree

height, canopy height and canopy radius in the N, S, E and W directions of each tree. The canopy volume [V (m^3)] was calculated according to the following formula (Burger et al., 1970):

$$V = r^2(\pi h - 1.046r)$$

r = canopy radius;

h = height of the fruit bearing canopy.

The same trees were used in both seasons. The alternate bearing index (I) of the two treatments with contrasting fruit loads was calculated using the following formula (Gur et al., 1969):

$$I = \frac{1}{(n-1)} \left[\frac{(a_2 - a_1)}{(a_2 + a_1)} + \frac{(a_3 - a_2)}{(a_3 + a_2)} + \dots \right]$$

n = number of seasons;

a = fruit yield in the corresponding season.

Branch experiments were set up in a randomised complete block design, in which a tree represented a block and a single branch represented a replicate ($n=8$). Due to a generally strong autonomous phenological growth habit of branches in mandarin trees, branches can be used to extrapolate results to alternate bearing in whole-tree scenarios (Monselise et al., 1983). All branches were located on the outside of the western side of the tree canopy at a height of ≈ 1.5 m above the orchard floor and had a fruit-to-leaf ratio of approximately one fruit per ten leaves and an average branch circumference of 55 mm. The following treatments were applied to single branches in moderate bearing trees on 20 Nov. 2014 in summer and 22 Apr. 2015 in autumn: 1) complete de-fruiting of branches; 2) de-fruiting and girdling of branches; 3) girdling of fruiting branches; and 4) fruiting branches left intact. For the girdling treatments a ring of bark approximately 3 mm in width was removed from around the branch by using a sharp knife. The branch treatments were repeated during the following season on the same dates, but on different branches.

Data collection

Tree and shoot phenology

The number of flowers per tree was estimated by counting the number of flowers within the limits of a $0.5 \times 0.5 \times 0.5$ m frame during full bloom in October. The tree canopy was divided into an East and West sector and an upper- and lower height. Four flower counts were performed in each tree, one in each quadrant. The total number of flowers was estimated by extrapolating the mean number of flowers per frame to the total tree volume. The same procedure was used to estimate the number of new vegetative shoots after cessation of periods of vegetative shoot flush in November, February and April.

The phenological pattern of different shoot types in “on” and “off” trees was followed by randomly selecting five vegetative (“off”) and five reproductive (“on”) shoots from each tree during full bloom in Oct. 2014. All shoots were approximately 12 months of age and had triangular internodes, a length of ≈ 15 cm and were located on the outside of the tree canopy at a height of ≈ 1.5 m above the orchard floor. On each shoot the number of nodes, the number of vegetative shoots and total number of flowers were counted in addition to the classification of inflorescence type. Inflorescences were classified as leafy, i.e. buds sprouting both flowers and leaves, or leafless, i.e. buds sprouting flowers only. In February and March the numbers of persistent fruit and new vegetative shoots that developed during the subsequent vegetative shoot flushes were recorded for each shoot, and return bloom and vegetative response were determined on the same shoots during the subsequent season.

For the branch experiments, the number of new vegetative shoots and the total number of flowers were counted on branch replicates subsequent to the cessation of the summer vegetative shoot flush in February and during full bloom in October.

For root growth observations, acrylic minirhizotron tubes were installed prior to winter in 2015. The tubes were installed parallel to the row direction at an inclined angle of 45° with the soil vertical for approximately 90 cm, thus exploring a vertical soil depth of approximately 60 cm. The bottoms of the tubes were sealed with a plug and the tops that protruded from the soil were capped with a white cap to reflect as much sunlight as possible

and prevent water from entering. Two tubes, one on the Eastern side and one on the Western side of the tree canopy were installed below the canopy of each of one representative “on” and “off” tree. The top of the tube was located about 50 cm from the trunk and near the canopy dripline. Digital images were captured in each tube with a root scanner (CI-600 In-Situ Root Imager, CID-BioScience Inc., Camas, WA, USA). Three incremental vertical colour images of 21.6 cm × 19.6 cm were captured down each minirhizotron tube and the number of new roots counted at monthly intervals. To confirm observations of the first season, additional tubes were installed in four separate “on” and “off” tree replicates prior to winter in 2015. Root growth evaluations started at the end of Aug. 2016 and continued at monthly intervals.

Soil and ambient temperatures were logged throughout the study using a soil probe and air temperature logger (TinyTag®, Plus 2, Gemini Data Loggers, Chichester, UK).

Yield

Commercial harvest of fruit commenced in the middle of August after fruit quality standards complied with specifications established by fruit export markets, and was completed by the end of August. To determine the total fruit yield, in kg per tree, all fruit were harvested from individual trees on the same day prior to the start of commercial harvest. A sample of 100 randomly collected fruit per tree was collected from each tree and the diameter of each fruit was measured with an electronic calliper. Each fruit was assigned to a fruit size category of which the average fruit weight was determined in order to estimate the total number of fruit per tree.

Leaf gas exchange

In each of the five “on” and “off” shoots in eight “on” and “off” tree replicates, one leaf was tagged for repeated measurements of different parameters of leaf gas exchange. The measurements in each of the five “on” and “off” shoots were pooled to represent each treatment replicate (n=8). The rates of leaf CO₂ assimilation (A_c , expressed as $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), leaf stomatal conductance (g_s , expressed as $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and leaf transpiration (E , expressed as $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were measured at monthly intervals on selected cloudless days using a portable infra-red gas analyzer (Li-6400, LI-COR, Lincoln, NE, USA). Data collection started at \approx 8:00 AM and was completed between 11:00 AM and 12:00 PM on each measurement date. Measurements were conducted using a closed chamber. The airflow rate was set at $300 \mu\text{mol}\cdot\text{s}^{-1}$, photosynthetic photon flux of $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the block temperature at 25 °C, with controlled CO₂ concentration of 380 ppm.

Leaf and root carbohydrates

A sample consisting of eight leaves was collected from each treatment replicate between 9:00 and 10:00 AM. The eight leaves consisted of two leaves sampled from each of four vegetative shoots. Only mature leaves were sampled from the third to fifth position on fully hardened, non-fruiting and purely vegetative shoots. All shoots had triangular internodes, a length of \approx 15 cm and were located on the outside of the tree canopy at a height of \approx 1.5 m above the orchard floor. The spring leaf samples were collected from vegetative shoots that developed during the previous season’s vegetative shoot flushes, the summer leaf samples were collected from vegetative shoots that developed during the current season’s spring vegetative shoot flush, and the autumn and winter leaf samples were collected from vegetative shoots that developed during the current season’s summer vegetative shoot flush. A sample of fibrous roots (<0.5 mm in diameter) was collected from representative, pooled root tissues that were sampled from four different areas around the trunk of each tree. The root and leaf samples were washed with distilled water, frozen at -80 °C and freeze-dried (Christ Beta 1–8 LD Freeze Dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), before being ground to a fine powder with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany).

Total sugars were extracted from 100 mg of each dried leaf and root sample with 5 mL 80% (v/v) ethanol at 80 °C for 1 h. The extraction process was repeated twice following the first extraction and the respective supernatants pooled. The pellets were then extracted three times with 5 mL de-ionized water at 80 °C for 24 h for the determination of total water-soluble polysaccharides. Total starch was determined from the remaining pellet by quantifying the glucose released following an enzymatic digestion of the residue for 17 h at 60 °C, with the amyloglucosidase enzyme (AMG) [Sigma Aldrich (Pty) Ltd, Aston Manor, South Africa].

The 80% ethanol, water and AMG enzyme extracts were analyzed for total soluble sugars using the phenol–sulphuric acid assay (Brummer and Cui, 2005). Briefly, a volume of 20 μL of each of the respective extracts was added to 180 μL de-ionized water, 200 μL phenol ($5 \text{ mL}\cdot\text{L}^{-1}$) and 1000 μL concentrated sulphuric acid. Absorbance was determined on a spectrophotometer (Cary 50 Series, Varian, Mulgrave, Australia) at 490 nm, precisely after 30 min against a blank prepared for the standard. A standard curve for glucose concentrations was prepared by diluting 0, 50, 100, 150 and 200 μL glucose stock solution ($0.10 \text{ mg}\cdot\text{mL}^{-1}$) with de-ionized water to a final volume of 200 μL . The sugar concentrations were expressed as milligrams per gram leaf or root dry weight and are respectively referred to as sugar concentration, polysaccharide concentration and starch concentration. The sum values of the three components collectively contribute to the total carbohydrate concentration.

Analysis of mineral nutrient concentration

Mineral nutrient analyses of individual elements in leaf and fruit samples were conducted by an accredited commercial chemical and microbiology analytical laboratory [Bemlab (Pty) Ltd., Strand, South Africa] according to published protocols (Hou and Jones, 2000). Briefly, 1 g of fruit or dried leaf tissue was made up to a volume of 50 mL with a 50:50 hydrochloric acid (50%) solution for extraction through filter paper. The P, K, Ca and Mg concentrations were analysed using inductively-coupled plasma-emission spectroscopy (Varian PRX–OEX, Varian Inc., Palo Alto, CA, USA) against suitable standards and subsequent to a nitric-hydrochloric total acid digestion step. For analysis of total N, 0.15 g of each sample was combusted at 850 °C and analysed using a LECO N analyzer (LECO FP528 Nitrogen analyzer, LECO cooperation, St. Joseph, MI, USA) by thermal conductivity. The concentrations of the mineral nutrients in the leaf and fruit were expressed as $\text{mg}\cdot\text{g}^{-1}$ leaf dry weight or $\text{mg}\cdot\text{g}^{-1}$ fruit fresh weight.

Analysis of phyto-hormones

Leaf and root samples were collected between 05:00 and 06:00 AM on each sampling date, viz. on 04 Jan. 2017 in summer, and on 27 Apr. 2017 in autumn. For each sample, eight fully-intact shoots were removed from each treatment replicate, placed in individual paper bags and transported on ice to the laboratory in Stellenbosch. In the laboratory, one leaf was removed from each of the eight shoots to compile a leaf sample that consisted of eight leaves per sample for fruiting “on” shoots of “on” trees and vegetative “off” shoots of “off” trees. Only fully-hardened leaves were sampled from the first to third position in the apical region of each shoot since flowering is most likely to occur at these positions (Abbott, 1935; Sauer, 1951). A sample of fine fibrous roots (<0.5 mm in diameter) was collected from a pooled root sample that was sampled from four different areas around the trunk of each tree. The leaves and roots were separated, immediately frozen in liquid nitrogen and lyophilised using a pestle and mortar. The ground leaf and root samples were immediately transferred to 15 mL centrifuge tubes sealed with screw caps and stored in a –80 °C freezer and freeze-dried (Christ Beta 1–8 LD Freeze Dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The samples were shipped to the Plant Biotechnology Institute of the National Research Council of Canada in Saskatoon, SK, Canada for determination of the hormone concentrations using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) with multiple reaction monitoring (MRM) (Waters Corp., Medford, MA, USA) (Ross et al., 2004).

The procedure for quantification of multiple hormones and metabolites, including 1 *H*-indole-3-acetic acid (IAA) and IAA-conjugates (IAA-aspartate, IAA-glutamate, IAA-alanine, and IAA-leucine), and indole-3-butyric acid (IBA), abscisic acid (ABA) and its metabolites [phaseic acid (PA), dihydrophaseic acid (DPA), 7'-hydroxy-ABA (7'-OH-ABA), neo-phaseic acid (neoPA), and ABA-glucose ester (ABA-GE)], cytokinins [isopentenyladenine (2iP), iso-pentenyladenosine (iPA), trans- and cis-zeatin (t- and c-Z), trans- and cis-zeatin riboside (t- and c-ZR), dihydrozeatin (dhZ), dihydrozeatin riboside (dhZR), and trans- and cis-zeatin-O-glucoside (t- and c-ZOG)], and gibberellins was described in detail by Chiwocha et al. (2003; 2005).

Briefly, a 100 mL aliquot containing all the hormone internal standards (ISs), each at a concentration of 0.2 $\text{pg}\cdot\text{mL}^{-1}$, was added to 50 mg of ground leaf or root sample, followed by 3 mL of the extraction solvent that consisted of isopropanol:water:glacial acetic acid (80:19:1, v/v/v). The samples were agitated in the dark for 24 h at 4 °C. After centrifugation, the supernatant was isolated and dried on a distillation evaporator (Syncore Polyvap, Büchi Labortechnik, Flawil, Switzerland) and reconstituted in 100 mL of acidified methanol, adjusted to 1 mL with acidified water, and partitioned against 2 mL hexane. After 30 min, the aqueous layer (bottom

phase) was isolated and dried as above. The dried sample was reconstituted in 800 mL of acidified methanol and adjusted to 1 mL with acidified-water, passed through equilibrated Sep-Pak C18 cartridge (Waters Corp.) and the eluates were dried in a centrifuge vacuum concentrator (Labconco Corp., Kansas City, MO, USA). An IS was prepared with 100 mL of the deuterated ISs mixture. A quality control standard (QC) was prepared by adding 100 mL of a mixture containing all the analytes of interest, each at a concentration of 0.2 pg·mL⁻¹, to 100 mL of the IS mix. Finally, the sample, IS and QC were reconstituted in an aqueous solution of 40% methanol (v/v), containing 0.5% acetic acid and 0.1 pg·mL⁻¹ of each of the recovery standards. The samples were analysed by injection onto an ACQUITY UPLC® HSS C18 SB column (2.1 x 100 mm, 1.8 mm, Waters Corp.) with an in-line filter and separated by a gradient elution of water containing 0.02% formic acid against an increasing percentage of a solution of acetonitrile and methanol (50:50, v/v).

The analysis uses the MRM function of the MassLynx v.4.1 control software (Waters Corp.) with the resulting chromatographic traces quantified off-line by the QuanLynx software (v.4.1, Waters Corp.). By this method, each trace is integrated and the resulting ratio of signals (non-deuterated/IS) is compared with a previously constructed calibration curve to yield the amount of analyte present [nanograms (ng) per sample]. Calibration curves were generated from the MRM signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding IS.

Statistical analysis

STATISTICA data analysis software (Dell Inc. 2015, Round Rock, TX, USA) was used to analyse the data. Analysis of variance (ANOVA) or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at $P \leq 0.05$. Relationships between two continuous variables were analysed by regression analysis and the strength of the relationship indicated by Spearman's correlation coefficient. The percentage variation explained is $100 \times R^2$ % which is indicated as $(-)R^2$ if the correlation was negative.

Results and discussion

Objective / Milestone	Achievement
Only data for one plot are presented.	
Objective A:	Two seasons of leaf and root carbohydrate measurements were completed.
Objective B:	Quantification of endogenous phytohormones in leaves and roots was completed.
Objective C:	Two seasons of leaf mineral nutrient analyses were completed.
Objective D:	Measurements of leaf photosynthesis for season 1 and 2 were completed.
Objective E:	Horticultural responses for two seasons were completed.

Summary of results:

The aim of this study was to improve the understanding of the mechanism perpetuating alternate bearing in Citrus spp. and to establish the underlying cause(s) in the context of the recognised nutritional and hormonal theories of alternate bearing in citrus. 'Nadorcott' mandarin (*C. reticulata* Blanco) was selected as a model cultivar to use in this study.

Fruit load in 'Nadorcott' mandarin trees was the central factor determining return bloom flowering in subsequent seasons [$R^2=(-)0.80$ and $R^2=(-)0.73$ in seasons 1 and 2, respectively; ($P<0.001$)]. The quantity of flowers and fruit also had a strong inverse relationship with the number of new vegetative shoots in spring [$R^2=(-)0.80$ and $R^2=(-)0.79$ in seasons 1 and 2, respectively; ($P<0.001$)], summer [$R^2=(-)0.81$ $R^2=(-)0.78$ in seasons 1 and 2, respectively; ($P<0.001$)] and with total new vegetative shoots [$R^2=(-)0.79$ and $R^2=(-)0.85$ in seasons 1 and 2, respectively; ($P<0.001$)]. The number of new vegetative shoots that developed in "off" trees was 2- to 3-fold

higher in spring and summer, and the number of total new vegetative shoots that developed in “off” trees was almost double that in “on” trees (“off” = 863 and 1439 vs. “on” = 306 and 766) (Table 1). Therefore, fewer new vegetative shoots developed when fruit load was high, i.e. in “on” trees, than when fruit load was low, i.e. in “off” trees.

The higher number of new vegetative shoots in “off” trees affected flowering in the subsequent spring; “off” trees had more nodes and more potential sites available from which flowers could develop. Hence, tree flower number was 1.7-fold higher in “off” trees in spring of season 1 (“off” = 51 097 flowers per tree vs. “on” = 30 034 flowers per tree) and \approx 230-fold higher in spring of season 2 (“off” = 37 712 flowers per tree vs. “on” = 165 flowers per tree) (Table 1). Besides more flowering positions, flowering intensity was consistently higher in individual vegetative (“off”) shoots in “off” trees, than in “off” shoots in “on” trees. From “off” shoots in “off” trees \approx 50% more nodes developed than from “off” shoots in “on” trees and, additionally, flower intensity in “off” shoots in “off” trees, i.e. the number of flowers that sprouted from a single node in an individual shoot, was about 5-fold that of “off” shoots in “on” trees (Tables 2 and 3). From these results it was concluded that alternate bearing in ‘Nadorcott’ mandarin trees perpetuates because of an inhibiting effect of fruit load on subsequent flowering. This mechanism firstly manifests because of reduced budbreak and new vegetative shoot growth, and secondly, by a lower number of flowers that develops from a single flowering position in newly developed vegetative shoots.

Neither of these mechanisms affecting return bloom as a result of heavy fruit load were related to parameters of leaf gas exchange, or to leaf carbohydrate concentration. Apart from some anomalies, photosynthesis, stomatal conductance and transpiration rates during spring and summer were always higher in leaves in fruiting (“on”) shoots and “on” trees, from which fewer new vegetative shoots developed, than in “off” shoots in “off” trees, which was unsurprising (Tables 4 and 5). The relationship between leaf sugar concentration and the number of new vegetative shoots, however, was non-significant and very weak [season 1: $R^2=0.49$, $P=0.050$; season 2: $R^2=(-)0.01$, $P=0.970$]. Due to a higher starch concentration in leaves in “off” trees, than in “on” trees [season 1: 98 vs. 72 $\text{mg}\cdot\text{g}^{-1}$ leaf dry weight (DW); season 2: 53 vs. 42 $\text{mg}\cdot\text{g}^{-1}$ leaf dry weight DW] leaf starch concentration and number of new vegetative shoots showed a stronger relationship and a positive correlation in summer (season 1: $R^2=0.53$, $P=0.040$; season 2: $R^2=0.71$, $P<0.001$). However, when testing the significance of the apparent relationship using branch experiments, results failed to provide confirmation of the tree-level results. When fruiting branches were girdled, leaf starch concentration increased \approx 3-fold compared to non-fruiting branches (298 vs. 112 $\text{mg}\cdot\text{g}^{-1}$ leaf DW), but very few new vegetative shoots sprouted per branch compared to non-fruiting branches (1.6 vs 8.6). Furthermore, the study showed that although high leaf starch concentration correlated with the number of new vegetative shoots, leaf starch concentration did not contribute to new vegetative shoot growth, but accumulated to near-toxic levels in the palisade mesophyll parenchyma cells, the spongy mesophyll parenchyma cells and in the phloem cells of the leaf vein (Fig. 1). This resulted in the development of an abiotic physiological phenomenon in “off” trees described for the first time by this study as “fruit-load-induced leaf chlorosis”.

Fruit load probably disturbed the balance between vegetative shoot development and root growth. In “off” trees, root growth and vegetative shoot flushes showed alternating growth patterns – two distinct root growth peaks and three vegetative shoot flushes occurred in an eloquently synchronised pattern. The first root flush started during early summer in November, after cessation of the first and spring vegetative shoot flush (Fig. 2). By mid-summer in December this root flush peaked, but growth ceased towards the end of January and prior to initiation of the second and summer vegetative shoot flush. A second and final root flush started when shoot growth stopped in March and a third, small vegetative shoot flush followed in April (Fig. 2).

In “on” trees, root growth was almost completely absent and the number of new vegetative shoots was half that of “off” trees. The lack of root growth in “on” trees appeared to be related to a source-limitation in carbohydrates caused by profuse flowering in spring, and excessive fruiting in summer. The up to 230-fold more flowers in spring and \approx 7.3-fold more fruit in summer in “on” trees used the majority of sugars, which likely limited carbohydrate availability in the roots during these periods. This was apparent in the \approx 3-fold higher root sugar concentration in “off” trees during full bloom in October (119 vs. 36 $\text{mg}\cdot\text{g}^{-1}$ leaf DW) and \approx 20% higher root sugar concentration during summer in December (61 vs. 49 $\text{mg}\cdot\text{g}^{-1}$ leaf DW) (Fig. 3). These results were

convincing in terms of the significance in the difference between carbohydrate concentrations, but results were of a correlative nature only and this may be a shortcoming of this aspect on the research on alternate bearing. The results and interpretation nevertheless strongly concur with the well-documented and important inter-dependent relationship between root growth and vegetative shoot flushes in citrus and for the first time points to a similar relationship under conditions of alternate bearing. This opens up new avenues for horticultural research and could also provide practical opportunities to explore as a potential cultural practice in citrus production, e.g. exploring means to stimulate root growth and vegetative shoot flush in heavy-fruited trees. More importantly, this paves the way for possible novel research opportunities and a better understanding of alternate bearing in general, e.g. does the same relationship exist in other alternate bearing citrus species and/or cultivars or fruit crops.

Fruit load affected leaf mineral nutrient concentration, but not to the detriment of vegetative shoot flush or flowering. The crop removal factor, i.e. the g mineral element removed per kg fruit per tree, was higher for each mineral element in “off” trees – one kg fruit removed 2.3 g N, 0.3 g P, 3.1 g K, 1 g Ca and 0.4 g Mg, compared to 1.3 g N, 0.2 g P, 1.7 g K, 0.6 g Ca and 0.2 g Mg per one kg fruit in “on” trees. Fruit loads of 84, 110 and 52 kg fruit per tree in “on” trees, however, removed 217 g N, 28 g P, 296 g K, 100 g Ca and 35 g Mg per tree, which were 1.5 to 7 times more than that removed by fruit loads of 14, 71 and 16 kg fruit per tree in “off” trees (Table 6). In “off” trees, macro-nutrients accumulated in leaves to concentrations between 20% and 30% higher compared with that in “on” trees (Fig. 4). In all the experiments, however, leaf mineral nutrient concentrations showed no consistent relationship with return bloom flowering and/or with fruit load in the subsequent season (Table 7). With the exception of some anomalies, there were no relationships between the concentrations of any of the leaf mineral nutrients and parameters of flowering and vegetative shoot flush in response to different defruiting treatments in “on” trees. In addition, results from foliar nutrient spray treatments dismissed the significance of any ambiguities regarding the role of nutrients. It should, however, be mentioned that foliar spray treatments in this study were applied relatively late in the alternate bearing cycle and that future research on mineral nutrients should target the induction of root and/or shoot flushes with foliar nutrient sprays applied at an earlier timing. The results on this aspect of the possible cause of alternate bearing nevertheless suggest that tree mineral nutrient status can be considered a consequence, rather than a cause, of fruit load in alternate bearing ‘Nadorcott’ mandarin trees.

The two primary triggers in the alternate bearing mechanism in ‘Nadorcott’ mandarin were related to high concentrations of specific endogenous phyto-hormones. High concentrations of 1 H-indole-3-acetic acid (IAA) and metabolites of abscisic acid (ABA) in leaves was related to reduced new vegetative shoot development during the summer vegetative shoot flush (Table 8). “Off” shoots sprouted more new vegetative shoots and had a $\approx 47\%$ lower IAA concentration in leaves compared with “on” shoots, from which very few new vegetative shoots sprouted. The concentration of the end-product of ABA catabolism, viz. dihydrophaseic acid (DPA) was higher in leaves in “on” trees than in “off” trees (761.6 vs. 530.3 ng·g⁻¹ leaf DW), as well as that of ABA-GE (113.1 vs. 0.0 ng·g⁻¹ leaf DW), an ABA glucose ester and ABA storage form. On the other hand, the lower number of new summer vegetative shoots in “on” trees was not related to the low concentration of endogenous cytokinins. On the contrary, the concentration of cis-zeatin O-glucoside (c-ZOG), a storage form of active cytokinin, was higher in leaves in “on” trees than in leaves in “off” trees (1862.7 vs. 1092.5 ng·g⁻¹ leaf DW) (Table 8). Results suggest that cytokinin availability in “on” trees was not limited, but merely unable to participate in bud sprouting because of high concentrations of IAA in the presence of fruit. Exogenous cytokinin application was unable to stimulate bud sprouting and new summer vegetative shoot growth from “on” parent shoots, and when fruit were removed from “on” parent shoots, new vegetative shoots sprouted freely. A second major outcome was that high gibberellin (GA) concentration in leaves during winter was related to less flower development from shoots in “on” trees. The concentration of gibberellic acid (GA₃) in “on” shoot leaves was high and no GA₃ was detected in “off” shoot leaves (Table 9). Treatments of “off” trees and shoots with 40 mg·L⁻¹ synthetic GA₃, inhibited flowering. May and June was the period when citrus buds were most sensitive to GA, i.e. when maximum inhibition on flowering was obtained by exogenous GA₃ application, but earlier applications during summer should be tested to determine their effects on flowering response. Nevertheless, soil and foliar treatments of “on” trees during the corresponding period with 1000 mg·L⁻¹ of the GA biosynthesis-inhibitors paclobutrazol and uniconazole increased flowering and fruit development in “on” shoots. Considering that alternate bearing in citrus perpetuates due to an inhibition on flowering by fruit, these

results on the effects of treatments with GA biosynthesis-inhibitors in May and June could provide a practical mean for citrus producers to overcome the inhibition of fruit on flowering under conditions of alternate bearing.

An overall model is presented that integrates the nutritional and hormonal theories in alternate bearing in 'Nadorcott' mandarin (Fig. 5).

Technology transfer

This project formed the basis of a PhD study and thesis, at the Department of Horticultural Science, at the University of Stellenbosch, which was successfully completed in February 2018. The first season's data was presented at the 2016 CRI research symposium in the Drakensberg, as well as at the ICC in Brazil 2016. Preliminary findings and progress were also presented at the various CRI production workshops. Two peer-reviewed research articles also resulted from this study. A oral presentation will be presented at the 2018 CRI research symposium.

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Table 1. Total fruit yield, vegetative response and return bloom of ten-year-old alternate bearing 'Nadorcott' mandarin (*C. reticulata*) trees for three seasons.

Tree fruiting status	Fruit yield in the current year (kg per tree)	Fruit per tree in the current year (no.)	Return bloom and vegetative response in the following year (no. per tree)			
			Total flowers	Total new spring shoots	Total new summer shoots	Total new shoots
<u>Season 1</u>						
B: "Off"	14 b ^y	126 b	51 097 a	163 b	144 b	306 b
W: "On"	84 a	918 a	30 034 b	493 a	369 a	863 a
<i>P</i> value	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<u>Season 2</u>						
B: "On"	110 a	1225 a	165 b	1018 a	420 a	1439 a
W: "Off"	71 b	657 b	32 712 a	598 b	167 b	766 b
<i>P</i> value	0.0005	<0.0001	0.0004	0.0007	<0.0001	<0.0001
<u>Season 3</u>						
B: "Off"	16 b	144 b				
W: "On"	52 a	621 a				
<i>P</i> value	<0.0001	<0.0001				

^z For easier interpretation of results over three seasons, treatments were assigned colours blue (B) and white (W).

^y Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher's LSD test; $n = 10$).

Table 2. The phenological pattern of different shoot types (“on” or “off”) in “on” and “off” treatments of ten-year-old alternate bearing ‘Nadorcott’ mandarin (*C. reticulata*) trees during season 1. Values are expressed as number per shoot or in percentage (%) for fruit set measurements.

Treatments	Nodes	New spring vegetative shoots	Dormant buds	Leafy inflorescences	Leafless inflorescences	Flowers	Fruit	Fruit set %	New summer vegetative shoots
<u>Tree</u>									
B: ^z “On”	11.1 ns ^y	-	6.4 ns	1.5 ns	-	-	0.9 ns	22.4 ns	-
W:“Off”	10.6	-	6.7	1.5	-	-	0.7	29.4	-
<u>Shoot</u>									
“On” shoots	11.4 ns	-	5.6 b	2.5 a	-	-	1.5 a	21.8 ns	-
“Off” shoots	10.7	-	7.5 a	0.5 b	-	-	0.1 b	30.1	-
<u>Tree x Shoot</u>									
B:“On” × “on” shoots		0.1 c ^x			3.4 a	12.7 a			0.3 c
B:“On” × “off” shoots		1.9 b			0.1 c	0.7 c			1.2 b
W:“Off” × “on” shoots		0.7 c			1.3 b	6.4 b			0.5 c
W:“Off” × “off” shoots		3.2 a			0.00 c	0.5 c			3.5 a
<u>P value</u>									
Tree	0.0705	0.0411	0.4531	0.9751	0.0012	0.0111	0.3748	0.4902	0.0478
Shoot	0.4895	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.4211	<0.0001
Tree x Shoot	0.7381	0.0186	0.3141	0.5100	0.0022	0.0162	0.4295	0.6198	0.0237

^z For easier interpretation of results from two seasons, treatments were assigned colours blue (B) and white (W).

^y No significant difference.

^x Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s least significant difference test; $n = 10$).

Table 3. The phenological pattern of different shoot types (“on” or “off”) in “on” and “off” treatments of ten-year-old alternate bearing ‘Nadorcott’ mandarin (*C. reticulata*) trees during season 2. Values are expressed as number per shoot or in percentage (%) for fruit set measurements.

Treatments	Nodes	New spring vegetative shoots	Dormant buds	Leafy inflorescences	Leafless inflorescences	Flowers	Fruit	Fruit set %
<u>Tree</u>								
B ^z :“Off”	-	3.3 ns ^x	7.1 ns	1.4 ns	4.4 ns	-	1.4 ns	26.8 ns
W:“On”	-	2.6	8.0	1.5	4.8	-	1.4	22.2
<u>Shoot</u>								
“On” shoots	-	4.3 a	6.9 ns	0.7 b	1.7 b	-	0.9 b	36.0 a
“Off” shoots	-	1.6 b	8.1	2.2 a	7.5 a	-	2.0 a	13.0 b
<u>Tree x Shoot</u>								
B:“Off” x “on” shoots	11.4 c ^y					1.92 c		
B:“Off” x “off” shoots	18.2 b					5.70 b		
W:“On” x “on” shoots	12.3 c					1.32 c		
W:“On” x “off” shoots	27.6 a					24.43 a		
<i>P</i> value								
Tree	0.0348	0.2991	0.1614	0.8332	0.7925	0.0187	0.9058	0.4589
Shoot	0.0002	<0.0001	0.0598	0.0005	<0.0001	<0.0001	0.0058	0.0015
Tree x Shoot	0.0251	0.2915	0.8032	0.2105	0.9533	0.0162	0.0714	0.7148

^z For easier interpretation of results over two seasons, treatments were assigned colours blue (B) and white (W)

^y Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s LSD test; $n = 10$).

^x No significant difference.

Table 4. The rates of photosynthesis, stomatal conductance and transpiration of leaves in different shoot types (“on” or “off”) in “on” and “off” treatments of ten-year-old alternate bearing ‘Nadorcott’ mandarin (*C. reticulata*) trees during summer of season 1.

Treatments	Leaf photosynthesis (A_c)				Leaf stomatal conductance (g_s)				Leaf transpiration (E)			
	$\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				$\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				$\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$			
	Oct.	Nov.	Dec.	Jan.	Oct.	Nov.	Dec.	Jan.	Oct.	Nov.	Dec.	Jan.
<u>Tree</u>												
B: ^z “On”	6.11 a ^y	3.66 a	-	6.51 a	0.09a	0.06 a	-	0.11ns	1.83a	1.18a	1.13b	2.18ns
W:“Off”	3.22 b	3.28 b	-	5.88b	0.07b	0.04 b	-	0.11	1.16b	0.94b	1.60a	2.12
<u>Shoot</u>												
“On” shoots	5.36 a	3.62 ns ^x	-	6.24ns	0.09a	0.05 ns	-	0.11ns	1.63a	1.06ns	1.50a	2.18ns
“Off” shoots	3.97 b	3.32	-	6.15	0.07b	0.05	-	0.11	1.36b	1.05	1.24b	2.13
<u>Tree x Shoot</u>												
B:“On” x “on” shoots			3.76a				0.06 a					
B:“On” x “off” shoots			2.72b				0.04 b					
W:“Off” x “on” shoots			3.45a				0.06 a					
W:“Off” x “off” shoots			3.52a				0.06 a					
<i>P</i> value												
Tree	<0.0001	0.0234	0.1858	0.0252	0.0002	0.0003	0.0072	0.4321	<0.0001	0.0002	<0.0001	0.4532
Shoot	0.0004	0.0675	0.0110	0.7178	0.0071	0.8699	0.0041	0.7031	0.0108	0.9209	0.0024	0.6503
Tree x Shoot	0.2501	0.2804	0.0022	0.9874	0.1947	0.6272	0.0238	0.9213	0.2759	0.8124	0.0847	0.8607

^zFor easier interpretation of results from two seasons, treatments were assigned colours blue (B) and white (W)

^y Different letters in the same column denote significant differences between values ($P<0.05$; Fisher’s LSD test; $n=8$).

^xNo significant difference.

Table 5. The rates of photosynthesis, stomatal conductance and transpiration of leaves in different shoot types (“on” or “off”) in “on” and “off” treatments of ten-year-old alternate bearing ‘Nadorcott’ mandarin (*C. reticulata*) trees during summer of season 2.

Treatments	Leaf photosynthesis (A_c)				Leaf stomatal conductance (g_s)				Leaf transpiration (E)			
	$\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$				$\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$				$\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$			
	Oct.	Nov.	Dec.	Jan.	Oct.	Nov.	Dec.	Jan.	Oct.	Nov.	Dec.	Jan.
<u>Tree</u>												
B ^z : “Off”	3.10 b ^y	1.15 b	1.28 b	2.34 b	0.03b	0.02 b	0.03 b	0.03 b	-	-	0.73 b	0.73 b
W: “On”	5.08 a	1.52 a	2.02 a	4.80 a	0.05a	0.03 a	0.02 a	0.07 a	-	-	0.99 a	1.37 a
<u>Shoot</u>												
“On” shoots	3.99 ns ^x	1.43 ns	1.68 ns	3.26 ns	0.04 ns	0.02 ns	0.02 ns	0.05 ns	-	-	0.88 ns	0.98 ns
“Off” shoots	4.20	1.24	1.62	3.89	0.05	0.02	0.02	0.06	-	-	0.85	1.13
<u>Tree x Shoot</u>												
B: “Off” x “on” shoots									0.94 b	0.72 c		
B: “Off” x “off” shoots									0.83 b	0.84 bc		
W: “On” x “on” shoots									1.09 b	1.10 a		
W: “On” x “off” shoots									1.39 a	0.93 ab		
<i>P</i> value												
Tree	<0.0001	0.0461	<0.0001	<0.0001	0.0061	0.0045	0.0083	<0.0001	0.0010	0.0032	0.0047	<0.0001
Shoot	0.5495	0.2955	0.6557	0.1152	0.3203	0.5027	0.5469	0.1062	0.3223	0.7656	0.6742	0.2180
Tree x Shoot	0.1470	0.2440	0.7150	0.5700	0.0810	0.0880	0.2710	0.2090	0.0472	0.0024	0.3370	0.1120

^zFor easier interpretation of results from two seasons, treatments were assigned colours blue (B) and white (W).

^y Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s LSD test; $n=8$).

^x No significant difference.

Table 6. The total amount (g) of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) removed by crop load from “on” and “off” ‘Nadorcott’ mandarin (*C. reticulata*) trees over three seasons.

Tree fruiting status	Mineral elements removed by fruit load (g per tree)				
	N	P	K	Ca	Mg
<u>Season 1</u>					
B ^z : “Off”	32b	4 b	43 b	14 b	5 b
W: “On”	106a	14 a	145 a	49 a	17 a
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<u>Season 2</u>					
B: “On”	217a	28 a	296 a	100 a	35 a
W: “Off”	152b	19 b	204 b	68 b	23 b
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<u>Season 3</u>					
B: “Off”	38b	5 b	52 b	17 b	6 b
W: “On”	93a	12 a	127 a	43 a	15 a
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^z For easier interpretation of results over two seasons, treatments were assigned colours blue (B) and white (W).

^y Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s LSD test; $n=8$).

Table 7. The relationship between measurements of tree phenological events and the concentrations of leaf nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) in alternate bearing 'Nadorcott' mandarin (*C. reticulata*) trees during season 1.

Month	Leaf mineral nutrient concentration (mg·g ⁻¹ leaf DW ^z)	Flowers		Spring vegetative shoots		Summer vegetative shoots		Total shoots		Total tree fruit yield		Return bloom		Spring vegetative shoots	
		R ²	P	R ²	P	R ²	P	R ²	P	R ²	P	R ²	P	R ²	P
September	N	(-)0.21	0.430	0.35	0.190	0.49	0.05	0.35	0.180	(-)0.45	0.080	0.62	0.010	(-)0.40	0.130
	P	(-)0.55	0.030	0.26	0.330	0.17	0.53	0.15	0.570	(-)0.16	0.560	0.28	0.300	(-)0.41	0.120
	K	(-)0.47	0.060	(-)0.20	0.460	(-)0.29	0.28	(-)0.25	0.360	0.19	0.480	(-)0.25	0.360	(-)0.14	0.600
	Ca	0.06	0.820	(-)0.13	0.630	(-)0.19	0.49	(-)0.17	0.520	0.26	0.340	(-)0.15	0.590	0.11	0.680
	Mg	0.05	0.860	0.15	0.590	0.17	0.53	0.18	0.510	(-)0.22	0.400	0.28	0.290	0.07	0.810
December	N	(-)0.11	0.670	0.01	0.980	0.21	0.44	0.16	0.560	(-)0.18	0.500	0.24	0.360	(-)0.07	0.800
	P	(-)0.46	0.070	(-)0.01	0.960	0.04	0.89	(-)0.08	0.760	0.10	0.700	(-)0.08	0.770	(-)0.29	0.280
	K	(-)0.20	0.450	(-)0.06	0.820	(-)0.22	0.42	(-)0.22	0.400	0.22	0.410	(-)0.18	0.500	0.00	1.000
	Ca	0.00	1.000	(-)0.19	0.490	(-)0.23	0.40	(-)0.21	0.440	0.14	0.610	(-)0.28	0.290	(-)0.01	0.970
	Mg	0.15	0.580	0.02	0.940	0.07	0.81	0.03	0.920	(-)0.16	0.560	0.15	0.570	0.06	0.810
March	N	-	-	-	-	0.23	0.40	0.14	0.590	(-)0.031	0.250	0.20	0.450	(-)0.45	0.080
	P	-	-	-	-	0.51	0.04	0.41	0.110	(-)0.59	0.020	0.48	0.060	(-)0.70	0.001
	K	-	-	-	-	0.45	0.08	0.40	0.130	(-)0.58	0.020	0.45	0.080	(-)0.76	0.001

June	Ca	-	-	-	-	(-)0.57	0.02	(-)0.48	0.060	0.62	0.010	(-)0.61	0.010	0.61	0.010
							0								
	Mg	-	-	-	-	0.14	0.60	0.08	0.760	(-)0.19	0.470	0.27	0.320	0.00	0.990
							0								
	N	-	-	-	-	-	-	-	-	(-)0.62	0.010	0.66	0.010	(-)0.51	0.050
	P	-	-	-	-	-	-	-	-	(-)0.63	0.010	0.52	0.040	(-)0.62	0.010
	K	-	-	-	-	-	-	-	-	(-)0.60	0.010	0.67	0.001	(-)0.50	0.050
Ca	-	-	-	-	-	-	-	-	0.63	0.010	(-)0.68	0.001	0.45	0.080	
Mg	-	-	-	-	-	-	-	-	(-)0.28	0.290	0.34	0.190	(-)0.05	0.850	

^z Dry weight.

The data were analysed using regression analysis. The strength of the relationship is indicated by Spearman's correlation coefficient. The percentage variation explained is $100 \cdot R^2$, which is indicated as $(-)R^2$ if the correlation was negative. Significance at the 95% level.

Table 8. The phyto-hormone profile of endogenous auxin (IAA), cytokinin, abscisic acid (ABA) and gibberellin (GA), during summer in leaves of heavy-fruiting, i.e. “on” and low-fruiting, i.e. “off” ‘Nadorcott’ mandarin (*C. reticulata*) trees representative of alternate bearing. Values are expressed in ng·g⁻¹ leaf dry weight.

Auxins	IAA			
“On” leaves	12.5 a ^z			
“Off” leaves	6.6 b			
<i>P</i> value	0.0239			
Cytokinins	t-ZR	iPA	t-ZOG	c-ZOG
“On” leaves	0.74 ns	5.4 ns	120.9 ns ^y	1862.7a
“Off” leaves	0.47	6.1	134.5	1092.5b
<i>P</i> value	0.6270	0.5640	0.7339	
Abscisic acid	ABA	ABA-GE	PA	DPA
“On” leaves	24.3 ns	113.1 a	17.4 ns	761.6a
“Off” leaves	23.9	0.0 b	20.7	530.3b
<i>P</i> value	0.9115	0.0001	0.5848	0.0261
Gibberellins	GA ₃			
“On” leaves	3.1 ns			
“Off” leaves	2.4			
<i>P</i> value	0.2645			

^z Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s LSD test; $n = 5$).

^y No significant differences.

Table 9. The phyto-hormone profile of endogenous auxin (IAA), cytokinin, abscisic acid (ABA) and gibberellin (GA), during winter in leaves of heavy-fruited, i.e. “on” and low-fruited, i.e. “off” ‘Nadorcott’ mandarin (*C. reticulata*) trees representative of alternate bearing during winter. Values are expressed in ng·g⁻¹ leaf dry weight.

Auxins	IAA				
“On” leaves	6.9 ns ^z				
“Off” leaves	5.6				
<i>P</i> value	0.6755				
Cytokinins	iPA	t-ZR	t-ZOG	c-ZOG	
“On” leaves	10.2 b ^y	0.51 ns	138.7 ns	2633.1 a ^y	
“Off” leaves	20.3 a	0.39	103.3	1353.6 b	
<i>P</i> value	0.0231	0.4110	0.3127	0.0018	
Abscisic acid	ABA	ABA-GE	PA	DPA	
“On” leaves	41.5 ns	195.7 ns	15.3b	671.7 a	
“Off” leaves	47.6	130.8	50.5a	445.3 b	
<i>P</i> value	0.2227	0.4495	0.0107	0.0252	
Gibberellins	GA ₃				
“On” leaves	5.3 a				
“Off” leaves	0.0 b				
<i>P</i> value	0.0270				

^z No significant differences.

^y Different letters in the same column denote significant differences between values ($P < 0.05$; Fisher’s LSD test; $n = 5$).

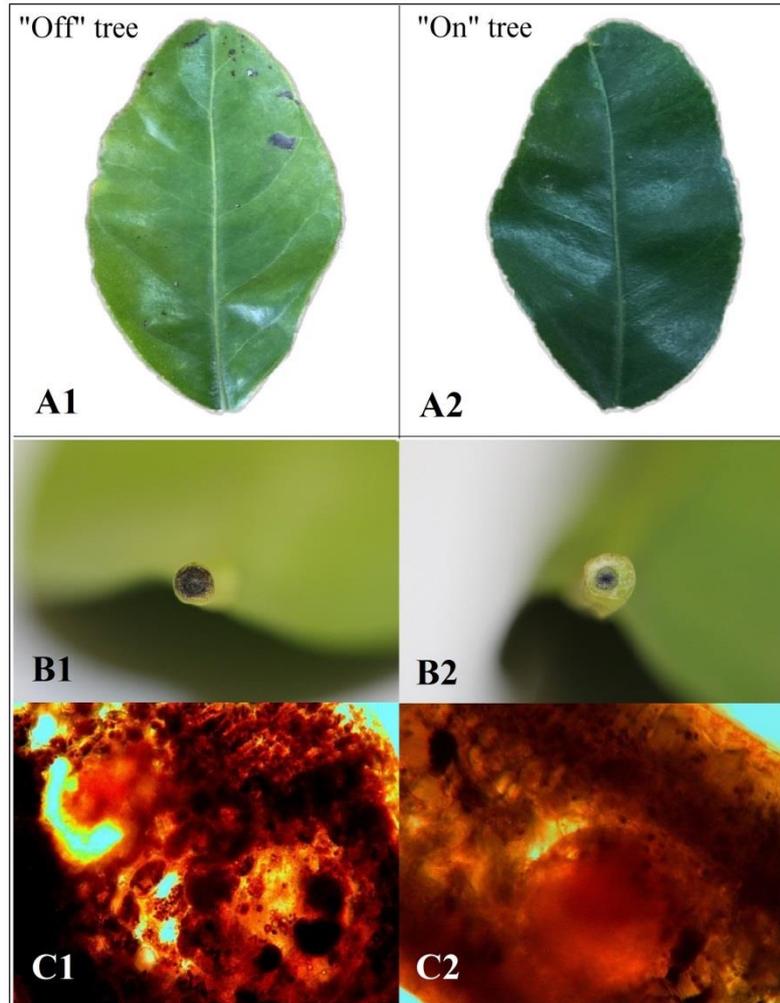


Fig. 1. The effects of fruit load ("off" = low fruiting; "on" = heavy fruiting) on leaf colour (A1 and A2) and the distribution of starch granules in the leaf petiole (B1 and B2) and the leaf blade (C1 and C2) of 'Nadorcott' mandarin (*C. reticulata*) trees during winter in 2015. Leaves were dissected, stained with a 2% (v/v) iodine solution and examined using microscopic (Carl Zeiss ERc5s; Göttingen, Germany) photographic comparisons.

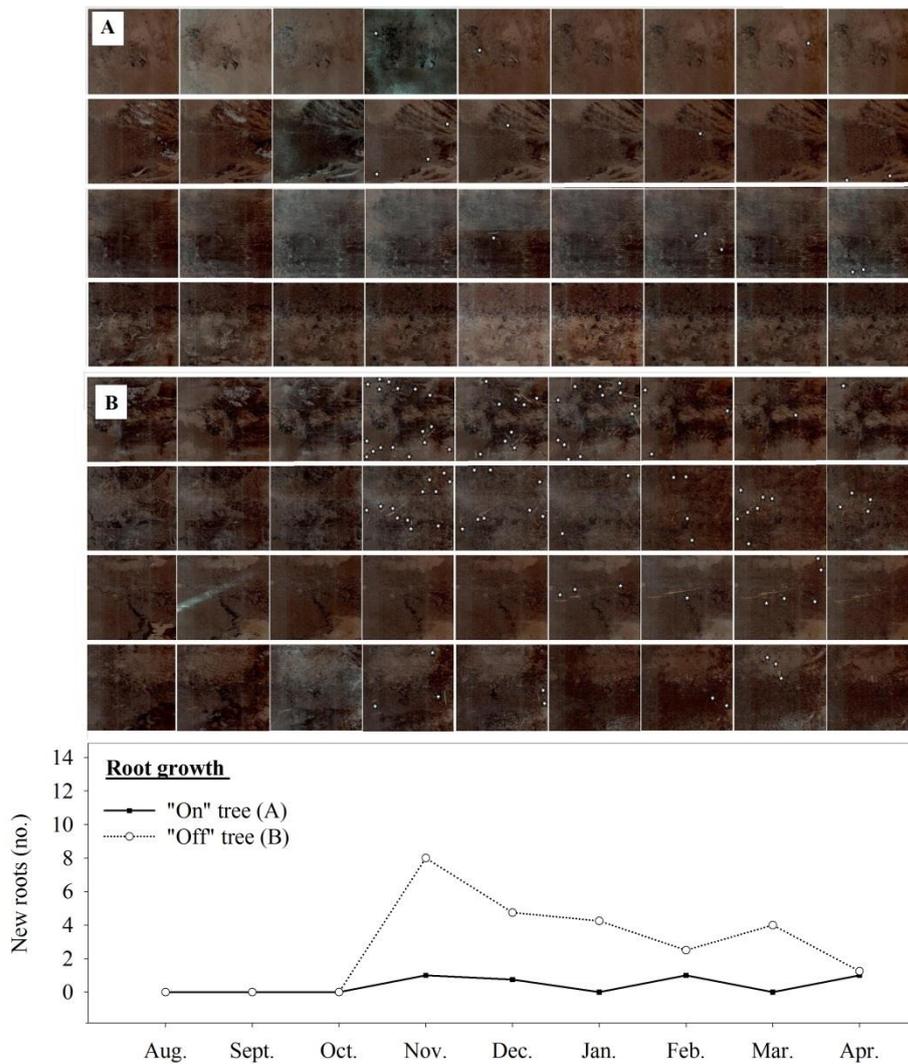


Fig. 2. The root growth pattern of four ten-year-old “on” (A) and “off” (B) ‘Nadorcott’ mandarin (*C. reticulata*) trees. Minirhizotron tubes were installed prior to winter in 2016 and evaluations started in Aug. 2016 and were continued at monthly intervals. Digital images were captured in each tube with a root imager (CI-600 In Situ Root Scanner, CID-BioScience Inc., Camas, WA, USA). Vertical images were captured down each minirhizotron tube and new roots counted at monthly intervals. A star indicates a new root within each observation.

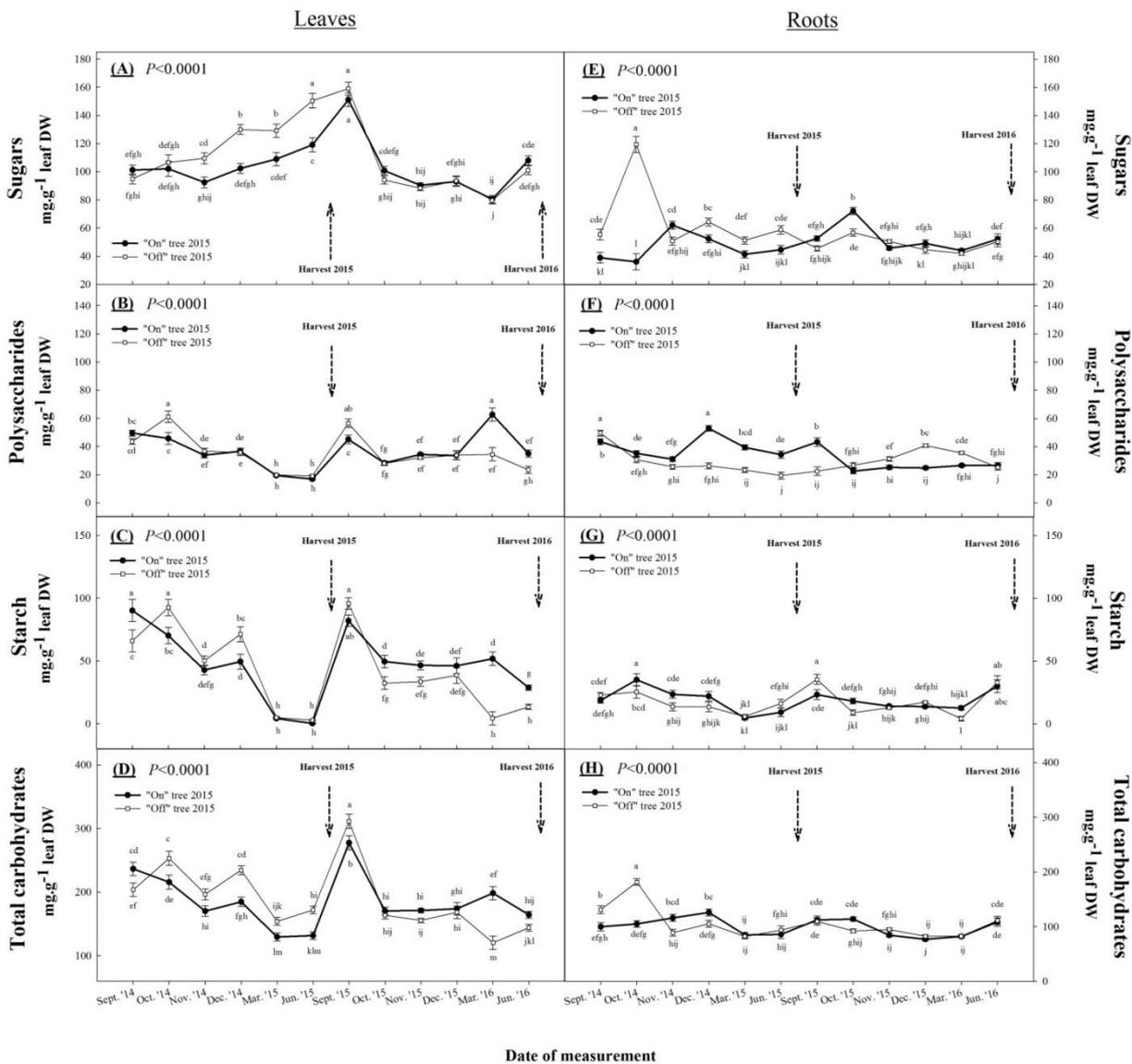


Fig. 3. The seasonal concentrations of different carbohydrate components in leaves and roots of ten-year-old “on” and “off” ‘Nadorcott’ mandarin (*C. reticulata*) trees: A and E) sugars; B and F) polysaccharides; C and G) starch; and D and H) total carbohydrates. The arrows indicate the time of harvest for each season. Bars denote standard errors of the means, and different letters, significant differences between values ($P < 0.05$; Fisher’s LSD test; $n = 10$). DW = dry weight.

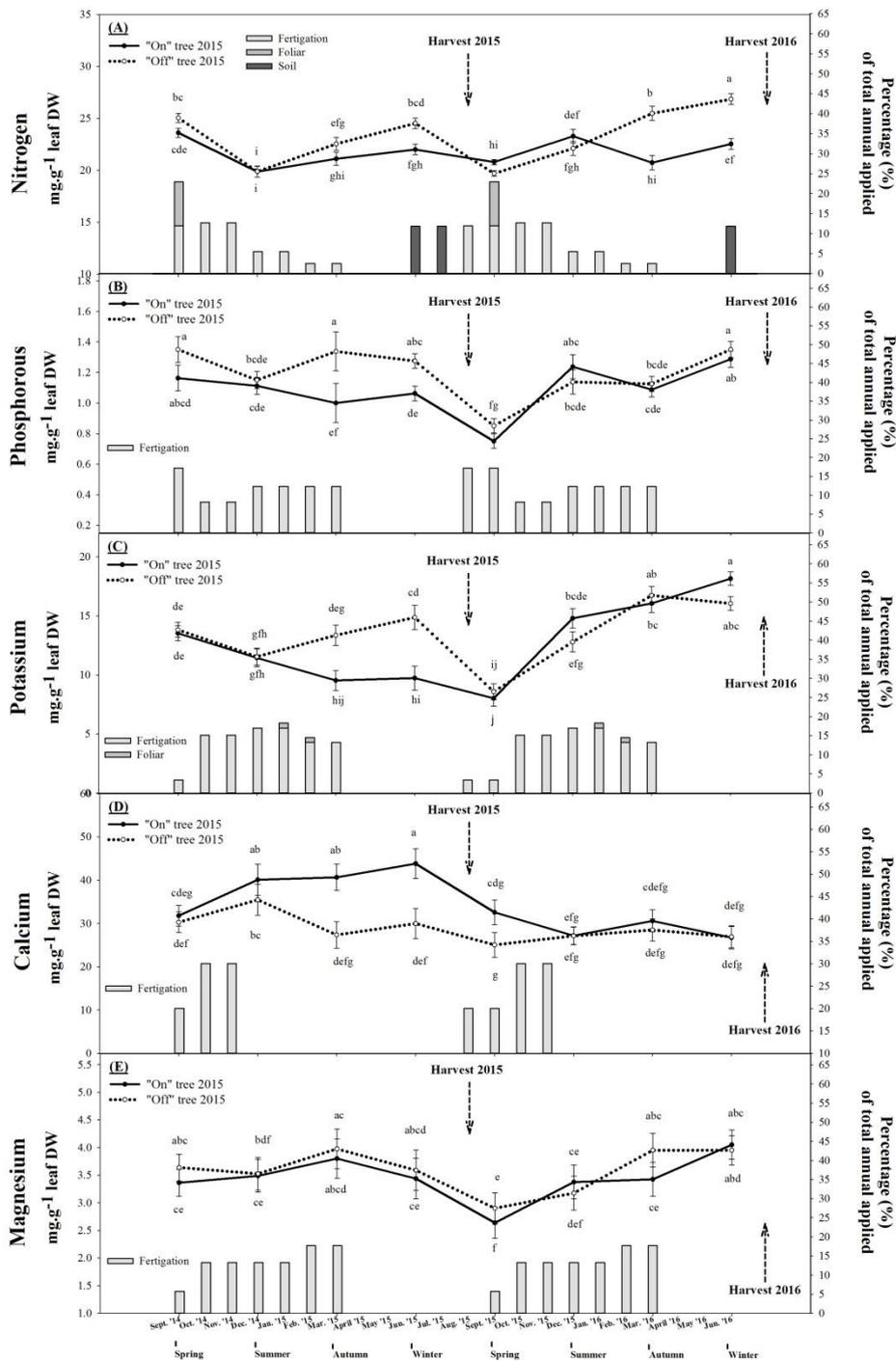


Fig 4. The concentrations of leaf nitrogen (A), phosphorous (B), potassium (C), calcium (D) and magnesium (E), determined at three-monthly intervals over two seasons in alternate bearing 'Nadorcott' mandarin (*C. reticulata*) trees. The line graph corresponds to the left Y-axis and represents the concentration of the mineral elements in the leaf expressed as $\text{mg}\cdot\text{g}^{-1}$ leaf dry weight (DW), whereas the bar graph corresponds to the right Y-axis and represents the rate and distribution of the annual nutrient application as a percentage of the total annual application. The arrows indicate the time of harvest. Bars denote standard errors of the means and different letters significant differences between values ($P < 0.05$; Fisher's LSD test; $n=8$). DW = dry weight.

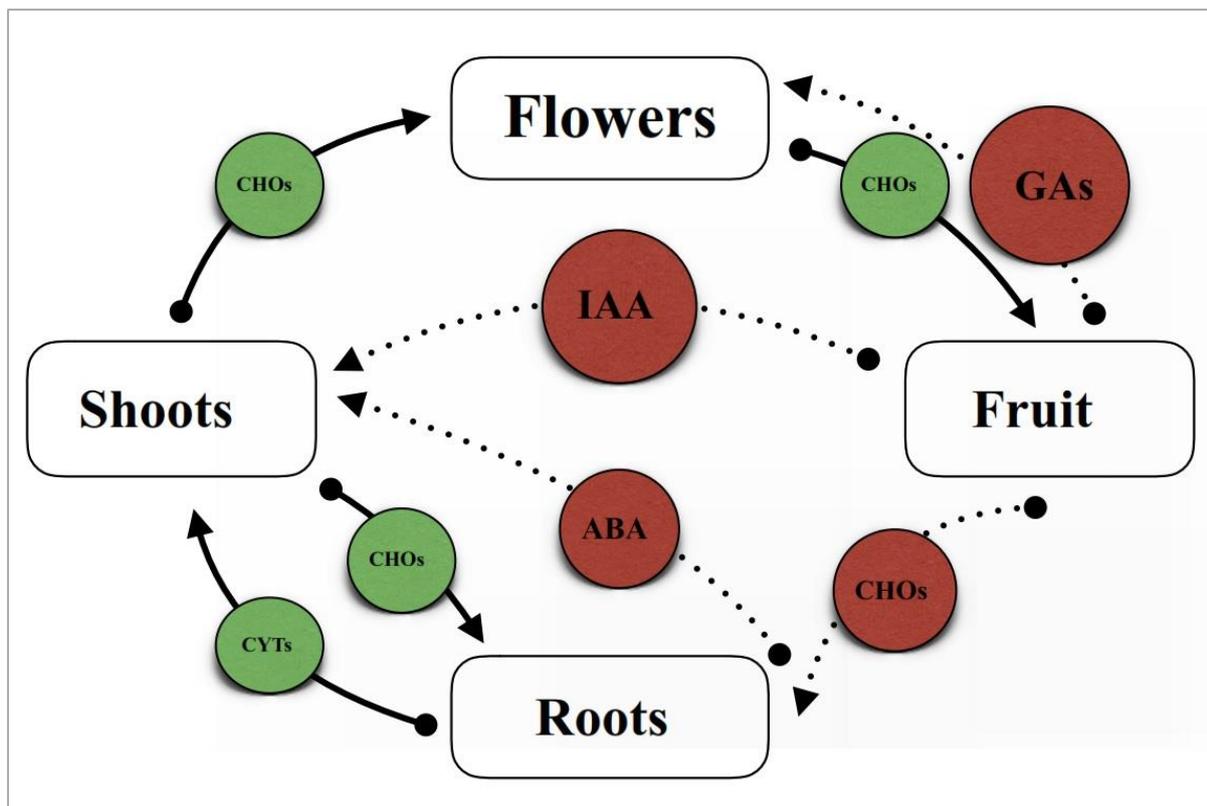


Fig 5. A schematic model proposed for the various factors affecting the alternate bearing habit of 'Nadorcott' mandarin, including carbohydrates (CHOs) in the nutritional theory, and the phyto-hormones abscisic acid (ABA), cytokinins (CYTs), gibberellins (GAs) and the auxin, 1 *H*-indole-3-acetic acid (IAA), in the hormonal theory of alternate bearing. Solid arrows indicate a positive relationship between the organs, viz. roots, vegetative shoots, flowers and fruit, and dotted arrows indicate a negative relationship. A factor in green is responsible for the endogenous stimulation (promotive action) of the organ to which its arrow is pointed, and a factor in red is responsible for the endogenous inhibition (inhibitive action) of the development of the organ to which its arrow is pointed.

4.3.5 FINAL REPORT: The benefits of shade netting for citrus fruit quality

Project RCE4 (Exp: 1125) (2015/6 – 2017/8) by Paul Cronje, Jakkie Stander, Teunis Vahrmeijer, Jade North, Martin Gilbert, Jan van Niekerk (CRI), Graham Barry (XLnt citrus) Remy Rosalie, Robert Brown, Johane Botes and Du Toit Prins (SU)

Summary

All planned aspects of this project have been successfully completed for the second season of data capturing and culminated into three MSc Agric theses being submitted in 2018. Due to the relevance of this topic the project was granted a further extension to enable answering further pertinent questions in one additional data season. The data collection started at fruit set in 2015 and will be finished during the harvest of 2018. All aspects regarding the development of the experimental site were successfully completed and a 2 ha Nadorcott orchard was covered with 20% shade netting. After completion of the structure and redesign of the irrigation system, various instruments were installed to measure environmental and soil conditions. The aim of the project was to determine if shade netting improved or reduced the export volume and quality of citrus fruit. Subsequently, data on various aspects which includes microclimate, insect species present, fruit quality, and horticultural practise and water use efficacy was gathered. It is clear that shade netting, in general, will improve fruit size and reduce the superficial blemishes such as wind and sun damage. In addition, the carbon accumulation and water use efficiency was also enhanced in the hot summer months (Dec-Feb) compared to

the control. Under these experimental conditions in Citrusdal, no negative impact was documented on any fruit quality attribute i.e., rind colour, susceptibility to physiological disorders, or reduction of TSS or TA.

Opsomming

Al die aspekte van die projek is suksesvol afgehandel in die tweede seisoen van data insameling en het gelei tot die inhandiging van drie MSc Agric tesisse in 2018. Na gelang van die projek se relevansie vir die bedryf, is daar verder befondsing verskaf vir 'n addisionele seisoen. Die eerste seisoen het in 2015 begin en sal afgesluit word tydens die oes in 2018. Alle ontwikkelingswerk t.o.v. die proefpreseel was suksesvol afgehandel en 'n 2 ha Nadorcott boord was met 'n 20% wit skadunet bedek. Na afhandeling van die strukture en besproeiings sisteem was verskeie instrumente geïnstalleer om die mikroklimaat verandering en die grond water kontinuum te meet. Die doel van die projek is om te bepaal of skadunet die volume en kwaliteit van sitrusvrugte vir die uitvoermark verhoog. Om hierdie doel te bereik, was data op verskeie aspekte soos mikroklimaat, insek spesie teenwoordigheid, vrug kwaliteit, hortologiese bestuursaspekte en water gebruik ingewin. Die data dui aan dat die skadunette onder hierdie toestand in Citrusdal, wel lei tot 'n verhoogde vruggrotte en verlaging van oppervlakletsels soos sonbrand en windskaad. Verder meer was die koolhidraat akkumulاسie en water gebruik verbeter gedurende die somer maande (Des-Feb). Onder hierdie toestande kon geen negatiewe effek soos interne en eksterne vrugkwaliteit, gedokumenteer word agv die skadunette nie.

Introduction

The sustainable economic success of the South African citrus export industry depends heavily on increased production and export of high quality citrus fruit to the valuable, but discerning northern hemisphere markets. It is evident from literature and extrapolating from other horticulture crops in different countries that various advantages could be gained by using shade netting in citrus orchards. This technology has received inadequate research focus in both the worldwide Citricultural industry as well as in South Africa. The high cost of the implementation of this technology asks for very clear advantages in fruit quality that would translate into a significant increase of export quality fruit and return on investment. Central to the use of shade netting stands the aspect of carbon fixation and allocation under the reduced photosynthetic active radiation (PAR) conditions.

Photosynthesis in sun-acclimated citrus trees reaches saturation point after about one third of full sunlight (Syvertsen, 1984). In high light environments this excess PAR can increase leaf and fruit temperature by as much as 9°C above air temperature (Syvertsen and Albrigo, 1980). Resulting in higher water loss (higher VPD) and reduced stomatal control. These physiological responses to high light and heat conditions cascade into a reduction of photosynthesis. As the closed stomata result in a drop in growth and fruit yield (Goldschmidt, 1999) due to lower carbohydrate accumulation and water use efficiency.

Various fruit quality aspects could benefit from the reduction of heat in citrus orchards covered by shade netting, i.e., fruit set and rind condition (colour and lack of blemishes). The reduced heat load under the shade netting could increase fruit set due to lower ambient temperature at this physiologically sensitive period, especially with the valuable seedless cultivars. The impact of shade netting on the fruit, foliar and soilborne pests and diseases is also unknown.

This research will be undertaken over the next three seasons using an orchard with and without shade netting in order to study the responses of both trees and fruit to the altered environment and determine whether implementation of this technology would make financial sense for harsh, marginal climates. The primary aim of this project would therefore be to improve yield as well as internal and external fruit quality of citrus in order to realise higher export volumes.

Objectives

To determine the impact of an altered ambient climate on:

1. Key tree and fruit physiological response that determine fruit quality due to microclimatic changes.
2. Water use efficiency.
3. Susceptibility of fruit/tree to damage by insects and infection by fruit pathogens.

The reporting will be done in separate sections for the different focus areas within the larger project and will only contain key results from the study. The three complete MSc thesis, from which the results were selected, will be available from the researchers on request.

Impact of shade netting on 'Nadorcott' mandarin tree's microclimatic

Due to a lack of comprehensive data on the microclimate changes under shade netting in citrus the aim of this part of the project was to measure and describe those changes occurring in a 'Nadorcott' mandarin orchard after covering it with 20% white shade net. It was hypothesized that 20% permanent white shade net will not alter the orchard microclimate and growing conditions in terms of ambient and soil temperature, soil water content, solar radiation, relative humidity, and wind speed.

Materials and methods

Two Campbell scientific weather stations were installed, one under shade net and the other in an open block. Hourly measurements of soil water content ($\text{m}^3\cdot\text{m}^{-3}$), relative humidity (%), irradiation ($\text{Mj}\cdot\text{m}^{-2}$) (Fig 1), wind speed ($\text{m}\cdot\text{s}^{-1}$), soil and air temperatures are measured using descriptive analysis. In addition to the data from the weather stations additional air temperature data was gathered in each replicate, by the use of TinyTag Plus 2 TGP-4510 data loggers (Gemini Data Loggers, Chichester, UK). The loggers were placed within the tree (1 ± 1.5 m above ground level). Loggers were placed in each treatment block consisting of four replicates per treatment. Calculations, as made for the weather stations for ambient air and soil temperatures, were also done with the data from the TinyTag loggers only with different time frames due to data being more complete during critical the growth stages of citrus.

A Li-COR LP 6400 was used to measure the difference in physiology of the trees under shade netting and without. A light meter was used to determine variation in the photosynthetic active radiation between treatments as well as variation within a tree. Monthly readings were taken from Feb 2016 until harvest and continued from June 2016 until harvest 2017 (full season). The 2016 season was used to identify which leaves to use in a tree to reduce the variation between treatments in order to interpret the differences in treatments accurately. Measurements were done between 9 and 11 in the morning on a monthly basis comparing the shade net with open blocks.

Results and discussion

The study showed differences between the shade net and open treatments in terms of seasonal variances for different parameters. Solar radiation was reduced by 17% but ambient air temperatures was not drastically affected (Fig. 1). Nonetheless, a change was seen with the amount of hours experienced within a specific physiological temperature range. Changes in monthly air temperatures under shade net within the canopy lead to an increase in effective heat units (EHU) accumulated throughout a season and can have an effect on vegetative and fruit growth (Fig. 2). A high reduction of wind speed was observed for both seasons, as well as an increased soil water content under the net. Lowered windspeed reduced air mixing under shade netting and lead to a slight difference in relative humidity. However, these small changes in temperature and RH resulted in a decrease in vapour pressure deficit (VPD) under the netting (Fig. 3). Citrus trees in an orchard covered by 20% shade netting will experience an alteration of the microclimate which could lead to a reduction in superficial damage and possibly improved carbon accumulation and water use efficiency (Fig. 4). A citrus tree's response to effectively conserve water during heat stress periods and high VPD is by reducing stomatal conductance and as a result, a reduction of A_c rate. This increase in VPD had a negative effect on stomatal conductance and photosynthesis in Feb. and Mar. 2017, causing stomatal closure and a decrease in CO_2 assimilation rates (Fig. 5). During the summer, i.e. Jan. to Mar. 2017, the shade net affected the photosynthetic capacity of the 'Nadorcott' mandarin trees positively, with a trend of higher A_c and g_s , whereas E and photosynthetic WUE remained fairly constant.

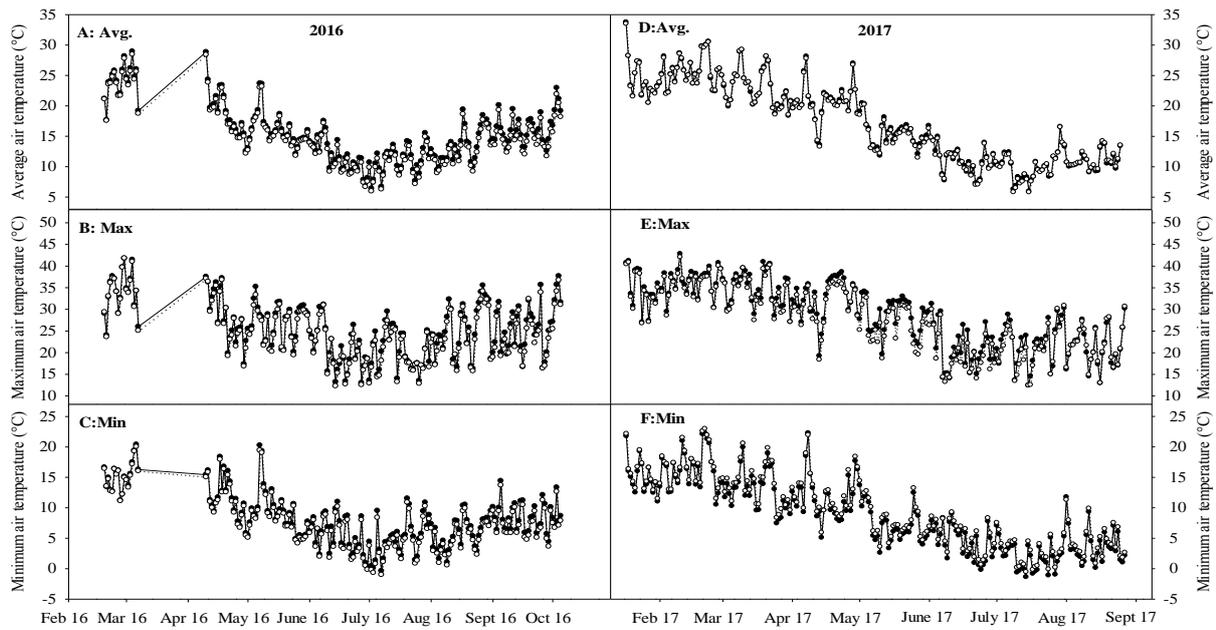


Fig. 1. The effect of 20% white shade net on the average (A and B) and maximum (C and D) solar radiation in a 'Nadorcott' mandarin orchard based in Citrusdal measured at 4 m height. (○ = Shade net; ● = Control).

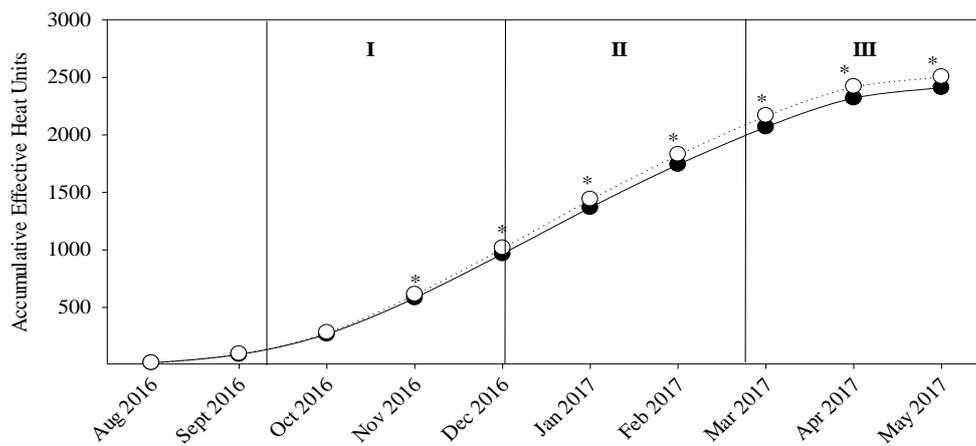


Fig. 2 The effect of 20% white shade netting on within tree canopy accumulative effective heat units during critical fruit growth stages I, II and III in a 'Nadorcott' mandarin orchard in Citrusdal (○ = Shade net; ● = Control). * indicates mean values ($n = 4$) within a month differing significantly at $P < 0.05$. Month*Treatment = $P < 0.0001$.

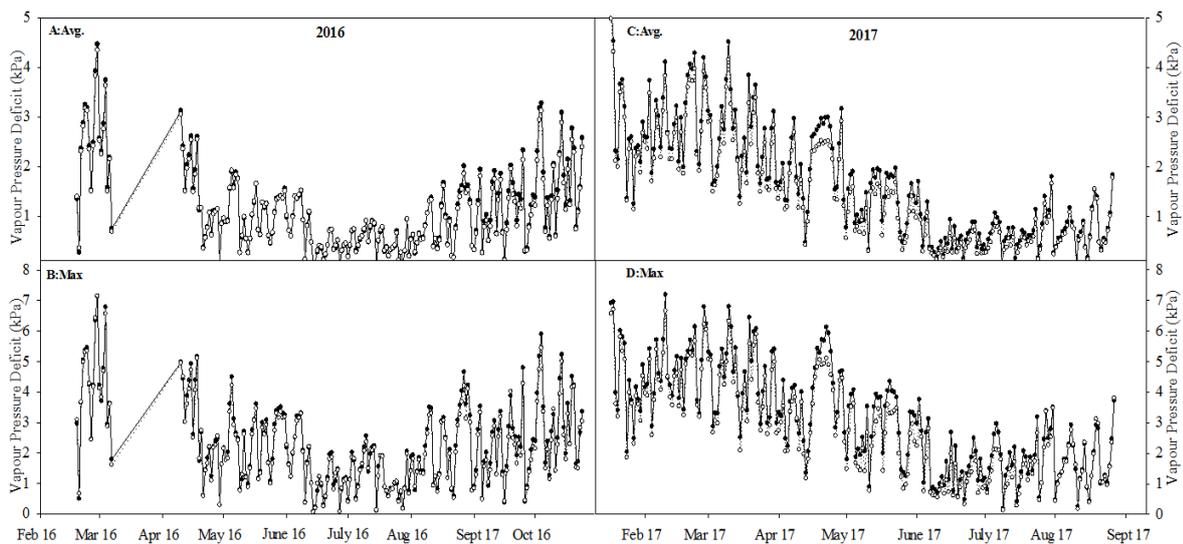


Fig. 3. Vapour pressure deficit of a 'Nadorcott' mandarin orchard in Citrusdal and how 20% white shade net (○) affects the daily average (A and C) and the maximum (B and D) compared to the control open environment

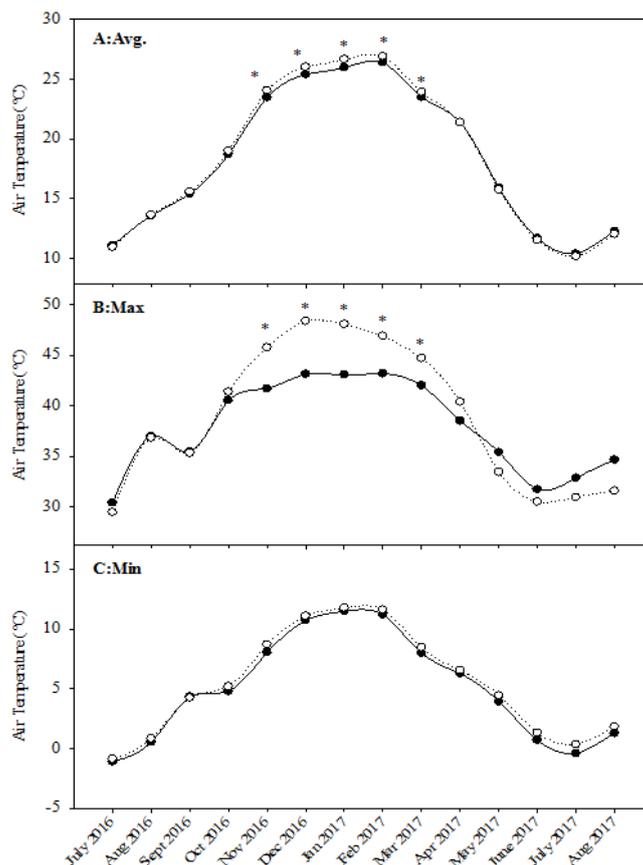


Fig. 4 The effect of 20% white shade netting on within-canopy air average - (A), maximum (B) and minimum (C) temperatures on a monthly basis from July 2016 until Aug 2017 in a 'Nadorcott' mandarin orchard in Citrusdal. (○ Shade net; ● Control). * Indicates mean values (n = 4) within a month differ significantly ($P \leq 0.05$).

Conclusion

Constructing a permanent 20% white shade net over a commercial 'Nadorcott' mandarin orchard in Citrusdal does change the orchard microclimate with regard to the important climatic parameters affecting citrus

physiology. In return the horticultural response could potentially be of significant importance for citrus growers as the change in global climate and catastrophic climatic events are becoming more severe which will increase the risk of developing new orchards. In addition, the lack of new resources, i.e. arable land, water and optimal growing climate availability, can lead to the use of shade netting in order to establish successful orchards in areas previously deemed marginal. An additional aspect to take into consideration for future research on shade netting would be the impact of these microclimatic changes documented in this study on the pest and disease pressures in a citrus orchard. The shade net reduced conditions considered stressful to the citrus trees throughout a season and as the technology and its understanding thereof increases, there would be a potential to increase cultivation of citrus in climatically challenged areas.

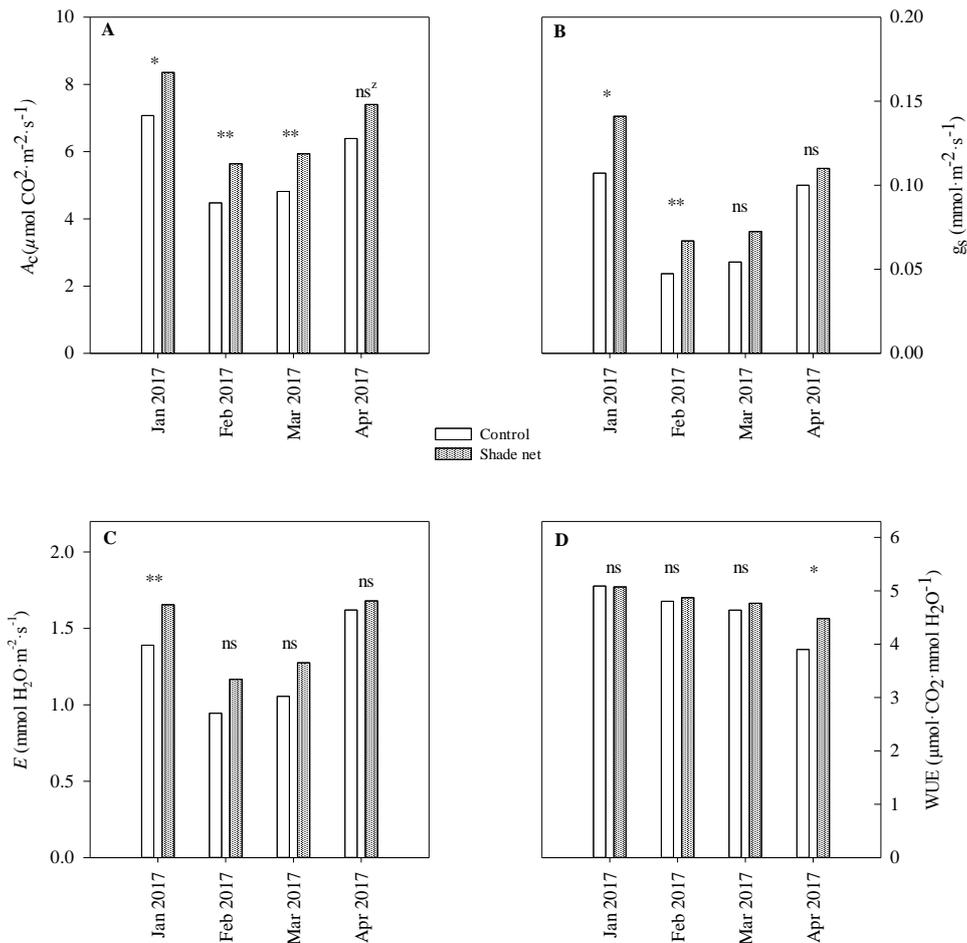


Fig. 5. The effect of 20% white shade netting during the summer months on physiological parameters of a ‘Nadorcott’ mandarin tree physiology, i.e. A_c (A), g_s (B), E (C) and WUE (D). ($n = 4$). * Indicates significant differences between treatments within a month as tested by Fisher’s LSD test ($P \leq 0.05$). ** Indicates significant differences between treatments within a month as tested by Fisher’s LSD test ($P \leq 0.10$). ^z Indicates no significant differences between treatments within a month

The impact of shade netting on the vegetative/reproductive balance of ‘Nadorcott’ mandarin trees

Permanent shade netting in citrus (*Citrus* spp.) is implemented to protect high-value fruit and trees from damaging natural elements. However, the use of the technology accompanies inevitable changes in orchard microclimate that impacts on the physiology and phenology of a citrus tree. A 20% white permanent shade netting treatment was evaluated for its effects on citrus tree phenology, its impact on the efficacy of chemical fruit thinning agents and the long-term profitability of the technology in a ‘Nadorcott’ mandarin orchard.

Materials and methods

Vegetative/Reproductive balance of citrus trees under shade netting

Five shoots per tree was tagged under the net and in the open for each shoot growth period (flush). The final length, number of nodes and number of leaves were evaluated for each of these shoots to compare treatment effects. In September, flowering was evaluated on the tagged shoots from the previous fruiting season to determine which flush exhibited the strongest flowering response. Fruit set was evaluated on these same flushes to determine fruit set % for the different treatments. At the end of the growing season (Jul. 2016), tree volume and trunk circumference were measured as additional vegetative parameters. For the 2017 season the same measurements will be repeated.

Efficacy of thinning agents (PGR's) under shade netting

During the 2016 season Corasil (2,4-DP) and Maxim (3,5,6-TPA) were applied at the label recommended timing. Fruit growth was measured, as well as final fruit yield, fruit size distribution and response to cold storage. At harvest, MRL fruit samples were collected taken and sent for analysis. For the 2017, season the trial was repeated and additional Corasil and Maxim treatments were applied. The two additional treatments were applied at the same time as the recommended Corasil treatment and consisted of 0.5X Corasil and an early Maxim treatment. In 2017, MRL fruit samples were collected every second month, starting in January and continued until harvest. All measurements at harvest was repeated for two season.

A 15-year budget model quantifying the influence of 20% permanent white shade netting on 'Nadorcott' mandarin orchard profitability

This part of the study was conducted to identify all known advantages and disadvantages of citrus shade netting, to quantify the impact thereof on the profitability of a high value 'Nadorcott' mandarin orchard. Financial evaluations were done by compiling an enterprise budget model based on standard accounting principles. The model was compiled in a spreadsheet programme, allowing for multiple assumptions and integrated calculations. All parameters and values assumed in the model are based on weighted averages from a South African citrus shade netting industry survey, in addition to information gathered in scientific studies in this project.

Results and discussion

Shade netting did not enhance the growth of individual vegetative shoots but did increase tree volume over time (Table 1). In general, flowering was not affected by the shade net treatment, but during the second season, flower intensity on summer vegetative shoots was higher in the shade net treatment. Fruit set, fruit yield and fruit internal quality were not affected by the shade net treatment (Table 2), but fruit diameter was increased in the second season (Fig. 1).

Table 1. Tree volume of 'Nadorcott' mandarin (*C. reticulata*) trees in a 20% white permanent shade netting treatment (net) and an untreated control (open) (n=4) in Citrusdal in the Western Cape Province of South Africa. Values of 2015 represent initial tree volume, before the shade netting treatment was applied.

Treatment	Tree volume (m ³)		
	2015	2016	2017
Open	3.57 d ^z	8.22 c	11.30 b
Net	5.44 d	10.73 b	15.10 a
P-value	0.0504	0.0101	0.0002

^z Means with a different letter within a column differ significantly at the 5% level (LSD).

^{ns} No significant differences within column.

Table 2. Fruit yield (kg· tree⁻¹) and number of fruit per tree (fruit·tree⁻¹) for ‘Nadorcott’ mandarin (*C. reticulata*) trees in a 20% white permanent shade net treatment (net) compared to an untreated control (open) (n=4), at time of commercial maturity, in Citrusdal in the Western Cape Province of South Africa.

Treatment	2016		2017	
	Fruit·tree ⁻¹	Kg·tree ⁻¹	Fruit·tree ⁻¹	Kg·tree ⁻¹
Open	367 ^{ns}	31.98 ^{ns}	550 ^{ns}	55.31 ^{ns}
Net	432	40.19	526	58.69
<i>P</i> -value	0.3894	0.2183	0.7908	0.7125

^{ns} No significant differences at the 5% level (LSD).

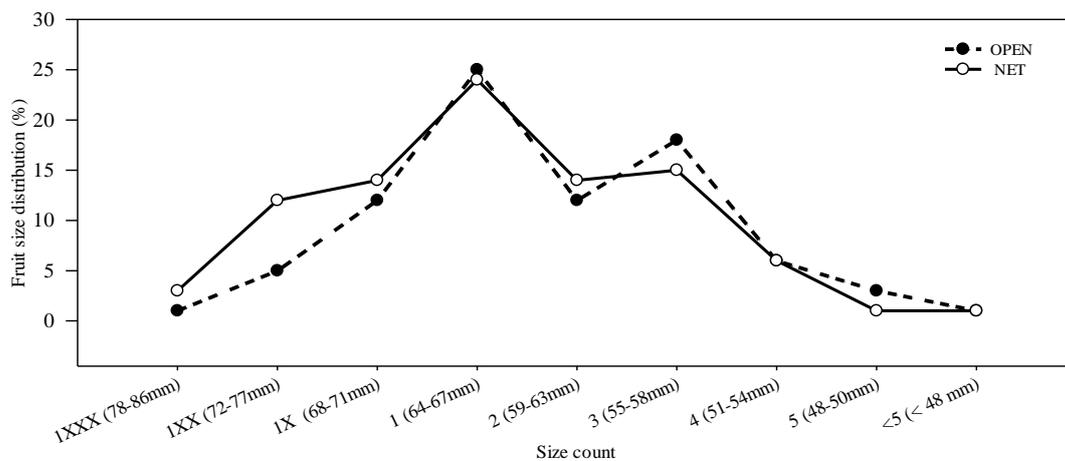


Fig 1. Fruit size distribution (%) of ‘Nadorcott’ mandarin (*C. reticulata*) in a 20% white permanent shade net treatment (net) and an untreated control (open) (n=4), in Citrusdal in the Western Cape province of South Africa. No significant differences were recorded at the 5% level.

The shade net treatment did not influence the efficacy of synthetic auxin fruit thinning agents to thin fruit (Table 3). The synthetic auxin fruit thinning treatments increased the concentration of selected mineral elements in fruit, and treatments resulted in a shift in fruit size distribution, with higher number of large, premium-sized fruit per tree. The effect on fruit size distribution was more pronounced in the shade net treatment. Apart from fruit size, a combination of shade netting and chemical fruit thinning treatments had no effects on other important fruit quality attributes.

Table 3. Fruit size distribution (%) of ‘Nadorcott’ mandarin (*C. reticulata*) during season 2 (2016/17), in reaction to synthetic auxin fruit thinning agent treatments, in a 20% white permanent shade net treatment (net) and an untreated control (open) (n=4), in Citrusdal in the Western Cape province of South Africa.

Factor	Size Count (% per tree)								
	1XXX (78-86mm)	1XX (72-77mm)	1X (68-71mm)	1 (64-67mm)	2 (59-63mm)	3 (55-58mm)	4 (51-54mm)	5 (48-50mm)	<5 (< 48 mm)
Treatment									
Open	12 b ^z	19 ^{ns}	20 ^{ns}	20 ^{ns}	19 a	6 ^{ns}	2 ^{ns}	1 ^{ns}	0 ^{ns}
Net	19 a	22	18	18	13 b	4	1	0	0
Thinning agent (PGR)									
Control	8 b	17 c	19 ^{ns}	25 a	20 a	7 a	3 a	1 ^{ns}	0 ^{ns}
2,4-DP	10 b	19 bc	19	23 a	18 a	6 a	2 a	1	0
3,5,6-TPA	22 a	23 ab	19	16 b	12 b	3 b	1 bc	0	0
0.5X 2,4-DP	9 b	17 bc	21	22 a	21 a	8 a	2 ab	1	0
Early 3,5,6-TPA *	28 a	27 a	18	11 c	8 c	2 b	1 c	0	0
<i>P</i> -values									
TMT	0.0365	0.1504	0.4447	0.1883	0.0113	0.1013	0.3224	0.5999	0.7608
PGR	<0.0001	0.0010	0.8766	<0.0001	<0.0001	0.0003	0.0172	0.4732	0.3983
TMT*PGR	0.8199	0.5313	0.2677	0.9548	0.0515	0.3119	0.3878	0.7137	0.0573

From the budget model generated in this study, it can be concluded that 20% white permanent shade netting resulted in increased orchard profitability, despite a high establishment cost and increase in production costs (Fig. 2). Shade netting resulted in a 10% increase in production costs per hectare, while gross income was 28% higher under shade netting. The result was a gross margin increase of 32% per annum for the shade netted orchard, during full bearing potential. It can therefore be concluded that under typical Mediterranean-type production conditions, 20% white permanent shade netting increased the productivity and profitability of a 'Nadorcott' mandarin orchard. The use of the technology can be recommended in areas that experience extensive yield losses due to climatic conditions or seeds and possibly also permit citrus production in non-traditional areas.

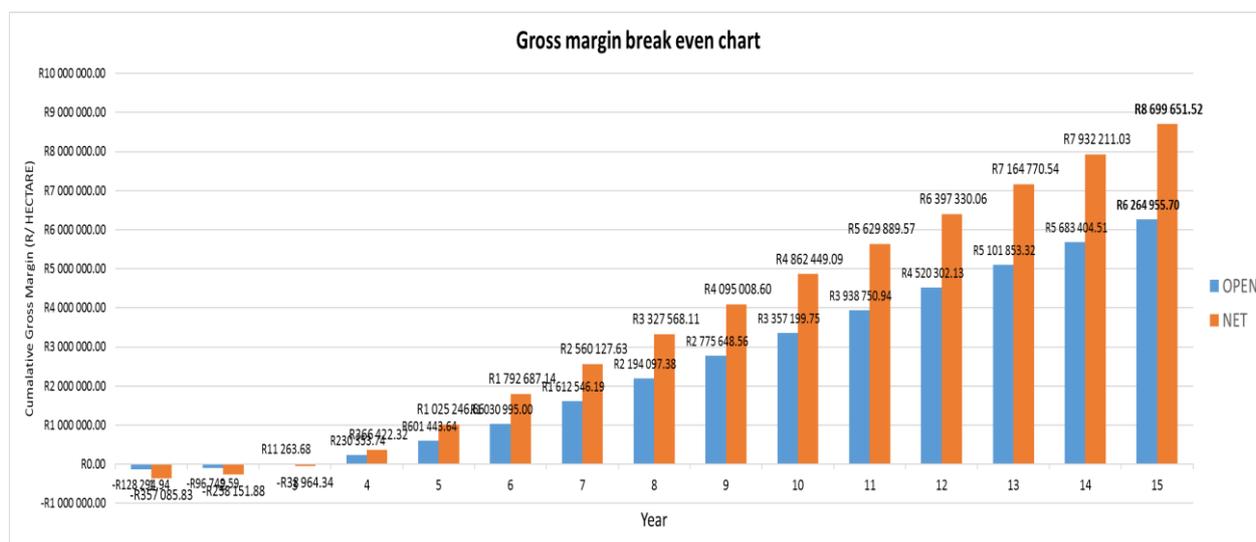


Fig 2. Gross margin – Break-even Chart of an open orchard compared to an orchard with seedless fruit under shade netting.

Conclusion

The main conclusions that can be drawn from this part of the study is that the use of 20% white permanent shade netting, in a Mediterranean-type climate such as Citrusdal, increased the vegetative growth of 'Nadorcott' mandarin without affecting any reproductive parameters. The efficacy of synthetic auxin chemical fruit thinning agents was not altered, while ultimately, shade netting has the ability to increase the effectivity and profitability of mandarin production.

The impact of shade netting on 'Nadorcott' Mandarin fruit quality

In the export-focused citrus industry of southern Africa, the production of high quality fruit i.e. good size, well-developed rind colour, blemish-free fruit and good taste is of utmost importance to remain sustainable. Shade netting is a new preharvest technique in Citriculture implemented to protect crops against excessive sunlight, wind and hail damage. Shade nets are effective in reducing sunburn, but inconsistent results from previous studies arise about fruit size, rind colour and internal quality. Furthermore, the impact on postharvest fruit quality with regard to developing rind physiological disorders and to maintain the physical integrity of the rind as a protection layer against moisture loss and decay development, is not known. Three experiments were conducted to determine how 20% white permanent shade netting would influence, firstly the fruit quality development, secondly the postharvest behaviour and lastly the rind physical properties of 'Nadorcott' mandarin fruit produced in Citrusdal, over two seasons (2016 and 2017).

Materials and methods

Monthly in-orchard measurements and destructive lab measurements will be done in order to analyse the internal and external quality of the fruit. Orchard measurements: 10 fruit/tree/block were tagged at random and measurements on the fruit done from fruit set until harvest. Measurement includes fruit size with a fruit size

data logger. The rind colour is measured with a colorimeter and the fruit surface temperature is measured with an infrared temperature gun.

Destructive lab analysis: Two fruit was sampled from 5 sample trees each/block for destructive quality analyses to determine juice %, TSS, TA, flavedo and albedo thickness, rind and pulp dry mass as well as colour and quantification of rind pigments: Spectrophotometric chlorophyll and carotenoid measurements was done on milled albedo and juice samples from the sampled fruit. This is done on fruit samples taken monthly. Fruit was evaluated (moderate / severe) to quantify the percentage sunburn. Harvested fruit was stored at 4°C and - 0.6°C for 14, 27 and 34 + 7days shelf life respectively where after external and internal quality evaluation occurred on the specific days. The chilling injury incidence was graded at each temperature and storage day.

The physical rind properties of the fruit as affected by shading was also evaluated at each storage temperature (4° & 6°C) after 14, 27, 34 days storage followed by a 7 day shelf life period. A texture analyser was used to perform a cutting, compression and puncture test which measures the rind Force (N).

Results and discussion

The fruit size, rind colour and internal quality development patterns were similar for both shade-net exposed and control fruit. Shade netting however resulted in an increased fruit size over the 2017 development period, but with no influence on rind colour or maturity (Fig. 1, 2). Sunburn incidence was effectively reduced by shade nets (Table1). During cold storage at 4 and -0.6 °C over a period of 34 days, storage duration did not influence the postharvest quality of shade net fruit differently compared to control fruit in terms of rind colour, internal quality parameters and fruit weight loss. In addition, no negative effect of shade netting was evident for the above-mentioned parameters or the incidence of staining (data not shown).

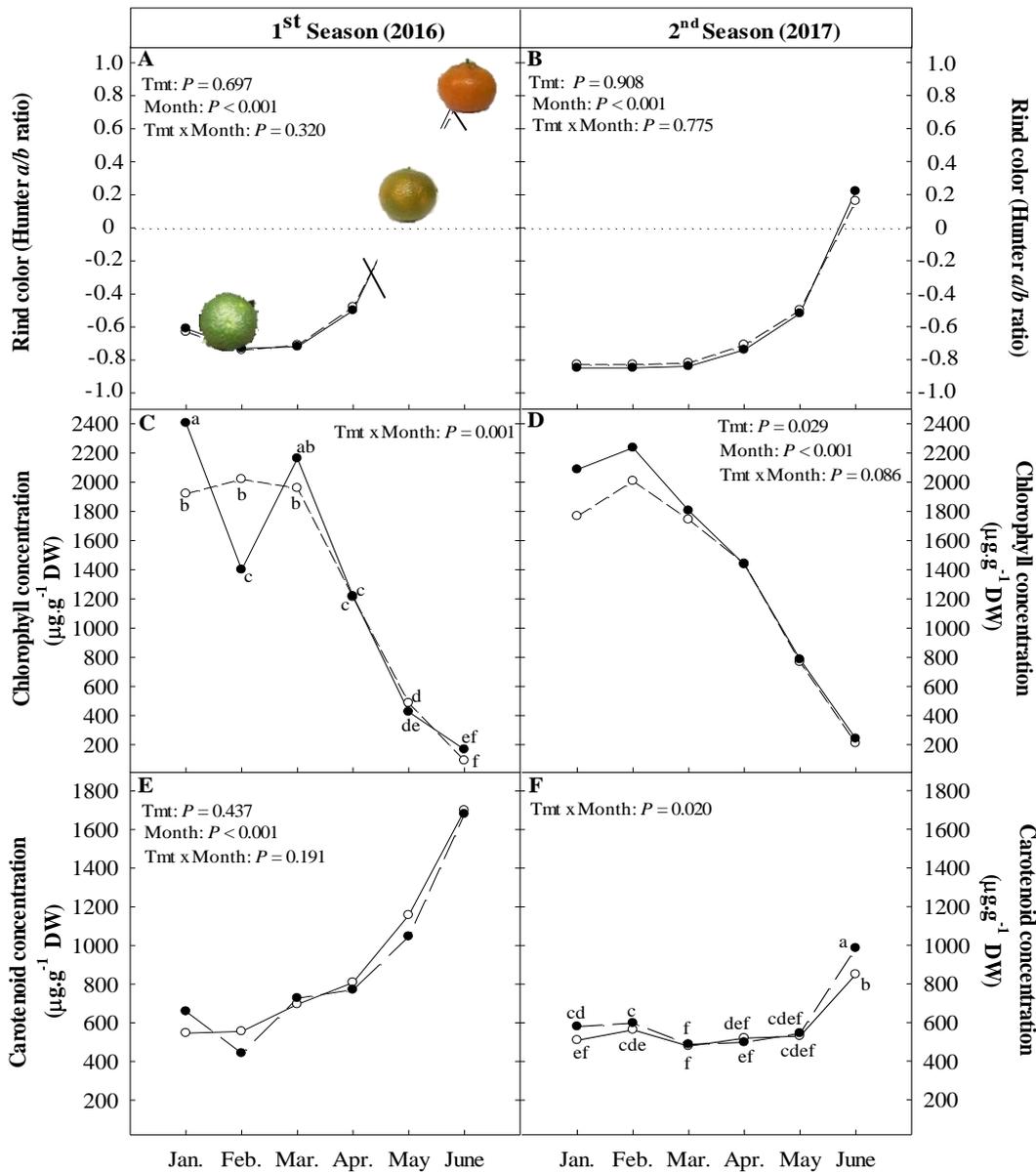


Fig. 1. Changes in rind colour development (A-B) and that of chlorophyll (C-D) and carotenoid content (E-F) of 'Nadorcott' mandarin fruit grown under 20% white shade net (solid line●) and control (no net, broken line○) over two consecutive seasons. The P -values depicts the main effects, Treatment (Tmt), Month and the interaction between Tmt x Month. Values reported are the means of four replications. Different letters at each point denote significant differences between means ($P \leq 0.05$) where the interaction was significant. Mean separation was done by means of Fisher's LSD test. Parallel bars in A indicates a missing value. The dotted line through the 0.0 axis indicates where colour break occurs and where the hunter *a/b* ratio changed from a negative to positive value.

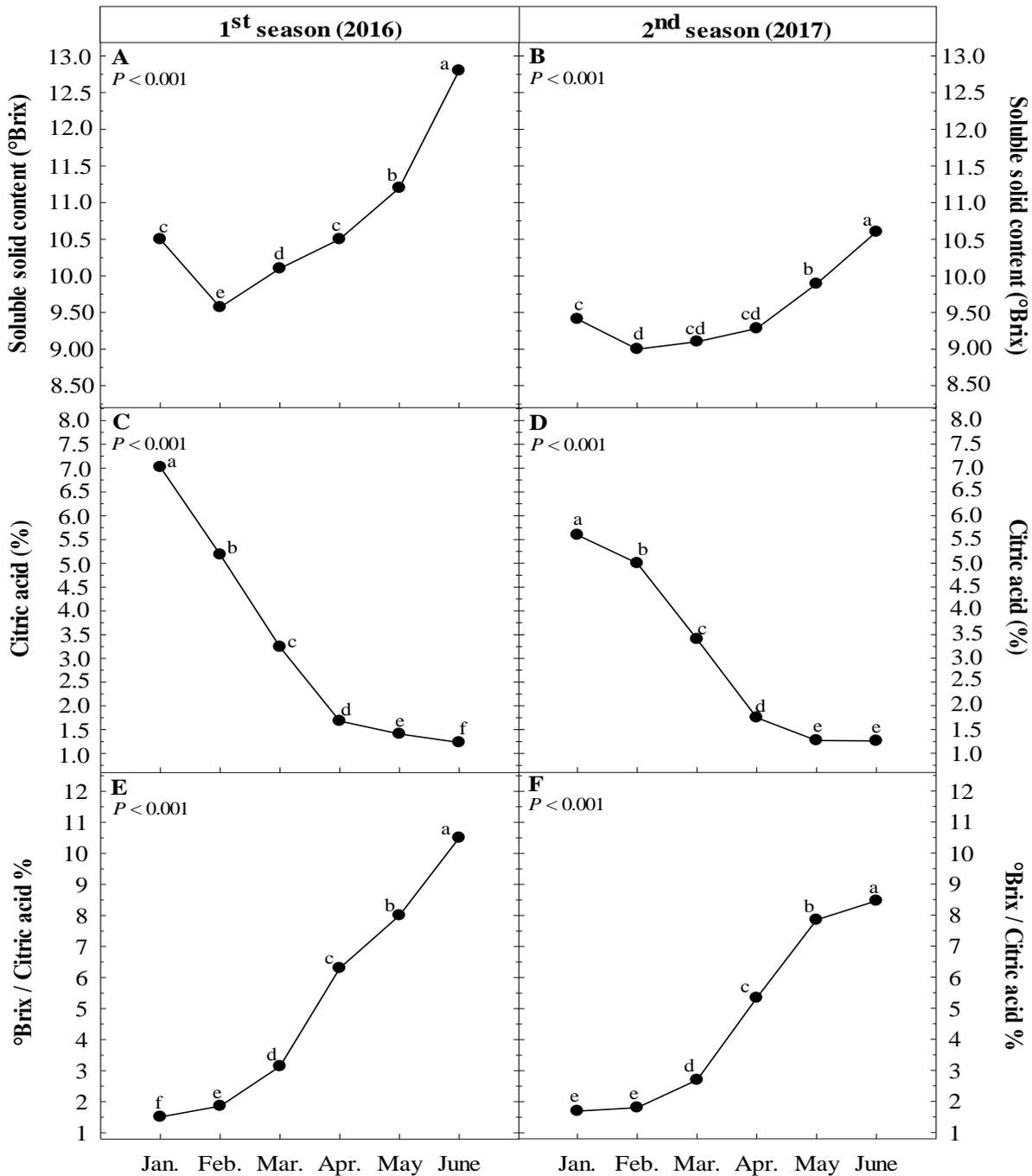


Fig. 2. Seasonal development of °Brix (A-B), citric acid percentage (C-D) and the °Brix:citric acid % ratio (E-F) of the combined control and 20% white shade net treatments for ‘Nadorcott’ mandarin fruit during the 2016 and 2017 season respectively. The *P*-value for the Month main effect is indicated on each graph. Different letters denote significant differences between means at 5% significant level according to Fisher’s LSD test.

Table 1. The influence of 20% white shade net application on the sunburn index and percentage (%) of ‘Nadorcott’ mandarin fruit. Values are means of four replications.

	Treatment	Index ^z	Percentage ^y (%)
2016 ^w	Control	0.37 a ^x	23.7 a
	Shade net	0.07 b	5.75 b
	<i>P</i> -value	0.005	0.002

2017 ^v	Control	0.28 a	20.8 a
	Shade net	0.05 b	5.00 b
	<i>P</i> -value	0.014	0.031

^z Degree of severity scale 0-2.

^y Percentage of fruit scored 1-2.

^x Different letter denotes significant difference between means within a column for each season at $P \leq 0.05$ according to Fisher's LSD test.

^w 200 fruit per replicate scored on the eastern side of the tree after color break.

^v 40 fruit per replicate scored (20 on the eastern and 20 on the western side) before color break.

Inconsistency occurred with regards to the effect of shade netting on fruit rind strength at harvest (Table 2). A higher firmness was recorded for shaded fruit in the first season. However, during cold storage, there was some indication that the shade net fruit was more susceptible to deformation and required a lower force over the whole storage duration to puncture the rind, compared to the control fruit. However, a lower force required may also be beneficial as it could be indicative that the fruit may be easier to peel. The firmness of shade net-produced fruit was differently influenced by cold storage in 2016, within the first 14 days of storage, compared to the control. In 2017, control fruit were recorded to have a higher firmness over the storage duration. Both results indicated that the control fruit possibly stored better than shade-net fruit.

Table 2. The influence of 20% white shade net on the rind properties of 'Nadorcott' mandarin fruit at harvest presented for two consecutive seasons, 2016 and 2017, as determined by means of three texture analyser tests namely rind cutting, fruit puncture and fruit compression, performed at a speed of 1 mm·s⁻¹ and at a trigger force of 0.049 N. Values reported are the means of four replications.

Season	Treatments	Rind cutting ^z	Fruit puncture ^y	Fruit compression ^x	
		Peak force (N)	Peak force (N)	Force (N) ^w	Area (N·mm ⁻¹)
2016	Control	23.6 ^{NS}	6.39 a ^v	58 ^{NS}	301 b
	Shade net	22.7	5.61 b	66	395 a
	<i>P</i> -value	0.528	0.020	0.133	0.021
2017	Control	23.8 ^{NS}	6.04 ^{NS}	69.8 ^{NS}	- ^u
	Shade net	21.7	6.05	68.5	-
	<i>P</i> -value	0.144	0.982	0.602	-

^z A HDP/BS blade set was used to cut a dissected rind piece at 100% strain.

^y A 2 mm cylindrical probe was used to puncture 15 mm into the fruit.

^x A 100 mm platen probe compressed fruit at 20% strain.

^w Tests performed in 2017 had a different parameter set than in 2016.

^v Different letters denote significant differences between means within a column in each respective season according to Fisher's LSD test at $P \leq 0.05$.

^u Test not performed.

NS Indicates non-significant differences between treatment means at a 5% confidence level.

Conclusion

Shade net was effective in reducing sunburn without negatively affecting any external and internal quality parameters. The postharvest storage potential of fruit from shade netting did not differ from the control at both storage regimes. Results regarding the impact of shade netting on the physical properties of the rind provides some first guideline threshold force values required before damage is inflicted on the fruit. Knowledge of typical forces applied during the commercial harvest- and pack house processes is however required before these values can be compared to commercial practices to determine its importance. The use of shade-netting shows

potential as a preventative technology ensuring high quality, unblemished fruit, but requires future studies taking into account the effect of various cultivars, tree age, bearing positions and the microclimatic effect on fruit production and postharvest storage behaviour.

Tree water use and reaction on change in soil water

The use of shade netting in agriculture in South Africa is rapidly increasing, primarily for the protection of crops from hail and wind damage. It is well documented that shade nets modify the microclimate of the orchard of which they cover by reducing incoming solar radiation, reducing the day time temperature, decreasing vapour pressure deficit (VPD), and decreasing the wind speed. The objectives of the study are to measure and compare water use and water use productivity of mandarins under shade netting and in the open while also gaining and understanding of the reasons for any of the observed effects of the shade netting

Materials and methods

The study in Citrusdal on an orchard of 'Nadorcott' mandarin on Carrizo rootstock planted in 2013 and with a spacing of 2.5 x 5.5m is being used in the trial. The trial is set up with shade net treatments arranged in a randomised complete block design. Soil moisture content at varying distances from irrigation drippers is measured by capacitance probes. Irrigation frequency and application amount is also measured using flow meters installed on dripper lines. Leaf and stem water potential, stomatal conductance, leaf area index and canopy dimensions of all the trees used in the trial are periodically measured.

To evaluate the effect of different irrigation treatments on tree water status as influenced by shade netting during fruit growth, the application of irrigation volumes was changed a month before full bloom on 3 Sept. 2016. Water was supplied through double row drip irrigation with each tree having four drippers of supplying a high (3.2L/h/dripper), low (0.8L/h/dripper) and control (1.6L/h/dripper) volume of water and designated 2X, X and 0.5X. The frequency of irrigation was not changed. This technique is commercially used to monitor the impact of irrigation scheduling on fruit size in orchards, and would create contrasting effects of under- and over-irrigation to be compared with standard irrigation volumes applied.

Results and discussion

A permanent 20% white shade net over a 'Nadorcott' mandarin orchard increased the soil water content and in return improved tree water potential (Fig 1,2). This decrease in water stress experienced under the shade net could lead to improved stomatal conductance for parts of the day, thereby, increasing the potential to assimilate CO₂ and improve carbohydrate status of 'Nadorcott' mandarin trees. Under the shade net the higher soil water availability, as well as tree water potential could have led to the increased fruit growth and increased the pack out of larger fruit. It can be hypothesised that the trees under shade net could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissues. On a practical level, the data indicate the possibility to reduce irrigation volumes under a shade net in citrus orchards without recuing yield or fruit quality. Therefore, during a restrictive water period, in a season or between seasons, shade net trees could receive less water and maintain a commercially valuable crop.

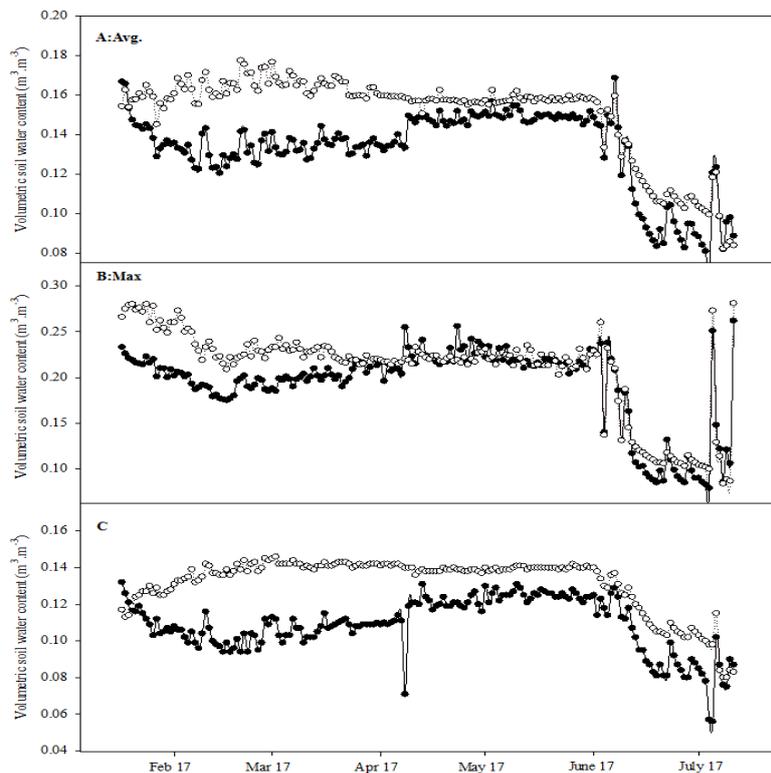


Fig. 1. Monthly daily mean (A), maximum (B) and minimum (C) volumetric soil water content ($\text{m}^3 \cdot \text{m}^{-3}$) of the second season and how 20% white shade net affect the water availability in a uniform clay loam soil of a 'Nadorcott' mandarin orchard in Citrusdal. (\circ = Shade net; \bullet = Control).

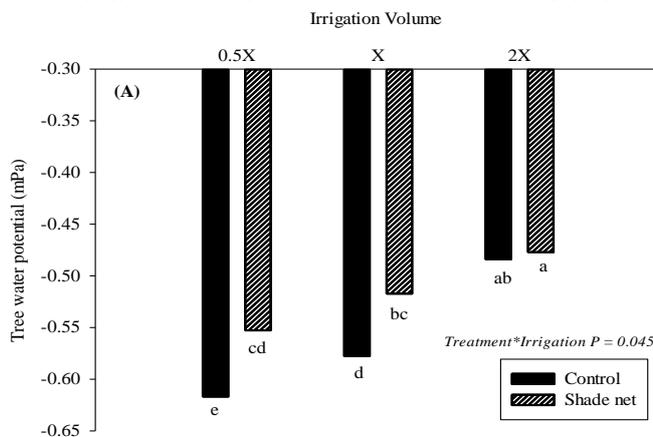


Fig. 2 The effect of 20% white shade netting combined with different irrigation treatments on the pre-dawn (after 0300_{HR}) tree water potential (mPa) 'Nadorcott' mandarin from physiological fruit drop (Nov. 2016) throughout fruit enlargement phase II (Jan. to Mar. 2017) in Citrusdal, South Africa. Different letters within and between irrigation treatments (0.5X, X, 2X) differ significantly at 95% confidence level as tested by Fisher's least significant difference (LSD) test; (n=4).

Soil health

Soil and root samples were collected during March 2016. These were analysed for the presence of juvenile citrus nematodes in the soil, female nematodes in the roots and *Phytophthora* presence in the soil.

To date no citrus nematodes or *Phytophthora* spp. have been detected in any of the samples. At this stage there are thus no differences between the covered and open trees.

Insect profile change

FCM and fruit fly monitoring continued as part of the entomological component of this project. Xsit releases sterile FCM over many citrus blocks in the Citrusdal area including the netting experimental site. Overall, a greater total number of sterile moths were caught in the open sites when compared to the area under netting. Wild FCM pest presence was generally very low but, so far, total catches have been slightly higher under the nets. No difference in FCM infestation has yet been observed. Mediterranean fruit fly numbers were also almost non-existent. Bollworm and thrips damage evaluations in 2017 showed no significant difference between netted and open areas. Further evaluations will be done close to harvest in July 2018. The monitoring of a netting site nearby which has full cover shade netting down to ground level will begin in the near future as a separate project. Drape nets were also included in the trial this season and monitoring of insects is ongoing with fruit blemish evaluations planned before harvest 2018.

Project milestones

Objective / Milestone	Achievement
A. Structural development phase of experimental site	Complete
B. Data System development	Complete
C. Baseline data (before net)	Complete
D. First season data	Complete
E. Second season data	
E.1. Tree physiological response (Pn + CHO)	Complete
E.2. Vegetative/reproductive balance	Co Ongoing
E.3. Fruit quality	Ongoing
E.4. Analytical methods development and optimisation	Complete
E.5. Water use efficiency	Ongoing
E.6. Soil pathogens	Complete
E.7. Insect Profile	Ongoing trapping and documenting
F. Comparative study on drape nett vs permanent	Ongoing

General conclusion

The shade netting project indicates that by reducing light level by 18-20% significant physiological change will occur in the tree as well as the fruit. It should therefore be noted that the use of shade netting is still in an early stage of development as a technology in citriculture. Some of the positive impacts such as improved yield and reduction in superficial scarring should be weighed over a longer term if a possible reduction on rind condition is found which reduces the postharvest potential of the fruit. At this stage shade netting under certain conditions does seem to improve the cropping efficiency of citrus trees.

Technology transfer

Shade netting research feedback day: 26 July 2017.

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4.4 PROGRAMME: COLD CHAIN & PACKAGING

Programme coordinator: Paul Cronjé (CRI-SU)

4.4.1 **PROGRESS REPORT: Precooling: ambient loading and forced air cooling of citrus for cold sterilisation markets**

Project 1125 (2014/15 – 2017/18) by Paul Cronje, Jade North (CRI) and Thijs Defraeye (EMPA-Zurich).

Summary

Ambient loading of citrus offers advantages to the SA citrus industry such as cost savings and the reduction in dwell time between packing and shipment. In addition, ambient loading of citrus reduces the demand on precooling facilities in the various ports. During the 2017/18 season, a project was completed to test the viability of an increase in the scope of ambient loading of citrus fruit. The viability to only partial precool fruit to 10°C, 15°C was compared to ambient loaded (20°C) fruit in terms of cooling rate to reach a target temperature. The results indicate a variation in cooling at various positions in the container to reach 4°C at all probe positions. However, carton type also added variation in cooling efficiency at the locations in the containers which were measured. In general it was shown that precooling to 15°C only added half a day to reach the target temperature compared to 10°C. If these results are confirmed they could lead to up-dating current cold chain regimes which would result in improved logistics in the complex citrus cold chain. The cooling performance of the container clearly depended on the way in which it was stowed and convectively cooled, and ways should be identified per fruit and packaging type to improve cooling rate and uniformity.

Opsomming

Warm-laai van sitrusvrugte m.a.w. sonder enige verkoeling direk in verskepingshouers bied verskeie koste besparings geleenthede vir die bedryf. Warm-laai kan ook bydrae tot 'n vermindering in druk op die verkoelingsfasiliteite in die hawes. Gedurende 2017/8 was 'n eksperimente afgehandel wat die wesenlikheid van uitbreiding van die warm-laai protokol getoets het. Daar was gepoog om te bepaal of gedeeltelike voorverkoeling and 10°C of 15°C in vergelyking met warm laai (20°C) die verkoelings tydperk betekenisvol nadeling beïnvloed. Daar was 'n duidelike variasie in verkoelings tempo om 4°C te behaal, soos verwag, tussen possies in die houer. Tipe karton het egter ook bygedra tot die verhoging in verkoelings tyd. Gesien oor die algemeen het verkoeling tot 15°C slegs 'n halwe dag bygedra ivm verkoeling tot 10°C. As hierdie resultaat bevestig kan word in kommersiële proewe kan die opdatering van koueketting regimes bydrae tot 'n verbetering van die komplekse sitrus logistieke ketting. Die verkoeling in 'n houer word bepaal deur hoe die pallette gelaai word en verkoel word deur konveksie en strategie moet geïdentifiseer word om per vrug en karton tipe te poog om verkoelings tempo en eenvormigheid in houers te verbeter.

Introduction

The volume of citrus fruit exported from South Africa under cold protocols continues to increase which has led to pressure on the limited pre-cooling facilities at the ports. The SA citrus industry is implementing a strategy to reduce the risk involved in phytosanitary insect pests during the export process by shipping fruit at various temperatures below 2°C to the EU markets. This new cold protocol form part of a system approach to mitigate the risk of live pest interception in the market but will increase the pressure on pre-cooling facilities as fruit needs to be pre-cooled down to lower temperatures. In addition, a higher risk of cold damage will exist in these shipments to the EU.

The large volume of citrus fruit produced and exported to Europe as well as the insufficient pre-cooling infrastructure available would result in logistical problems at the ports. The practice of ambient loading can mitigate this problem and involves loading the fruit warm, i.e., at ambient temperature, in the container, and using the cooling unit of the reefer containers to remove the field heat. It is currently used for citrus exports to non-cold sterilization markets has led to significant cost savings for producers and exporters and could supply the additional cooling capacity. Containers have the ability to cool fruit. However, it is unknown whether containers could reduce the pulp temperature within a sufficient time to below 4, 1.2 or 0°C (depending on the target temperature) in order to allow for adequate exposure days from the port to the EU markets.

Aspects involved in the ambient loading of fruit, such as initial pulp temperature and variation in the air flow/speed in the pallets, could reduce the cooling rate uniformity of the fruit. It is thought that the cooling rate could be improved by manipulating the airflow in the container to ensure no short-circuiting of colder air which slows down the removal of heat from the fruit. The possibility to use the refrigeration capabilities of a container to cool the fruit to the required protocol temperature could lead not only to a reduction in the pressure on the limited pre-cooling facilities but also to a reduction in handling time, which implies faster shipping.

The citrus cold chain differs from most other fruit in that temperature mismanagement for short durations does not - as a rule - result in a drastic reduction in fruit quality as seen in fruit such as apples, mangos and berries. However, fruit pulp temperatures that go out of protocol offer two distinct problems for the SA citrus cold chain. Firstly incorrect temperatures are more often associated with too low air temperatures causing chilling and freeze damage. Secondly, an increase in pulp temperature due to equipment failure or cold chain mismanagement during precooling or the voyage could result in failure to comply with cold sterilisation protocols, which has serious consequences.

Objectives

The aim of this multi-season project is to improve the cooling rate and uniformity of citrus fruit by manipulating vertical airflow in containers in order for fruit to reach the target temperature in a timeous manner in order to comply with cold protocols without quality loss.

The specific objectives for 2017 were:

1. Determine if the ambient loading cooling processes in reefers are efficient enough to reduce fruit temperature to 2°C in a period complying with the FSM for the EU.
2. To ascertain whether cooling rates could be influenced by temperature at which cooling in the container was started i.e. 10, 15 or 20°C as well as carton type.

Materials and methods

In all container experiments the following protocol was followed: **Data recording:** Insert iButton data loggers within fruit positioned in the middle, top and bottom cartons of 6 pallets (distributed from back to front and 3 on each side of container). iButtons to be inserted into fruit for pulp temperature and fixed onto fruit for air temperature. Place Temp/RH recorders in middle carton of each data pallet (6). Measure carton weight before and on arrival or completion of experiment, remove data loggers and score for decay and rind condition. **Quality evaluation:** All the fruit in each of the data cartons pallet will be evaluated for rind disorders and decay incidence as well as change in colour development. If possible 10 fruit per carton will be removed and kept at ambient for 7-14 days for shelf life evaluation. **Fruit:** Navel orange fruit was picked in same week and packed at the same packhouse (SRCC, Addo E-Cape) in order to reduce fruit quality variation. In addition, fruit within a similar size range will be used (peak i.e.72 plus-minus one count). **Treatments:** Fruit was pre-cooled to either 10°C, 15°C or left at Ambient ($\pm 20^\circ\text{C}$) prior to loading. All containers will support the same software as well as having similar refrigeration equipment. Containers will be ordered and supplied by Maersk to comply with these software and equipment requests. **Set-point:** For all six containers 2°C was used. **Carton type:** The navel orange fruit were packed into either Supervent or E15D cartons. **Statistical analysis:** To compare cooling rate the seven-eighths cooling time was calculated for all the data points as well as the duration to reach below 4°C in the containers. This enabled comparing cooling rate between treatments.

Results

The cooling patterns seen during the 2017 season in commercial shipments from Sundays River to Rotterdam concur with previous reports (Defraeye et al., 2016; CRI annual reports 2015, 2016). It is clear that cooling take place in the container of fruit loaded at a higher temperature than the target temperature and cooling at the refrigeration unit is faster than at the door side (Fig.1 and 2; Table 1 and 2). In Figure 1 the cycling of the DAT (delivery air) around the set point (2°C) are evident and which is the result of the regulating software in reefer temperature control units. In addition it should be noted that the initial DAT of ambient loaded fruit is not the set

point of close to the set point but also a gradually reduced value as the RAT (return air temperature) value decrease. In Figure 1 the impact of carton type on cooling profile can be seen with the Opentop carton having a reduced cooling rate compared to the SuperVent carton.

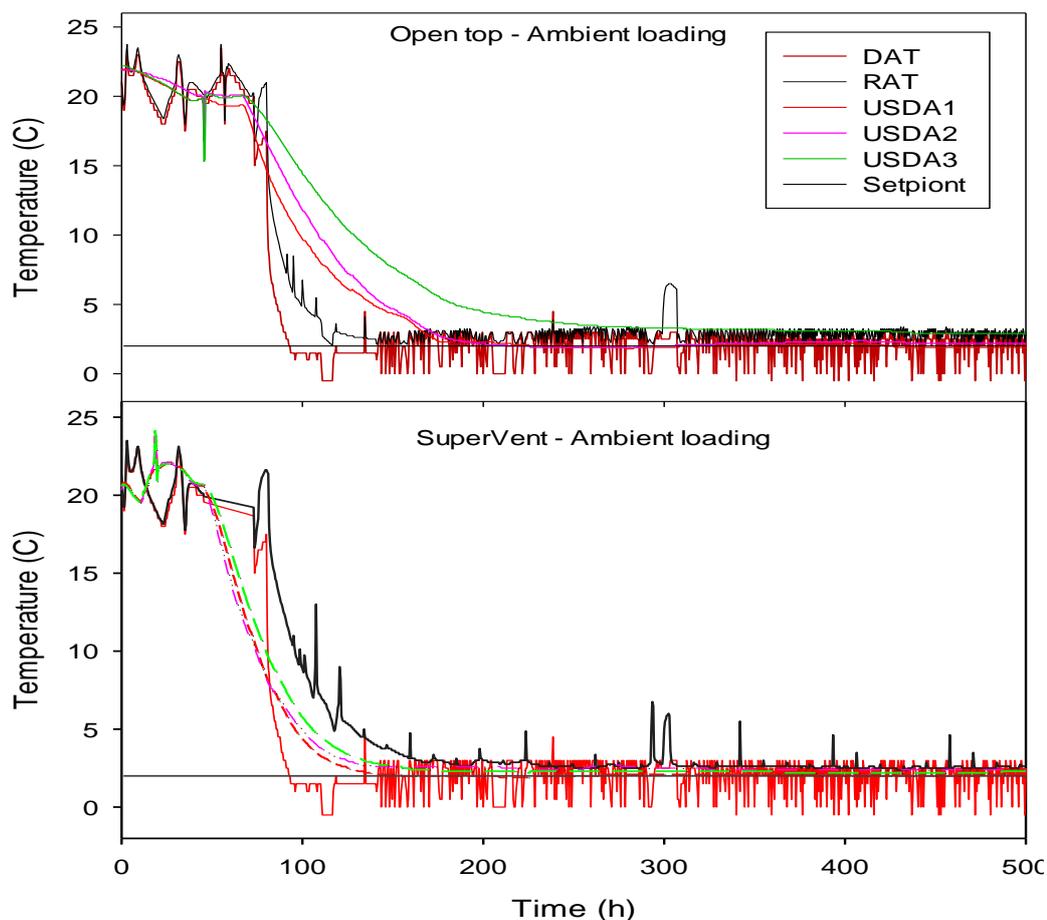


Fig. 1. Cooling pattern of 'Navel' orange fruit pre-cooled to 20°C (ambient loaded) prior to loading in refrigerated reefers (Maersk StarCool) at set point 2°C. In the top graph the fruit were packed in Opentop and in the bottom graph in SuperVent cartons. The data points were measured by the cargo probes at USDA1/2/3 positions.

Higher pulp temperature prior to ensuring the cooling in the refrigerated containers indicate that the duration to a target temperature would be decreased at lower temperatures i.e. 20°C vs. 10°C which took twice as long, 64 compared to 32 hours to reach 4°C for SuperVent (Fig.2; Table 1). However, the aim of the trial was to determine if cooling to 15 in place of 10°C could be considered to be adequate and not add undue cooling duration. From the SuperVent data it could be seen that for the 15°C treatment the cooling duration until 4°C was only 10 hours longer. This is not deemed problematic in terms of the cold chain to the EU and will be confirmed in large scale commercial shipments to the EU in 2018. The 7/8th cooling times for these two treatments also confirmed that no undue cooling duration was added by the extra 5°C warmer fruit.

What is also seen in the trial was the impact of carton type on cooling rate. Whereas the SuperVent carton responded in terms of cooling as expected viz. increased duration as pulp temperature increase, the same was not evident for the Opentop (Fig.2; Table 1 and 2). For the OpenTop the 15C treatments had to longest duration to reach the target temperature or the 7/8th cooling time. This was unexpected and the only explanation could be that this packaging type can have a larger negative impact on vertical cooling if a palletisation or loading was not optimal. It is suspected that the column resulting from the OpenTop carton stacking result in air only moving in this column within a pallet and is not forced to move laterally in a pallet due to the brick staking seen in SuperVent. In Table 2 the large variation between pallets and within these positions in these pallets can be seen. The extended cooling duration of some pallets (10 and 19 in which USDA 2 and 3 are positioned) resulted in the 15°C treatments being extended longer on average than the 10 and 20°C treatments. These data will form the base for subsequent projects to determine the impact of OpenTop cartons on cooling uniformity and rate.

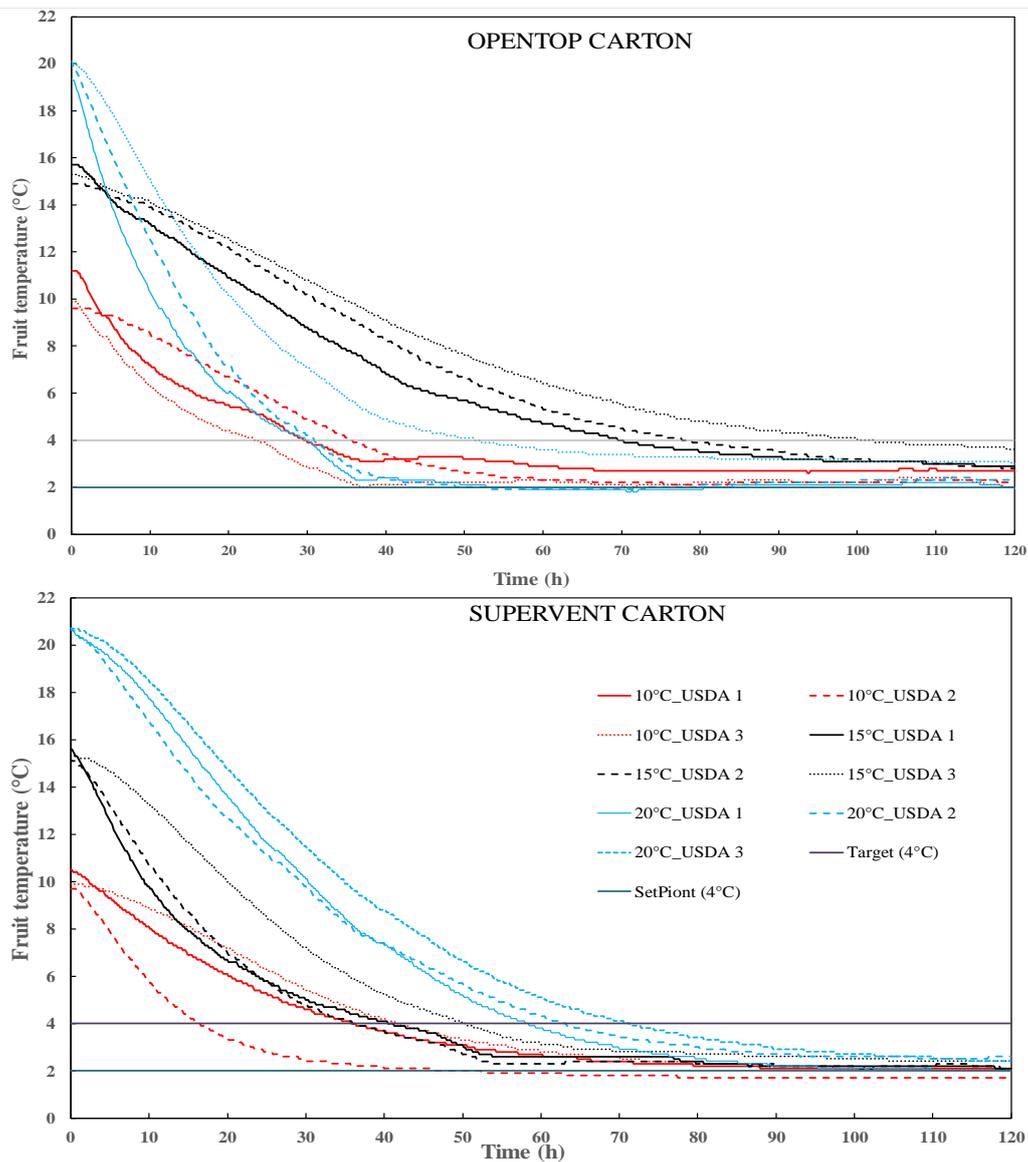


Fig. 2. Cooling pattern of 'Navel' orange fruit precooled to 10°C, 15°C or 20°C (ambient loaded) prior to loading in refrigerated reefers (Maersk StarCool) at set point 2°C. In the top graph, the fruit were packed in Opentop and in the bottom chart in Supervent cartons. The data points were measured by the cargo probes at USDA1/2/3 positions.

Table 1. The 7/8th cooling times (hours) of 'Navel' orange fruit precooled to 10°C, 15°C or 20°C (ambient loaded) prior to loading in refrigerated reefers (Maersk StarCool) at set point 2°C.

Treatment	Opentop (E15D) -Hours to 7/8 th -	SuperVent - Hours to 7/8 th -	Opentop (E15D) -Hours to 4°C-	SuperVent - Hours to 4°C -
10°C – USDA 1	58.1	50.6	36.3	36.8
10°C – USDA 2	44.5	23.5	35.8	18.1
10°C – USDA 3	29.2	55.8	23.8	42.2
Average	43.9	43.3	32.0	32.4
15°C – USDA 1	76.7	45.5	70.3	41.3
15°C – USDA 2	86.5	40.2	79.2	36.7
15°C – USDA 3	119.1	50.3	102.2	50.5
Average	94.1	45.3	83.9	42.8
20°C – USDA 1	28.5	55.3	30.3	58.5
20°C – USDA 2	30.1	59.5	31.1	63.3
20°C – USDA 3	47.5	66.8	52.2	71.5
Average	35.37	60.53	37.87	64.43

Table 2. The cooling duration (days) of 'Navel' orange fruit pulp - pre-cooled to 10°C, 15°C or 20°C (ambient loaded) prior to loading - in refrigerated reefers (Maersk StarCool) at set point 2°C to reach <4°C. ** Missing data point.

Treatment (Temp. + Carton)	1Top	1Mid	1Btm	6Top	6Mid	6Bot	10Top	10Mid	10Bot	12Top	12Mid	12Bot	19Top	19Mid	19Bot	20Top	20Mid	20Bot	AVRG (day)
10°C + SVent	1.67	**	0.23	0.98	0.58	0.42	1.35	0.77	0.60	1.50	1.50	0.56	2.04	1.73	0.56	**	1.39	1.13	1.06
15°C + SVent	2.29	1.51	**	2.23	1.60	1.57	2.05	1.56	1.54	2.04	2.04	0.67	2.04	1.96	1.13	1.50	0.00	4.42	1.77
20°C + SVent	2.79	2.83	1.8	2.23	1.90	1.60	3.54	2.69	2.04	3.28	2.21	**	4.08	3.08	2.48	1.33	**	1.58	2,54
10°C +OTop	1.44	1.33	1.10	1.42	1.42	1.10	1.90	2.27	1.35	**	1.69	0.75	3.29	2.33	1.04	1.27	1.67	1.73	1.59
15°C +OTop	2.8	4.35	1.02	2.08	2.21	**	5.31	4.08	3.19	2.85	2.19	0.65	3.04	4.71	3.48	3.00	2.94	2.90	2.99
20°C +OTop	1.92	2.92	0.79	1.63	1.69	1.35	1.88	1.25	1.00	1.90	2.58	1.42	1.83	1.71	1.65	**	3.04	2.00	1.19

Conclusion

The cooling efficiency of containers add considerable cooling capacity to the SA citrus industry in light of the lack of adequate precooling facilities. It is vital to make best use of this capacity without losing control of the cold chain in terms of quality and phytosanitary cold regimes.

It can be concluded that loading citrus fruit packaged in superVent cartons and precooled to 15°C, into containers would add only half a day to the cooling duration if compared to 10°C. If large scale commercial trials confirm these data, current regimes could be updated. What is clear that more research is needed to determine the impact of each packing type on vertical air movement in refrigerated containers. However, in all treatments, the duration of cooling never exceeded 3 days, until reaching below 4°C which is a good indication of the cooling capacity of refrigerated containers.

Acknowledgements

The authors would like to thank Sundays River Citrus Company (SRCC, Eastern Cape, South Africa) to offer the experimental materials, sites and aid, especially appreciation goes to André Mouton, Tina Oelofse and Jeanine Joubert. The authors would like to thank the Coop Research Program of the ETH Zurich World Food System Centre and the ETH Foundation for supporting this project.

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Technology transfer

CRI annual Postharvest workshops- Jan-Feb 2017

Further objectives and work plan

The specific objectives for 2018 are as follows:

1. Determine if the ambient loading cooling processes in reefers are efficient enough to reduce fruit temperature to 1°C or 0°C in a period complying with the FSM for the EU.
2. To ascertain whether cooling rates could be improved by applying the Oflow (www.otflow.eu) to current stowing practices in order to ensure better vertical air movement in a pallet.
3. Compare the impact on 'Star Ruby' grapefruit and possible advantage of different two cold chains from Letsitele viz. track transport vs train transport, as this cold chain route is considered one of the most critical ones at this point. In addition, two cartons types and pallet bases will be evaluated for their impact.

4.5 PROGRAMME: NUTRITION AND WATER MANAGEMENT

Programme coordinator: Pieter Raath (CRI)

4.5.1 FINAL REPORT: A novel approach to water and nutrient management in citrus

Project 986 (2013/4 – 2017/8) by Teunis Vahrmeijer, Nicolette Taylor, Colin Everson, Mpaballeng Sam, Mathew Banda, Ncamsile Shongwe, John Annandale (University of Pretoria), Sebinasi Dziki and Mark Gush (Council for Scientific and Industrial Research)

Summary

The objective was to measure and model citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size. The research was conducted in both the winter and summer rainfall regions on selected Valencia, Navel, grapefruit and soft citrus cultivars. Canopy dimensions, canopy cover and leaf area indexes were integrated with weather and transpiration/evapotranspiration data. Physiological measurements, e.g. leaf stomatal conductance and leaf water potentials were also conducted to try and explain water use dynamics of citrus trees.

Measurement of citrus water use in the winter rainfall region was completed in March 2017. While full season data for all eight orchards in Letsitele was only obtained by mid-February 2018. In general, the water use patterns of citrus trees are similar for the summer and winter rainfall regions. Progress was also made in modelling citrus tree transpiration, using a canopy conductance model which requires radiation interception by the canopy and vapour pressure deficit as main input variables.

Transpiration figures that can be reported are as follows: *Summer rainfall region*: For a single season, i.e. 2016/17, 'Midnight' Valencia planted in 1995 and 2008 respectively, transpired an estimate of 556 mm and 237 mm. While the 2006, 2010 and 2011 planted 'Star Ruby' orchards transpired an estimate of 424 mm, 319 mm and 152 mm of water. *Winter rainfall region*: For a single season, i.e. 2016/17, 'Nadorcott' mandarin and 'Mclean' Valencia orchards transpired an estimate of 938 mm and 163 mm of water respectively. While the 2000 and 2008 planted 'Midnight' Valencia orchards respectively transpired an estimate of 655 mm and 378 mm of water in 2015/16 season.

More correct figures for water use in the summer rainfall region were obtained from recent stem staining and wound width determinations. Together with other physiological measurements such as stomatal conductance and leaf water potential, it will be presented in the final WRC report, due August 2018.

Opsomming

Die doel was om watergebruik en water gebruikseffektiwiteit te meet en modelleer vir al die fenologiese stadiums van plant tot die bome volwasse is. Die navorsing is in beide die winter- en somerreënval streke deurgevoer op gelekteerde valencia, navel, pomelo en sagte sitrus kultivars. Boom dimensies, lowerbedekking en blaaroppervlak indekse is met weer- en transpirasie/evapotranspirasie data geïntegreer. Fisiologiese metings, te wete stomatale geleiding en blaarwaterpotensiaalmetings, is ook geneem om die watergebruiksdinamika van bome te ondersoek.

Meting van sitrus se waterverbruik is in Maart 2017 vir die winterreëval streek afgehandel, terwyl data vir 'n volle seisoen eers in mid-Februarie 2018 vir Letsitele verkry is. Waterverbruikspatrone van sitrus is soortgelyk vir die twee reënvalstreke. Vordering is ook gemaak om sitrusboom transpirasie te modelleer deur gebruik te maak van die lower geleidingsmodel wat radiasie onderskepping deur die lower, asook waterdampdruktekorte, as die belangrikste insette benodig.

Transpirasie waardes wat gerapporteer kan word is soos volg: *Sommerreënvalstreek*: Vir een seisoen, naamlik 2016/17, het 'Midnight' Valencia wat onderskeidelik in 1995 en 2008 geplant is elk naastebly 556 mm en 237 mm getranspireer. Die 2006, 2010 en 2011 geplante 'Star Ruby' boorde het weer ongeveer 424 mm, 319 mm en 152 mm water getranspireer. *Winterreënvalstreek*: Vir een seisoen, naamlik 2016/17, het 'Nadorcott' mandaryn en 'Mclean' valencia boorde onderskeidelik 938 mm en 163 mm water getranspireer. Die 2000 en 2008 geplante 'Midnight' valencia boorde het onderskeidelik 655 mm en 378 mm water in die 2015/16 seisoen getranspireer.

Meer akkurate watergebruikswaardes is onlangs met die stamvlek en wondwydte metings verkry. Tesame met ander fisiologiese metings, soos stomatale geleiding en blaarwaterpotensiale, sal dit in die finale WNK se verslag in Augustus 2018 gerapporteer word.

Introduction

The project is being finalised by the research team to submit a final report to the WRC in August 2018. Due to the resignation of the CRI's researcher a complete final report, that includes this section, could not be submitted at this time.

Stated objectives

General

To analyse the water use, yield, fruit size and quality of a selected Valencia, Navel, grapefruit and/or soft citrus cultivar for different canopy architectures in summer and/or winter rainfall regions; including a detailed analysis of water stress in relation to yield and quality for a selected cultivar at a single location.

Specific

1. To validate citrus water use by comparing different sap flow techniques with an appropriate technique such as lysimetry, cut stem and/or eddy covariance.
 2. To measure and model citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size.
 3. To determine the influence of water stress on fruit set, fruit yield, and pre- and post-harvest fruit quality for a selected cultivar and single location.
- 1.3 Specific objective 2: To measure and model citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size

This report focusses on specific objective 2 and the measurement of water use in the summer and winter rainfall areas of South Africa from planting to a mature canopy size. Transpiration measurements have been finalised in Citrusdal (winter rainfall) region, but in Letsitele, measurements in the Valencia and soft citrus orchard will continue until March 2018. We will therefore report on finalised transpiration data from all the measurement orchards in Citrusdal and the vast majority of orchards in Letsitele. From the data it is evident that we have captured variation in transpiration rates as a result of both canopy size and changing weather conditions in both a winter and summer rainfall region. This data also allows a comparison of transpiration characteristics of the different citrus species/varieties. In the winter rainfall region transpiration was quantified in Valencia, Navels and soft citrus, whilst in the summer rainfall region transpiration was quantified in Valencia, grapefruit and soft citrus. This comparison is important in order to ascertain if citrus water use can be modelled generically or if species/variety calibration is required. As a result of having both seasonal water use and yield, water use efficiency can be calculated and comparison can be made between navels and soft citrus in both regions.

The final aspect of the report is the desk top model to predict citrus water use according to the different seasonal growth stages. In this section we will focus on the modelling of solar radiation interception of the various orchards, which is a key input for the canopy conductance model we have previously parameterised for two orchards.

Materials and methods

Plant material and experimental sites

The aim of the project was to measure water use of orchards from planting to mature canopy size. Mature canopies were defined as those orchards in which a hedgerow had formed and where canopy cover exceeded 0.7. Where canopy cover is defined as the proportion of the area allocated to a tree which is shaded at solar noon. Intermediate-sized orchards were orchards in which a hedgerow had not formed and canopy cover varied between 0.4 and 0.6. Finally, newly planted orchards were defined as those orchards in which canopy cover was less than 0.4. Due to equipment limitations, a minimum stem diameter was required for transpiration measurements using both the heat ratio (HR) method and the stem heat balance system.

Winter rainfall region

The first phase of sap flow equipment took place in early August 2013 in four orchards on Patryberg farm (32° 27' 15" S, 18° 58' 03" E), which consisted of Valencia and Navel orchards of two different ages, as defined by canopy size. Sap flow equipment was also installed periodically during 2013 and 2014 in a small navel orchard using heat balance collars, which cannot be left on stems for long periods of time as the constant heat causes damage to the stems. Reinstallation in three of the four orchards took place in both March and April 2014 due to excessive gum production by the trees and failure of the sap flow systems. Monitoring in a mature Navel orchard was moved in November from the 23 year-old 'Bahianinha' Navel block to a 22 year-old 'Newhall' Navel block to facilitate soil water measuring and from a 13 year-old 'Bahianinha' to a 8 year-old 'Washington' Navel because the grower removed the 'Bahianinha' trees due to poor yields. Unfortunately, very poor data was collected in the 'Newhall' Navel orchard despite numerous attempts to improve the data. As a result, this data is excluded from the final report.

The second phase of measurements involved removing equipment from Navel orchards and installing the equipment in soft citrus orchards. As a result, two 'Nadorcott' Mandarin (Nadorcott) orchards were installed in August 2015 on the farm Brakfontein (32°30'27.63" S and 18°59'49.13" E).

Valencia: Three Valencia orchards were instrumented during the course of the study; 1) 'Midnight' Valencia planted in 2000 (14 years at the start of measurements), 2) 'Midnight' Valencia planted in 2008 (6 years at the start of measurements) and 3) 'McLean' Valencia planted in 2010 (5 years at the start of the study). These represented a mature, full bearing orchard, an intermediate-sized orchard and an orchard that had just started to bear fruit. Details of the Valencia orchards planted on the farm Patryberg farm in the winter rainfall region are given in Table 1. The average LAI for the 2000 and 2008 'Midnight' Valencia were 5.84 m² m⁻² and 4.33 m² m⁻², while for the 'McLean' Valencia was 3.28 m² m⁻².

Throughout the measuring period there has been an increase in the canopy volume in all trees (Figure 1). Canopy volume for 2000 'Midnight' Valencia ranged between 22.9 – 32.6 m³ with an average of 26 m³ and 2008 'Midnight' Valencia ranged between 16.8 – 20.2 m³ with an average of 18.5 m³. Whereas the 'McLean' Valencia ranged between 8.2 – 10.8 m³ with an average of 9.2 m³.

Table 1. Orchard details for 'Midnight' Valencia planted in 2000 and 2008 and 'McLean' Valencia planted in 2010.

Cultivar	'Midnight' Valencia	'Midnight' Valencia	'McLean' Valencia
Rainfall region	winter		
Measurement period Start End	07-Nov-2014 24-Oct-2016	07-Nov-2014 14-Sep-2016	31-Oct-2015 09-Jun-2017
Age	15 years old (planted 2000)	Planted in 2008	5 years old (planted 2010)
Rootstock	Troyer/Carizzo		Swingle/Carrizo
Orchard block area	3.3 ha	3.4 ha	2.3 ha
GPS co-ordinates	32°27'22.49" S, 18°58'10.76" E	32°27'55.31" S, 18°58'54.77" E	32°27'53.95" S, 18°58'58.41" E
Tree spacing	2.5 m x 5 m (12.5 m ²) planted on ridges (40 cm high)	3 m x 4.8 m (14.4 m ²), planted on ridges (50 cm high)	3 m x 5 m (15 m ²) planted on ridges
Row orientation	East-West	North-South	
Irrigation	Drip irrigation (2 drip lines per tree row. Drippers are spaced 0.8 m apart and has a delivery rate of 1.6 L hr ⁻¹ = 6.3 drippers per tree)	Drip irrigation (2 drip lines per tree row. Drippers are spaced 0.8 m apart and has a delivery rate of 1.6 L hr ⁻¹ = 7.5 drippers per tree)	
Canopy dimension	Height – 4.92 m Width – 2.68 m Breadth – 4.17 m	Height – 3.38 m Width – 3.08 m Breadth – 2.59 m	Height – 2.53 m Width – 2.40 m Breadth – 2.18 m
Canopy cover	0.83	0.54	0.35
Leaf area index – orchard (x̄ = 5 measurements)	5.63 m ² m ⁻² 5.84 m ² m ⁻²	4.51 m ² m ⁻² 4.33 m ² m ⁻²	3.28 m ² m ⁻² 3.31 m ² m ⁻²

- individual trees ($\bar{x} = 4$ measurements)			
Experimental trees	4	4	4
Tree circumferences	1 – 48.7 cm 2 – 50.9 cm 3 – 47.0 cm 4 – 48.5 cm	1 – 34.0 cm 2 – 33.8 cm 3 – 45.4 cm 4 – 29.1 cm	1 – 20.8 cm 2 – 20.4 cm 3 – 17.2 cm 4 – 21.2 cm
Yield (orchard)		25.5 t ha ⁻¹	9.8 t ha ⁻¹

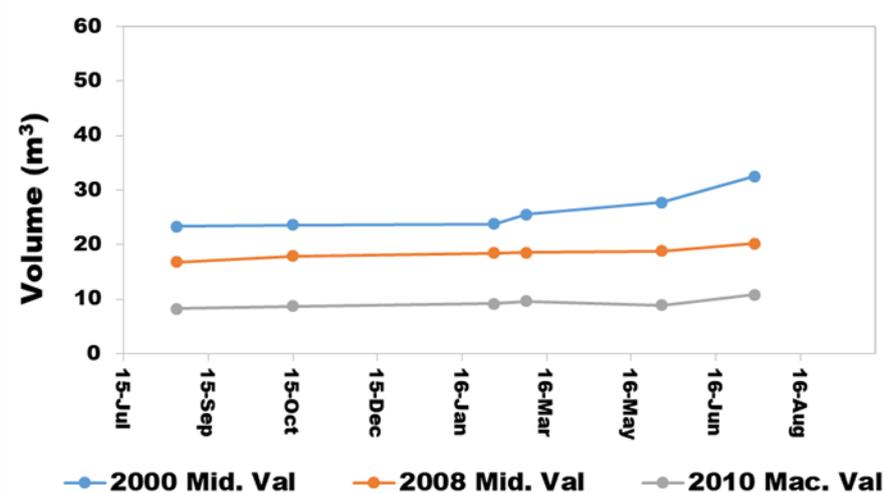


Figure 1. Canopy volume calculated using the ellipsoid volume method for three Valencia orchards. Each point represents 4 trees.

Navels: Over the course of the study three Navel orchards were instrumented; 1) ‘Bahianinha’ Navel planted in 1990 (23 years at the start of measurements), 2) ‘Washington’ Navel planted in 2006 (8 years at the start of measurements) and 3) ‘Cambria’ Navel planted in 2010 (4 years at the start of measurements). Details of the Navel orchards planted on Patrysborg farm in the winter rainfall region are given in Table 2. The average LAI for the 1990 ‘Bahianinha’ Navels was 2.5 m² m⁻², for 2006 ‘Washington’ 2.03 m² m⁻² and for ‘Cambria’ Navels 2.39 m² m⁻².

‘Nadorcott’ Mandarins: Over the course of the study two soft citrus orchards were instrumented: 1) ‘Nadorcott’ mandarin planted in 2002 and 2) ‘Nadorcott’ mandarin planted in 2013. These represented a mature and newly planted orchard. No intermediate-sized mandarin orchard was instrumented in Citrusdal as we could not find a suitable orchard on any of the farms with whom we worked regularly. Details of the Mandarin orchards planted on Brakfontein farm in the winter rainfall region are given in Table 3. Canopy volume for 2002 ‘Nadorcott’ Mandarin ranged between 20.2– 39.6 m³ with an average of 35.0 m³ and 2013 ‘Nadorcott’ Mandarin ranged between 3.6 – 12.4 m³ with an average of 9.0 m³.

Table 2. Orchard details for ‘Bahianinha’, ‘Washington’ and Cambria Navels planted in 1990, 2006 and 2010 respectively

Cultivar	‘Bahianinha’ Navels	‘Washington’ navels	‘Cambria’ Navels
Rainfall region	winter	winter	winter
Measurement period			
Start	05-Feb-2016	05-Feb-2016	23-Mar-2014
End	08-Sep-2017	05-Aug-2017	02-May-2015
Age	23 years old (planted 1990)	8 years old (Planted 2006)	4 years old (planted 2010)
Rootstock	Troyer/Carizzo		
Orchard block area	3 ha	4.1 ha	2.1 ha
GPS co-ordinates	32°26'52.40" S, 18°58'17.88" E	32°27'43.31" S, 18°59'1.46" E	32°27'51.14" S, 18°58'58.42" E
Tree spacing	3 m x 5.4 m (16.2 m ²) planted on ridges	3 m x 5.2 m (12 m ²)	3 x 5.5 m (16.5 m ²) planted on ridges
Row orientation	North-South		
Irrigation	Drip irrigation (2 drip lines per tree row. Drippers are spaced 0.8 m apart and has a delivery rate of 1.6 L hr ⁻¹ = 7.5 drippers per tree)		
Canopy dimension	Height – 3.2 m Width – 2.9 m Breadth – 2.6 m	Height – 2.57 m Width – 2.80 m Breadth – 2.61 m	Height – 2.3 m Width – 2.1 m Breadth – 2.2 m
Canopy cover	0.54	0.54	0.28
Leaf area index* – orchard (\bar{x} = 5 measurements) – individual trees (\bar{x} = 4 measurements)	1.4 m ² m ⁻² 2.5 m ² m ⁻²	1.71 m ² m ⁻² 2.03 m ² m ⁻²	3.17 m ² m ⁻² 2.39 m ² m ⁻²
Experimental trees	4	4	6
Tree circumferences	1 – 48.0 cm 2 – 40.0 cm 3 – 35.5 cm 4 – 37.0 cm	1 – 25.8 cm 2 – 29.5 cm 3 – 26.2 cm 4 – 27.8 cm	1 – 21.3 cm 2 – 19.2 cm 3 – 20.1 cm 4 – 24.8 cm 5 – 21.0 cm 6 – 22.3 cm

*These data points need to be confirmed in final WRC report.

Table 3. Orchard details for 2002 and 2013 ‘Nadorcott’ Mandarin

Cultivar	‘Nadorcott’ Mandarin	‘Nadorcott’ Mandarin
Rainfall region	winter	
Measurement period		
Start	11-Dec-2015	05-Feb-2016
End	30-Mar-2017	08-Sep-2017
Age	13 years old (planted 2002)	Planted in 2008
Rootstock	Swingle	
Orchard block area	5 ha	
GPS co-ordinates	32°32'28.39" S 19°00' 44.70" E	32°30'30.73" S 18°59' 31.77" E
Tree spacing	2.0 x 5.0 m (10.00 m ²) planted on ridges	2.5 x 5.5 m (13.75 m ²) planted on ridges
Row orientation	North-South	
Irrigation	Drip irrigation (2 drip lines per tree row. Drippers are spaced 0.8 m apart and has a delivery rate of 1.6 L hr ⁻¹ = 7.5 drippers per tree)	
Canopy dimension	Height – 4.92 m Width – 2.68 m Breadth/depth – 4.17 m	Height – 2.32 m Width – 1.90 m Breadth/depth – 1.53 m
Canopy cover	0.81	0.28
Leaf area index – orchard (\bar{x} = 5 measurements) – individual trees (\bar{x} = 4 measurements)	5.56 m ² m ⁻² 5.65 m ² m ⁻²	2.44 m ² m ⁻² 2.39 m ² m ⁻²
Experimental trees	4	5
Tree circumferences	1 – 52.5 cm 2 – 3 stems 34, 23, 31 cm 3 – 51.0 cm 4 – 3 stems 41, 33, 40.5 cm	1 – 24.2 cm 2 – 23.8 cm 3 – 22.9 cm 4 – 25.5 cm

		5 – 26.5 cm
Yield (orchard)	75 t ha ⁻¹	21 t ha ⁻¹

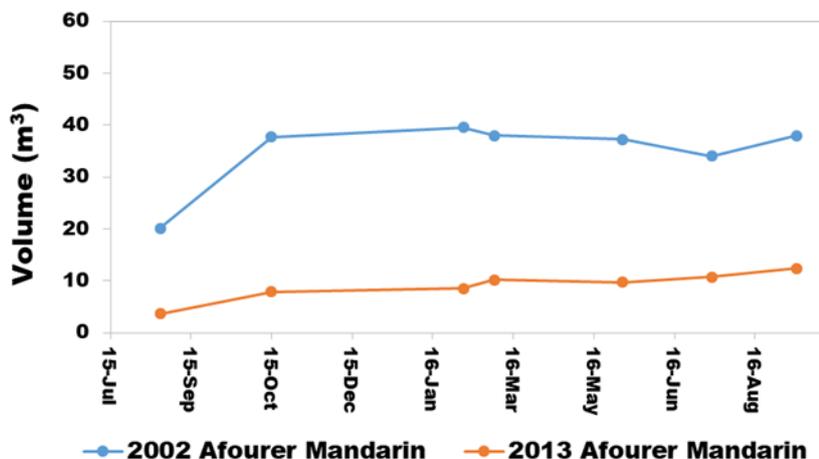


Figure 2. Canopy volume calculated using the ellipsoid volume method for two soft citrus orchards. Each point represents 4 trees.

Summer rainfall region

The campaign to measure water use in citrus in the summer rainfall region commenced in 2015 on various farms owned by Mahela Boerdery in Letsitele. A total of 8 orchards were instrumented with the sap flow equipment. In compliance with the terms of reference of the project, three different sized orchards of each species was instrumented, except for in the case of the 'Valley Gold' mandarins where we could not locate a suitable mature orchard on the farm.

'Star Ruby' grapefruit: Transpiration measurements in 'Star Ruby' orchards commenced in February and March 2016 and was terminated in July 2017 for the 2006 and 2010 'Star Ruby'. The HPV system installed in 2011 'Star Ruby' is still running and this was terminated in January 2018. In compliance with the terms of reference of the project, three different sized canopies for the 'Star Ruby' of were instrumented and are presented in Table 4.

Table 4. Orchard details for 'Star Ruby' orchard planted in 2006, 2010 and 2011.

Cultivar	'Star Ruby' grapefruit	'Star Ruby' grapefruit	'Star Ruby' grapefruit
Rainfall region	Summer		
Measurement period			
Start	05-Feb-2016	05-Feb-2016	23-Mar-2016
End	08-Sep-2017	05-Aug-2017	Still running
Age	11 years old (planted 2006)	7 years old (planted 2010)	6 years old (planted 2011)
Rootstock	Swingle Citrumelo		
Orchard block area	4.84 ha	3.40 ha	2.03 ha
GPS co-ordinates	23°48'16.09" S 30°28' 12.03" E	23°48'18.69" S 30°28' 08.60" E	23°49'12.75" S 30°25' 47.63" E
Tree spacing	7.0 x 3.0 m (21.00 m ²) planted on ridges		
Irrigation	Micro sprinklers (1 sprinkler per tree). 30 l h ⁻¹		
Canopy dimension	Height – 4.20 m Width – 4.96 m Breadth/Depth – 3.72 m	Height – 3.77 m Width – 4.15 m Breadth/Depth – 3.25 m	Height – 2.87 m Width – 2.87 m Breadth/Depth – 3.02 m
Canopy cover	0.71	0.59	0.41
Leaf area index			
– orchard (\bar{x} = 5 measurements)	4.2 m ² m ⁻²	4.6 m ² m ⁻²	3.55 m ² m ⁻²
– individual trees (\bar{x} = 4 measurements)	6.26 m ² m ⁻²	4.71 m ² m ⁻²	4.09 m ² m ⁻²
Experimental trees	4		
Tree circumferences	1 – 54.0 cm 2 – 51.0 cm	1 – 39.9 cm 2 – 38.8 cm	1 – 29.0 cm 2 – 27.0 cm

	3 – 42.0 cm 4 – 56.2 cm	3 – 38.0 cm 4 – 36.8 cm	3 – 27.0 cm 4 – 28.2 cm
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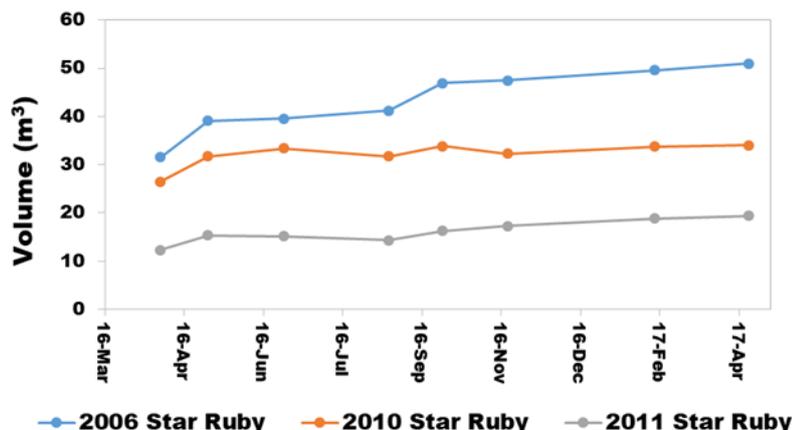


Figure 3. Canopy volume calculated using the ellipsoid volume method for three ‘Star Ruby’ orchards. Each point represents 4 trees.

As observed in the winter rainfall region, there was an increase in canopy volume over the measuring days (Figure 3). Canopy volume ranged between 12.3 – 21.0 m³, 26.5 – 34.0 m³, 31.6 – 51.0 m³ for the 2011, 2010 and 2006 ‘Star Ruby’ trees with an average of 16.7, 32.2 and 43.3 m³ respectively (Figure 3). As observed in the winter rainfall region, there was an increase in canopy volume over the measuring days (Figure 3). Canopy volume ranged between 12.3 – 21.0 m³, 26.5 – 34.0 m³, 31.6 – 51.0 m³ for the 2011, 2010 and 2006 ‘Star Ruby’ trees with an average of 16.7, 32.2 and 43.3 m³ respectively (Figure 3).

“Midnight’ Valencia: Transpiration measurements in ‘Midnight’ Valencia orchards commenced in April 2016 for the orchard planted in 1995 and 2008, whereas the 2014 ‘Midnight’ Valencia were only instrumented in March 2017. Full season data was collected for the 1995 and 2008 Valencia and the trial will be terminated in February 2018. As previously done for the ‘Midnight’ Valencia also three different sized orchards were instrumented in compliance with the terms of reference of the project. The different orchard details are presented in Table 5.

Table 5. Orchard details for ‘Midnight’ Valencia orchard planted in 1995, 2008 and 2014.

Cultivar	Midnight’ Valencia	Midnight’ Valencia	Midnight’ Valencia
Rainfall region	Summer		
Measurement period			
Start	08-Apr-2016	08-Apr-2016	19-Mar-2017
End	Still running	Still running	Still running
Age	22 years old (planted 1995)	9 years old (planted 2008)	4 years old (planted 2014)
Rootstock	MXT	Carizzo Citrange	
Orchard block area	2.58 ha	2.11 ha	10.33 ha
GPS co-ordinates	23°42’00.95” S 30°34’58.72” E	23° 41’57.61” S 30° 34’ 47.05” E	23°41’05.10” S 30°34’ 18.75” E
Tree spacing	7.0 x 3.0 m (21.00 m ²) planted on ridges		
Irrigation	Micro sprinklers (1 sprinkler per tree). 30 l h ⁻¹		
Canopy dimension	Height – 4.30 m Width – 5.2 m Depth/Breadth – 3.51 m	Height – 3,23 m Width – 3,75 m Depth/Breadth – 3.56 m	Height – 2.16 m Width – 2.42 m Breadth/Depth – 2.32 m
Canopy cover	0.74	0,51	0.27
Leaf area index			
– orchard (\bar{x} = 5 measurements)	2.54 m ² m ⁻²	3.53 m ² m ⁻²	2.24 m ² m ⁻²
– individual trees (\bar{x} = 4 measurements)	4.26 m ² m ⁻²	3.14 m ² m ⁻²	1.09 m ² m ⁻²
Experimental trees	4		

Tree circumferences	1 – 61.0 cm	1 – 32.5 cm	1 – 19.0 cm
	2 – 59.0 cm	2 – 32.0 cm	2 – 19.0 cm
	3 – 58.5 cm	3 – 33.0 cm	3 – 18.0 cm
	4 – 56.2 cm	4 – 36.0 cm	4 – 18.5 cm

Canopy volume ranged between 7.8 – 18.2 m³, 13.4 – 24.6 m³ and 40.9 – 50.6 m³ for the 2014, 2008 and 1995 'Midnight' Valencia trees with an average of 12.1, 17.4 and 46.1m³ respectively (Figure 4).

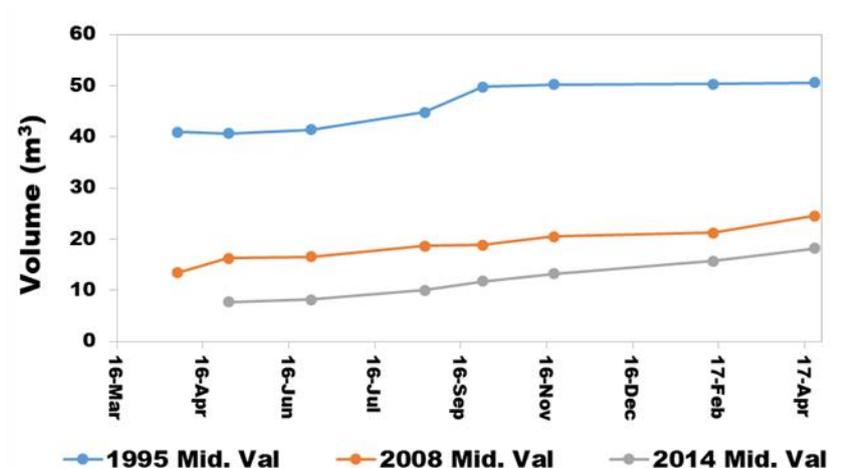


Figure 4. Canopy volume calculated using the ellipsoid volume method for three 'Midnight' Valencia orchards. Each point represents 4 trees.

'Valley Gold' mandarin: Transpiration measurements for the 'Valley Golds' planted in 2013 and 2015 only commenced in February 2017 and this will be terminated in February once full season data is obtained for both orchards. Unlike the other species only two different sized canopies were installed as we could not locate a suitable mature orchard on the farm. The orchard details are presented in Table 6.

Table 6. Orchard details for soft citrus orchards planted in 2013 and 2015

Cultivar	'Valley Gold'	'Valley Gold'
Rainfall region	Summer	
Measurement period		
Start	08-Feb-2017	09-Feb-2017
End	Still running	Still running
Age	4 years old (planted 2013)	2 years old (planted 2015)
Rootstock	Carizzo Citrange	
Orchard block area	4.89 ha	1.4 ha
GPS co-ordinates	23°51'34.72" S 30°21' 27.40" E	23°51'28.27" S 30°21' 11.21" E
Tree spacing	7.0 x 3.0 m (21.00 m ²) planted on ridges	
Irrigation	Micro sprinklers (1 sprinkler per tree). 30 l h ⁻¹	
Canopy dimension	Height – 2.87 m Width – 2.87 m Depth/Breadth – 2.81 m	Height – 2.28 m Width – 2.5 m Breadth/Depth – 2.43 m
Canopy cover	0.42	0.34
Leaf area index		
– orchard (\bar{x} = 5 measurements)	3.53 m ² m ⁻²	2.24 m ² m ⁻²
– individual trees (\bar{x} = 4 measurements)	3.14 m ² m ⁻²	1.09 m ² m ⁻²
Experimental trees	4	
Tree circumferences	1 – 32.5 cm 2 – 32.0 cm 3 – 33.0 cm 4 – 36.0 cm	1 – 19.0 cm 2 – 19.0 cm 3 – 18.0 cm 4 – 18.5 cm

Canopy volume ranged between 9.4 – 13.2 m³ and 11.6 – 16.3 m³ 40.9 – 50.6 m³ for 2015 and 2013 ‘Valley Gold’ mandarin trees with an average of 11.4 and 13.8 m³ respectively.

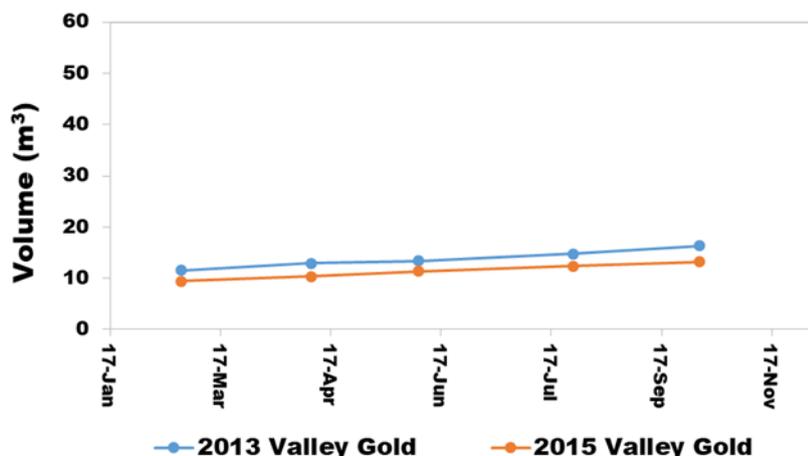


Figure 4. Canopy volume calculated using the ellipsoid volume method for three ‘Valley Gold’ Mandarin orchards. Each point represents 4 trees.

Water use measurements

Transpiration

The heat ratio method: Sap flow measurements were performed using the heat ratio (HR) method as described by Burgess *et al.* (2001) on four trees in each orchard in Citrusdal and Letsitele. Trees were selected in the centre of each block, with the objective of selecting trees with different stem circumferences, which represent the variation found within the orchard. Four heat pulse probe sets were inserted to four different depths in each tree trunk to account for the radial variation in sap flux within the conducting sapwood. These probe sets were inserted above the rootstock in the scion and below the first branch, with the probes being equally spaced around the trunk and randomly arranged, taking care to avoid any abnormalities in the trunk. Each probe set consisted of two Type T (copper/constantan) thermocouples (embedded in 2 mm outside diameter PTFE tubing) placed equidistantly (0.5 cm) upstream and downstream of the stainless steel heater probe (1.8 mm) which was inserted into a brass collar (2.5 mm) to avoid problems associated with resin causing corrosion of the probes. The heat pulse velocity (V_h) in cm h⁻¹ for each probe set was calculated following (Marshall, 1958) as:

$$V_h = \frac{k}{x} \ln\left(\frac{v_1}{v_2}\right) * 3600 \quad \text{Equation 1}$$

where k is the thermal diffusivity of green (fresh) wood (assigned a nominal value of $2.5 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$, (Marshall, 1958), x is distance in cm between the heater and either the upper or lower thermocouple, v_1 and v_2 are increases in temperature after the 0.4 s heat pulse is released (from initial temperatures) as measured by the upstream and downstream thermocouples and 3600 converts seconds to hours. Heat pulse velocities were measured and logged on an hourly basis using a CR10X or CR1000 data logger and an AM16/32B multiplexer (Campbell Scientific Ltd, Logan, Utah, USA). Conversion of heat pulse velocities to sap flux densities, taking into account wounding, were performed according to Burgess *et al.* (2001). Wound width was determined at the end of measurements in each orchard by chiselling out a wood sample for a minimum of four probe sets per orchard. Whole stem sap flux (assumed to be equal to transpiration) was calculated as a product of sap flux density and weighted sapwood cross-sectional area represented by each probe set. The presence of heartwood was determined by staining conducting tissue *in situ* using safranin and then using a corer to extract a core sample from the tree. Integrated volumetric sap flow of the individual trees (L day⁻¹) was converted to transpiration (mm day⁻¹) using the ground area allocated to each tree in the orchard. Transpiration was calculated as an average of the sample trees. Wood samples were taken from all the study orchards to determine sapwood properties (density, water content and xylem depth) and wound widths. Additional samples were taken from the ‘Delta’ Valencia orchard to examine wood anatomy prior to and after probe insertion to determine the impact of probe insertion on tissue wounding.

Validation of the orchard transpiration measurements was performed according to Taylor et al. (2013) using evapotranspiration (ET), determined by the eddy covariance data, and soil evaporation (E_s) determined using micro-lysimeters in the 9 year-old 'Washington' Navel orchard in March 2015. With this method of calibration orchard transpiration was underestimated by 5 % on average per day, which is considered reasonable. The close match of the HR method to micrometeorological method (Figure) shows that if the parameters (wound width, sapwood depth and heartwood radius) for determining SFD with the HR method are measured accurately, accurate measurements of transpiration in *Citrus sinensis* can be achieved.

Similar sap flow calibrations have been performed by Williams et al. (2004) in an olive orchard assuming evaporation from the soil is negligible and by using a combination of eddy covariance and micro-lysimeter measurements in a vineyard (Poblete-Echeverría *et al.* 2012), pear orchard (Conceição and Ferreira, 2008) and broad-leaved forest (Köstner *et al.* 1992). The advantage of this method of calibration is that it can be performed in field without the use of weighing lysimeters, which although ideal, are expensive to install and require a number of years for the tree planted in the lysimeter to reach an adequate size for measurement. It is also non-destructive as compared to stem perfusion and potometer calibration methods. However, eddy covariance measurements can be associated with some degree of error, but these can be assessed and minimised through careful analysis of the data (Allen *et al.* 2011). In addition, whilst water use of a single tree is determined in a lysimeter, eddy covariance measurements represent a much larger area and a number of trees. The size and the shape of the area sampled are not fixed in time and vary with wind speed and direction (Horst and Weil, 1992; Baldocchi *et al.* 1997). However, there was adequate fetch and as measurements in this study were conducted in a large orchard of clonal trees this variation is expected to be minimal. As the need for calibration arises largely due to the variation in the distribution of sap conducting vessels within the sapwood (Green and Clothier, 1988), which is assumed to be conservative for a species, a single calibration for a species for a specific sap flow technique should be sufficient (Smith and Allen, 1996) and it should be transferable to other citrus orchards. In addition, a single calibration should also be sufficient as (Pernice, 2009; Fernandez *et al.* 2001; Giorio, 2003) and Poblete-Echeverria, 2012) showed no reduced sensitivity due to injury effects caused by probe implantation over a period of 2 months to 3 years.

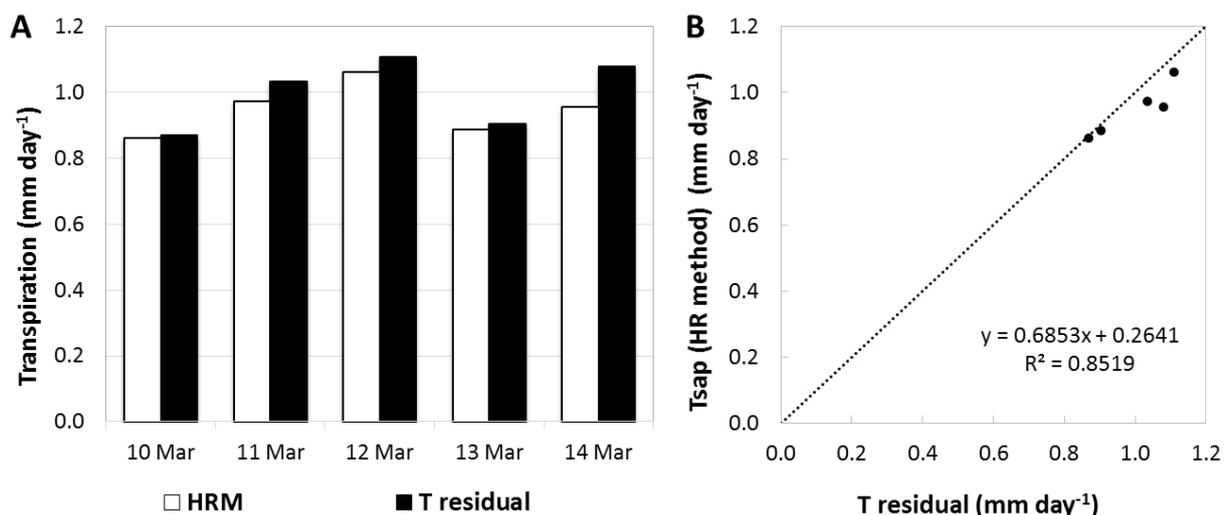


Figure 5. (A) Daily total residual transpiration and sap flow by the heat ratio (HR) method and (B) Regression analysis of daily T_{res} with the HR method 'Washington' Navel orchard. The dashed line is a 1:1 line.

Stem steady state heat energy balance method: Transpiration in an immature 4 year-old 'Cambria' Navel orchard was determined using the stem steady state heat balance method (Dynagage™, Dynamax, Houston, Texas, USA). This method estimates sap flow (g h^{-1}) and is therefore very useful for determining whole plant water use (Vandegehuchte and Steppe, 2013). In addition, it does not require calibration (Baker, 1987) which is seen as a major advantage. However, this method can only be used for small trees with fairly straight and round trunks and is therefore, although ideal, not suited for larger citrus trees. Additional disadvantages of this method include the high power requirements and the cost of the collars. SGB50 collars (45-65 mm diameter) were fitted to five trees. These collars were logged using a standard Flow 32A-1K system from Dynamax, consisting of a CR1000 logger, AM16/32B multiplexer and an AVR voltage regulator. Sap flow was estimated according to Figure . Data was processed using an Excel spreadsheet developed by Dynamax. A preliminary stem thermal conductivity (K_{st}) value of $0.42 \text{ W m}^{-1} \text{ K}^{-1}$ was used, but this must still be determined experimentally in the citrus trees. Gauge thermal conductance (K_{sh}) used for calculation of sap flow was determined each day by using the average K_{sh} values for each collar between 02 00 and 04 00 when sap flow was considered to be zero. The energy balance was computed for every sap flow measurement, only days on which energy balance was achieved for more than 80 % of the individual sap flow measurements were used to calculate the water use from the 'Cambria' Navel orchard.

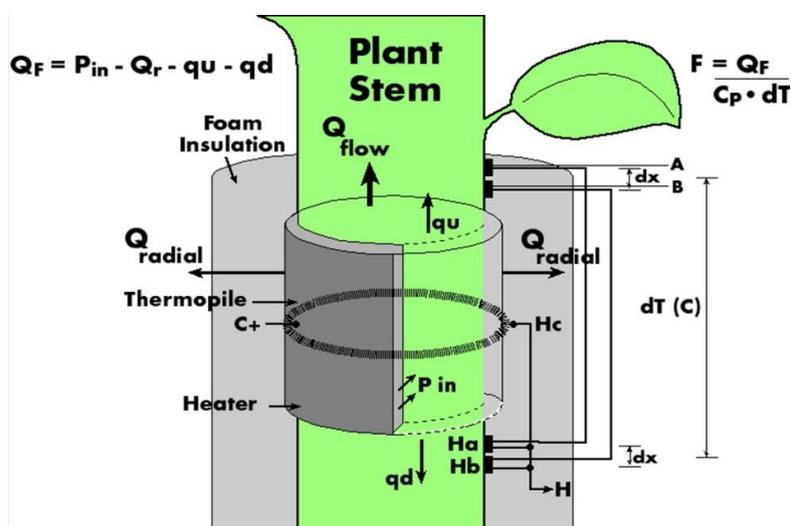


Figure 6. Sap flow sensor operation and the calculation of sap flow.

Eddy covariance

Citrusdal: An extended Open Path Eddy Covariance (OPEC) system, comprising a CSAT3 (Campbell Scientific Inc., Logan, Utah, USA) three-dimensional sonic anemometer for sensible heat flux (H) and an LI-7500 open path infrared gas analyser (IRGA) (LI-COR Inc., Lincoln, NE, USA) for latent heat flux (LE), was mounted at 5 m above ground (2 m above average canopy height) to determine total evaporation (ET) for the orchard. Additional measurements of air temperature and humidity were sampled using an HMP45C Vaisala temperature and humidity probe (Campbell Scientific Inc., Logan, Utah, USA) mounted at 4.5 m above ground. Net irradiance (R_n) was measured using a NR-Lite (Kipp and Zonen, Delft, The Netherlands) net radiometer mounted at 8 m above ground. Soil heat flux (G) was determined using HFP-01 (Hukseflux, Delft, Netherlands) soil heat flux plates buried 80 mm below the soil surface. In addition, TCAV-L soil temperature averaging probes (Campbell Scientific Inc., Logan, Utah, USA) were installed at 2 locations representing within-row and between-row conditions, and were positioned 20 mm and 60 mm below the soil surface to correct the measured soil heat flux data for the energy stored above the plates. A CS616 time domain reflectometer water content sensor (Campbell Scientific Inc., Logan, UT, USA) linked to the OPEC system was positioned in the upper 60 mm of the soil. Measurements were sampled at a frequency of 10 Hz and logged on a CR5000 data logger (Campbell Scientific Inc., Logan, Utah, USA) every 30 minutes.

Micro-meteorological instruments, utilising the shortened energy balance approach were used for the measurement of total evaporation (ET) of the orchard during this short-term seasonal measurement campaign.

Measurements took place from 12 to 25 November 2013, and were representative of early summer season conditions. Instruments were mounted on a lattice mast, installed in the centre of the orchard (Figure 7). The fetch was approximately 200 m based on the prevailing N-S wind direction and position in the centre of the orchard (Figure 7).



Figure 7. Installation of the eddy covariance system in the 'Washington' navels.

Letsitele: Fluxes of latent (LE) and sensible heat (H) were measured with an extended open path eddy covariance (OPEC) system, comprising an IRGASON open-path analyser and sonic anemometer (Campbell Scientific Inc., Logan, Utah, USA), which was mounted on a lattice mast 7.5 m above the soil surface (1.5 m above the canopy). Upwind and downward fetch of the prevailing northerly westerly and south easterly winds was 150 m. Air temperature and humidity were measured using a HygroClip2 HC2-S(3) thermohygrometer probe (Rotronic Instruments, Bassersdorf, Switzerland). Net radiation (Rn) was measured using an NR-Lite net radiometer (Model 240-110 NR-Lite, Kipp & Zonen, Delft, Netherlands) 7.5 m above ground. Four soil heat flux plates (model HFT-S, REBS, Seattle, Washington, USA) were used to measure soil heat flux (G) at a depth of 80 mm under the trees and between the rows, and four TCAV-L soil temperature averaging probes (Campbell Scientific Inc., Logan, Utah, USA) at depths of 20 and 60 mm were used to calculate the heat stored above the plates. Volumetric soil water content in the first 60 mm of the soil surface was measured using two-time domain reflectometer (CS616, Campbell Scientific Inc., Logan, Utah, USA) placed near the heat flux plates. Measurements were sampled at a frequency of 10 Hz and logged on a CR3000 data logger (Campbell Scientific Inc., Logan, Utah, USA) using the Easyflux-DL software from Campbell Scientific. The program applies the most common open-path EC corrections to fluxes.

Weather data

In Citrusdal hourly and daily weather data were obtained from the Campbell Scientific Automatic Weather Station (AWS) on Patrysburg (32°27'2.57"S and 18°58'6.23"E) and from October 2015 on Brakfontein (32°29'30.46"S and 18°59'48.79"E) (Figure 8), which were both installed according to standard conditions specified in FAO-56 and was situated over a short vegetated surface. Irrigated orchards (2-3 m in height) were found within 10 m west, 60 m north, 30 m east and 50 m south of the AWS at Patrysburg. Whilst the irrigated orchards would have conditioned the boundary layer, the height of the orchards may impact wind speed. This may result in an underestimation of ET_0 (Allen, 2008). At Brakfontein the AWS was installed in an open field, with an avenue of tall trees 50 m to the South. There were no irrigated fields within 1 km of the AWS in any direction. Under these fairly dry conditions, calculated ET_0 is likely to be slightly overestimated, as compared to data collected over a reference surface (Allen, 2008). The Constantia and Letsitele weather stations were surrounded by irrigated orchards and buildings. Whilst the irrigated orchards would have conditioned the boundary layer, the height of the orchards may impact wind speed. This may result in an underestimation of ET_0 (Allen, 2008). Both the weather stations at Letsitele are operated by Laeveld Agrochem.

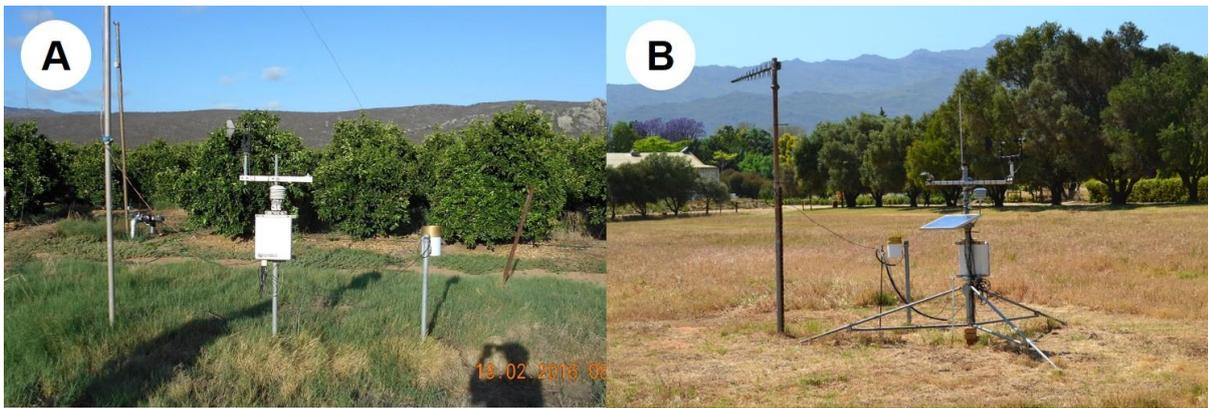


Figure 8. Automatic weather stations at A) Patrytsberg and B) Brakfontein.

Daily and hourly reference evapotranspiration for both a short and tall reference surface were calculated according to the FAO-56 Penman-Monteith equation (Allen, 1998 and Pereira, 2015) from weather data. The weather parameters recorded were wind speed, solar radiation, temperature, relative humidity and rainfall. Quality assessment and quality control of the data was performed according to the procedures described by (Allen, 2008). Solar radiation was found to be routinely underestimated at both Citrusdal weather stations and was corrected according to the procedure outlined by (Allen, 2008). No corrections were made to the other variables measured.

Tree characteristics and physiological measurements

Tree size and canopy volumes: Tree height, width (across the tree row) and breadth (within the tree row) of each orchard were routinely measured every 6 to 12 weeks. Canopies are approximately ellipsoidal therefore; the canopy volume was calculated using the formula for an ellipsoid.

Leaf area index and fractional interception of photosynthetically active radiation: Leaf area index of individual trees and the orchard as a whole was determined using an LI-2200 Plant Canopy Analyser (Li-Cor Biosciences, Lincoln, Nebraska, USA) under diffuse light conditions. For individual tree LAI, measurements were made at the four cardinal points under each tree, with clear sky readings taken in the open next to the orchard. For orchard LAI, measurements under the canopy were made across the work row from the trunk of one tree to the trunk of the tree in the next row. Once again clear sky readings were taken in the open next to the orchard. Fractional interception of photosynthetically active radiation (FI-PAR) was determined with a Decagon AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA) in a grid pattern around a representative tree in each orchard. The grid consisted of transect lines across the tree row with 1 m in between transect and with 1 m between the grid points. The number of measurements in each orchard depended on the planting density of the orchard. Measurements were conducted throughout the course of the day on the hour every hour under clear sky conditions. Full sun readings were taken in an open area next to the orchard.

Five calibrated tube solarimeters (Delta-T) were used to continuously measure solar radiation received at the orchard floor for all the study orchards. The solarimeters were positioned in the work row on both the east and west sides of the tree row, with the 5th solarimeter placed directly under the canopy in the tree row as shown in Figure 9.



Figure 9. Tube solarimeters used to continuously measure solar radiation received at the orchard floor.

Results and discussion

SUMMER RAINFALL REGION

Weather variables

Weather data for the field trials in the Letsitele region were obtained from two weather stations that are operated and maintained by Laeveld Agrochem. These weather stations are located at Letsitele junction and Constantia. The weather station at the Letsitele junction is the closest to the field trials, so the weather data from this station is considered. The lowest temperature, from 20 November 2015 to 15 October 2017, observed on all weather stations was 0 °C, whilst the maximum was 42.1 °C. Warm summers and mild winters are experienced in this region, with an average air temperature of 32.0 °C in summer and 18.8 °C in winter. The total rainfall for the period 1 December 2015 – 15 October 2017 was 1 037 mm.

A clear seasonal trend exists for VPD, ET_o and ET_r , with the lowest values in winter and highest values in summer. For the measuring period, 1 December 2015 – 15 October 2017, the average VPD for the summer seasons was 1.58 kPa, with a daily maximum of 3.57 kPa and the average for winter seasons was 1.01 kPa, with a daily minimum of 0.16 kPa. The average ET_o for the summer seasons was 4.71 mm (5.18 for ET_r), with a daily maximum of 7.77 mm (9.58 for ET_r), while the average ET_o for the winter seasons was 1.80 mm (2.02 for ET_r), with a daily minimum of 0.54 mm (1.04 for ET_r).

Transpiration and plant water relations

Grapefruit orchards: Transpiration in all orchards showed large day-to-day variation, which was largely determined by the prevailing climatic conditions as seen from the ET_o data. In the summer rainfall region, the ET_o in the 2016/17 season ranged between 0.54 mm day⁻¹ and 6.34 mm day⁻¹. Transpiration in the 2006 'Star Ruby' orchard was 1.57 mm day⁻¹ when ET_o was a maximum and 0.84 mm day⁻¹ when ET_o was a minimum, whilst for the 2010 'Star Ruby' orchard the transpiration was 1.19 mm day⁻¹ and 0.70 mm day⁻¹ respectively on the days when the maximum and minimum ET_o was recorded. For the 2011 'Star Ruby' orchard transpiration was 0.48 mm day⁻¹ and 0.30 mm day⁻¹ respectively for the maximum and minimum ET_o . The atmospheric evaporative demand was highest during summer, with no proportional increase in transpiration at this time. Transpiration from all the 'Star Ruby' orchard followed a similar trend lower transpiration values were observed in winter and higher transpiration values in summer. Daily transpiration coefficient values showed a large variation ranging from 0.03 -1.56, 0.08 -1.29 and 0.01 - 0.87 for the 2006, 2010 and 2011 'Star Ruby' orchards. Differences in the Kt values can be observed when average monthly Kt values were determined (Figure 10). Transpiration coefficients were fairly constant in summer for all the orchards. In Figure 10 it is evident that the water use is proportional to the canopy size as the 'Star Ruby' planted in 2006 with big canopy had higher Kt values followed by medium and small 'Star Ruby'.

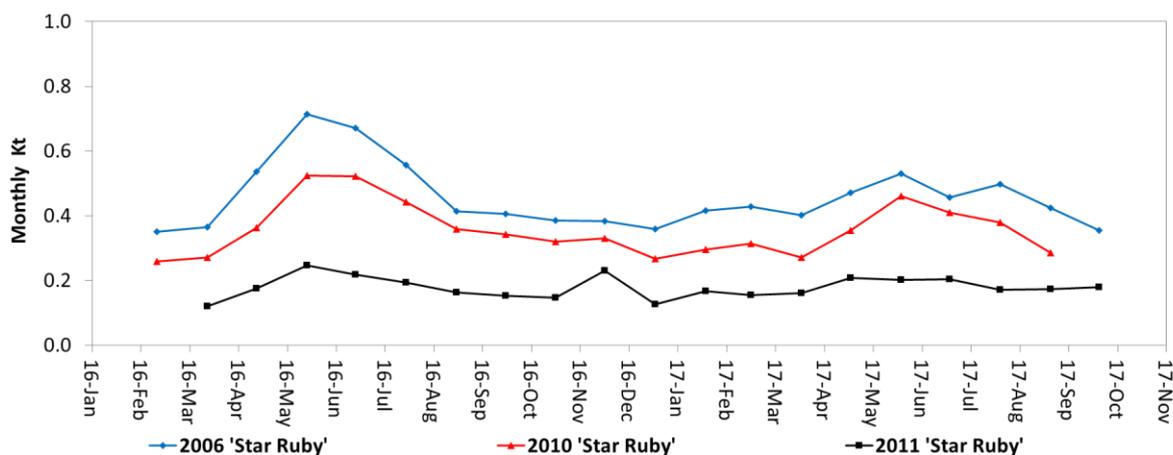


Figure 10. Monthly transpiration coefficient values for the ‘Star Ruby’ planted in 2006, 2010 and 2011.

Transpiration was measured for periods of 580, 547 and 616 days in the 2006, 2010 and 2011. Total transpiration for the measurement periods were 628, 450 and 249 mm in 2006, 2010 and 2011 ‘Star Ruby’ orchard. On average 1.08, 0.82 and 0.40 mm of water were transpired per day Table 7.

Table 7. Average tree water use for the four measuring trees in ‘Star Ruby’ orchard planted in 2006, 2010 and 2011

	2006 ‘Star Ruby’		2010 ‘Star Ruby’		2011 ‘Star Ruby’	
Transpiration	mm	L	mm	L	mm	L
Total	628	13 184	450	9 448	249	5 221
Maximum per day	1.97	41.29	1.26	26.44	0.74	15.63
Minimum per day	0.12	2.48	0.33	6.83	0.04	0.74
Average per day	1.08	23	0.82	17	0.40	8
Measurement period (days)	580		547		616	

Valencia orchards: As observed in the ‘Star Ruby’, transpiration in ‘Midnight’ Valencia orchards also showed large day-to-day variation, which was largely determined by the prevailing climatic conditions. For the period of 2016/17 season ET_o ranged between 0.54 mm day⁻¹ and 7.01 mm day⁻¹. Transpiration in the 1995 ‘Midnight’ Valencia orchard was 2.09 mm day⁻¹ on the day when maximum ET_o was measured and 0.94 mm day⁻¹ when minimum ET_o was measured. Transpiration in the 2008 ‘Midnight’ Valencia orchard, on the days when the maximum and minimum ET_o was recorded, was 0.83 mm day⁻¹ and 0.36 mm day⁻¹. For the 2014 ‘Midnight’ Valencia no transpiration value was recorded at maximum ET_o since measurements only commenced in February 2017. The transpiration for this orchard when minimum ET_o was recorded, was 0.56 mm. Like the ‘Star Ruby’ orchards, it is evident that even though the atmospheric evaporative demand was highest during summer, there was no proportional increase in transpiration at this time. Transpiration from all the ‘Midnight’ Valencia orchards followed a similar trend lower transpiration values were observed in winter and higher transpiration values in summer.

Similar to the ‘Star Ruby’, Kt values for the ‘Midnight’ Valencia orchards had a large variation. Transpiration coefficient values ranged from 0.11 -1.73, 0.03 - 0.67 and 0.06 - 0.56 for the 1995, 2008 and 2014 ‘Midnight’ Valencia orchards. Average monthly Kt values showed a similar trend between all the ‘Midnight’ Valencia orchards, which are well correlated (Figure 11) and indicates a proportional water use to canopy size. Transpiration coefficients were also fairly constant in summer for all the orchards with large variations in the winter months, this observation is consistent in many citrus cultivars. Presently transpiration has been measured for 601 days for both 1995 and 2008 ‘Midnight’ Valencia and 256 days for 2014 ‘Midnight’

Valencia. Total transpiration for the measurement periods were 835, 365 and 86 mm in 1995, 2008 and 2014 'Midnight' Valencia orchards respectively. On average 1.39, 0.61 and 0.34 mm of water were transpired per day in 1995, 2008 and 2014 'Midnight' Valencia orchards respectively (Table 8).

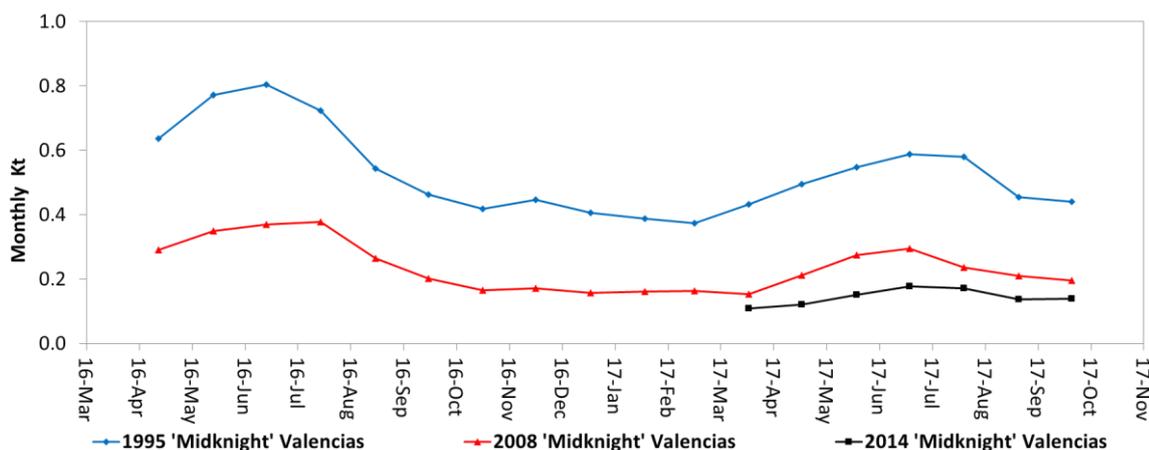


Figure 11. Monthly transpiration coefficient values for the 'Midnight' Valencia orchard planted in 1995, 2008 and 2014.

Table 8. Average tree water use for the four measuring trees in 'Midnight' Valencia orchard planted in 1995, 2008 and 2014.

	1995 'Midnight' Valencia		2008 'Midnight' Valencia		2014 'Midnight' Valencia	
	Mm	L	mm	L	mm	L
Transpiration						
Total	835	17 542	365	7 672	86	1 821
Maximum per day	2.33	49.00	0.90	18.81	0.51	10.79
Minimum per day	0.15	3.09	0.04	0.88	0.06	1.16
Average per day	1.39	29.14	0.61	12.74	0.34	7.09
Measurement period (days)	601		601		256	

Mandarin orchards: Transpiration data has been collected from February to November 2017 for the 2013 and 2015 'Valley Gold' orchards. For that period ET_o ranged between 0.54 mm day⁻¹ and 5.73 mm day⁻¹. Transpiration in the 2013 'Valley Gold' orchard on the days when the maximum and minimum ET_o was recorded, was 0.56 mm and 0.29 mm respectively. Also transpiration in the 2015 'Valley Gold' orchard for the maximum and minimum ET_o , was 0.20 mm and 0.18 mm. Daily transpiration coefficient values showed variation ranging from 0.04 - 0.54 and 0.02 - 0.32 for the 2013 and 2015 'Valley Gold' orchards. Differences in the Kt values can be observed when average monthly Kt values were determined (Figure 12).

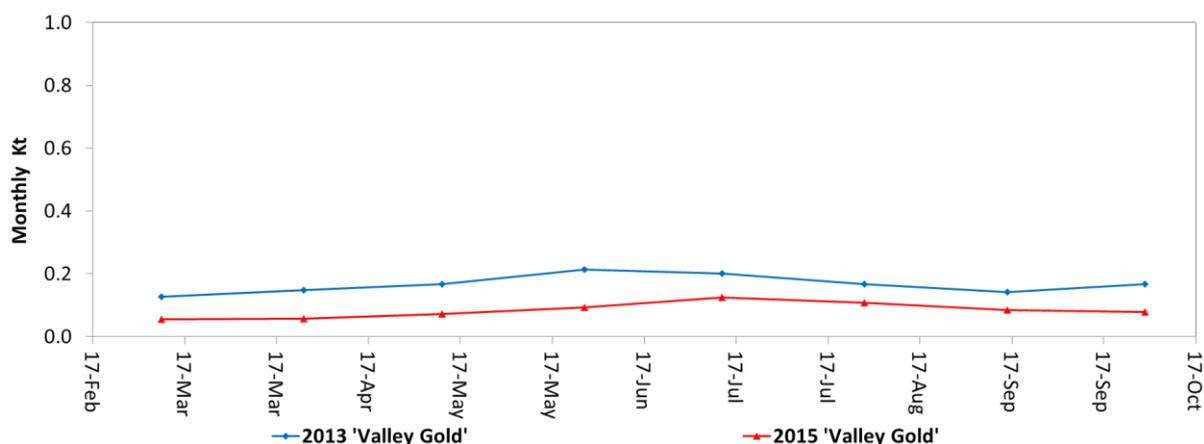


Figure 12. Monthly transpiration coefficient values for the ‘Valley Gold’ Mandarins planted in 2013 and 2015.

Transpiration was measured for 295 and 294 days in the 2013 and 2015 ‘Valley Gold’ orchards. Total transpiration for the measurement periods were 131 and 62 mm with an average of 0.44 and 0.21 mm of water being transpired per day in 2013 and 2015 ‘Valley Gold’ orchards respectively (Table 9).

Table 9. Average tree water use for the four measuring trees in ‘Valley Gold’ Mandarin orchard planted in 2013 and 2015.

	2013 ‘Valley Gold’		2015 ‘Valley Gold’	
	mm	L	mm	L
Transpiration				
Total	130.75	2745.73	61.76	1296.87
Maximum per day	0.75	15.80	0.38	7.90
Minimum per day	0.03	0.69	0.02	0.32
Average per day	0.44	9.28	0.21	4.40
Measurement period (days)	295		294	

The response of all 8 different citrus orchards in the summer rainfall region (i.e. 3 ‘Star Ruby’ orchards, 3 ‘Midnight’ Valencia orchards and 2 ‘Valley Gold’ orchards) had similar response to weather variables. The response of transpiration to increases in solar radiation and temperature was linear in all the orchards, this relationship could have improved if the weather station was in close proximity to the trial site. Whilst order 2 polynomial relationship was observed for the response of transpiration to ET_0 and VP. More than 43% of the variation in transpiration was explained by changes in solar radiation in the citrus orchards. Transpiration increased with VPD up to a certain point, with no further increase in transpiration once VPD had exceeded 2.0 kPa. A similar response was observed for ET_0 and no further increase in transpiration was observed when atmospheric demand exceeded approximately 6 mm. This confirms mechanisms in citrus that prevent a catastrophic decline in plant water status at high atmospheric demands.

WINTER RAINFALL REGION

Weather variables

Weather data from the AWS at Brakfontein and Patryberg indicate that the minimum temperature from 7 October 2015 (initial installation of equipment) to 4 December 2016 was 0.2 °C, whilst the maximum was 43.2 °C. Warm summers and cool winters are experienced in this region, with an average air temperature of 23.1 °C in summer and 14.6 °C in winter. The total rainfall recorded at Brakfontein weather station was 379.5 mm during the period of 7 October 2015 – 7 July 2017 and a total of 587.7 mm was recorded on Patryberg weather station during the period of 1 August 2013 – 28 October 2016.

Vapour pressure deficit (VPD) and reference evapotranspiration (ET_o – short crop and ET_r – tall crop) are important determinants of water use in plants and therefore these parameters should be considered when estimating water use and determining the drivers of water use. A clear seasonal trend exists for VPD, ET_o and ET_r , with the lowest values in winter and highest values in summer. For the measuring period (7 October 2015 – 29 October 2016) the average VPD for the summer seasons was 2.07 kPa, with a daily maximum of 4.35 kPa and the average for winter seasons was 1.00 kPa, with a daily minimum of 0.08 kPa. The average ET_o for the summer seasons was 4.56 mm (6.92 for ET_r), with a daily maximum of 8.09 mm (10.79 for ET_r), while the average ET_o for the winter seasons was 2.09 mm (2.72 for ET_r), with a daily minimum of 0.23 mm (0.75 for ET_r).

Valencia orchards: Water use from the winter rainfall region has been finalised. The trend for water use of citrus in the summer rainfall is the same as the winter rainfall. Also in winter rainfall region all ‘Midnight’ Valencia orchards also showed large day-to-day variation, which was largely determined by the prevailing climatic conditions (as per the ET_o data). For the period of 2014/16 ET_o ranged between 0.61 mm day⁻¹ and 8.85 mm day⁻¹. Transpiration in the 2000 ‘Midnight’ Valencia orchard on the days when the maximum and minimum ET_o was 2.69 mm day⁻¹ and 0.28 mm day⁻¹ respectively. Also transpiration in the 2008 ‘Midnight’ Valencia orchard on the days when the maximum and minimum ET_o was 1.80 mm day⁻¹ and 0.1 mm day⁻¹. ‘Mclean’ Valencia planted in 2010 were installed later than the other 2 orchards. The maximum and minimum ET_o recorded for which water use measurements was being conducted in ‘Mclean’ Valencia was 9.81 mm day⁻¹ and 0.66 mm day⁻¹ respectively. The transpiration values recorded on the days when the ET_o was maximum and minimum was 0.59 mm day⁻¹ and 0.2 mm day⁻¹.

Daily Kt values for the ‘Midnight’ Valencia orchards showed a lot of variation. Transpiration coefficient values ranged from 0.02 – 0.3, 0.05 – 1.01 and 0.26 – 1.62 for the 2010 ‘Mclean’, 2008 and 2000 ‘Midnight’ Valencia orchards. Average monthly Kt values showed a similar trend between all Valencia orchards which are well correlated (Figure 13) also indicating proportional water use to canopy size. In September and October 2015 same Kt values were recorded ‘Midnight’ Valencia planted in 2010 and 2008, this was a result of heavy pruning in 2000 ‘Midnight’ Valencia and reduced water use.

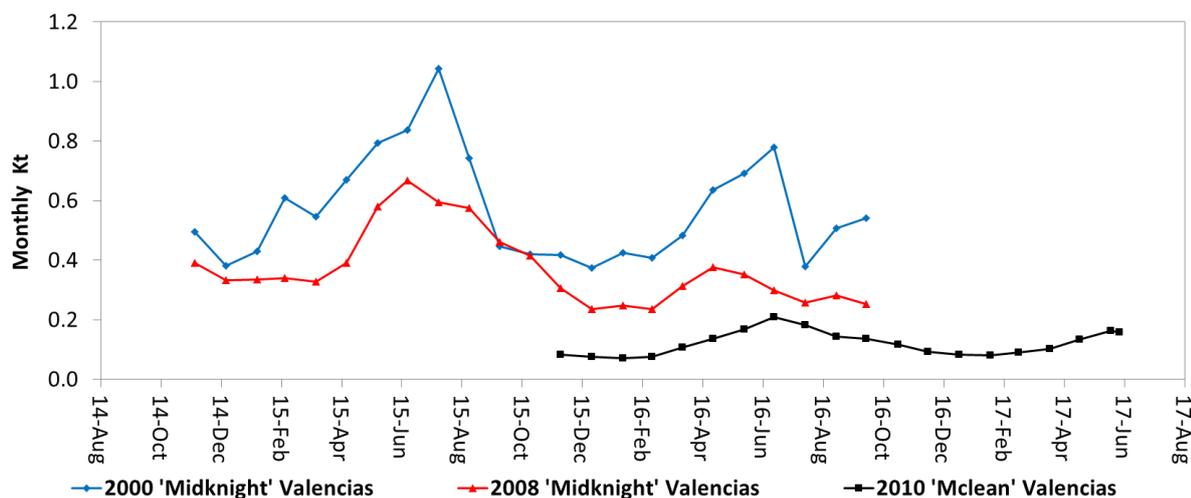


Figure 13. Monthly transpiration coefficient values for the 2010 ‘Mclean’ Valencia, 2008 and 2000 ‘Midnight’ Valencia orchards.

Transpiration was measured for 717 and 677 days for both 2000 and 2008 ‘Midnight’ Valencia and 587 days in 2010 ‘Mclean’ Valencia. Total transpiration for the measurement periods were 1295, 684 and 262 mm in 2000, 2008 and 2010 Valencia orchards respectively. On average 1.8, 1.1 and 0.45 mm day⁻¹ of water were transpired in 2000, 2008 and 2010 Valencia orchards respectively (Table 10).

Table 10. Average tree water use for the four measuring trees in 2010 'Mclean' Valencia, 2008 and 2000 'Midnight' Valencia orchards.

	2000 'Midnight' Valencia		2008 'Midnight' Valencia		2010 'Mclean' Valencia	
	mm	L	mm	L	mm	L
Transpiration						
Total	1 295	19 417	684	10 256	262	3 927
Maximum per day	3.7	54.8	2.1	31.0	0.72	10.83
Minimum per day	0.3	4.0	0.1	1.6	0.04	0.64
Average per day	1.8	27	1.1	16.1	0.45	6.68
Measurement period (days)	717		677		587	

Navel orchards: Due to equipment problems and gumming in the trees in the early stages of the project, water use in the navel orchards was only measured for 9 months in the 'Washington' navels and for 4 months in the 'Bahianinha' navels. For the period of 2014/15 season ET_o ranged between 0.65 mm day⁻¹ and 6.12 mm day⁻¹ for the period of measurements in the 'Bahianinha' navels and transpiration on the days when the maximum and minimum ET_o observed was 1.31 mm day⁻¹ and 0.65 mm day⁻¹ respectively. On the other hand, for the whole period of measurement of transpiration in the 2006 'Washington' navels ET_o ranged between 0.4 mm day⁻¹ and 8.8 mm day⁻¹ and transpiration on the days when the maximum and minimum ET_o observed was 1.04 mm day⁻¹ and 0.12 mm day⁻¹ respectively.

Daily Kt values for the navel orchards also exhibited excessive variation. Transpiration coefficient values ranged from 0.18 – 0.94 and 0.06 – 0.57 for the 1990 'Bahianinha' navels and 2006 'Washington' navels respectively. Average monthly Kt values showed a typical trend like all other citrus orchards (Figure 14) also indicating proportional water use to canopy size.

Transpiration was measured for 108 and 279 days for both 1990 'Bahianinha' navels and 2006 'Washington' navels. Total transpiration for the measurement periods were 90.25 and 200.6 mm in 'Bahianinha' and 'Washington' navels respectively. On average 0.83 and 0.7 mm day⁻¹ of water were transpired in 'Bahianinha' and 'Washington' navels respectively (Table 11).

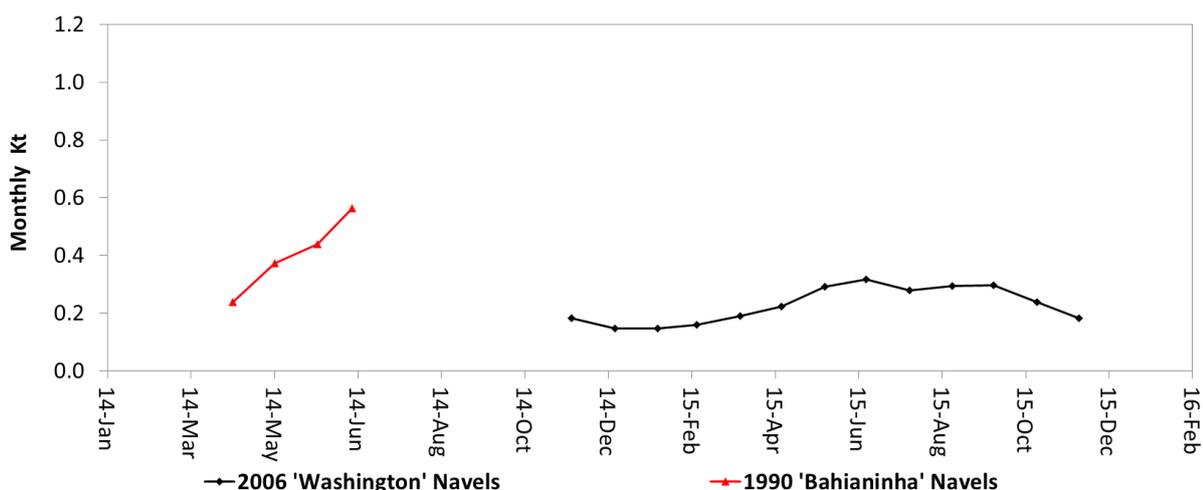


Figure 14. Monthly transpiration coefficient values for the 2006 'Washington' navel and 1990 'Bahianinha' navel orchards.

Table 11. Average tree water use for the four measuring trees in 2006 ‘Washington’ navel and 1990 ‘Bahianinha’ navel orchards.

	1990 ‘Bahianinha’ navels		2006 ‘Washington’ navels	
	mm	L	mm	L
Transpiration				
Total	90.25	1 895	200.6	3 009
Maximum per day	1.33	28	1.2	17.3
Minimum per day	0.25	5.28	0.1	0.9
Average per day	0.83	17.4	0.7	10.8
Measurement period (days)	108		279	

Mandarin orchards: For the whole period from 2015 – 2017 ET_o ranged between 0.66 mm day^{-1} and 9.81 mm day^{-1} . Transpiration in the 2002 ‘Nadorcott’ mandarin followed a typical seasonal trend and the transpiration measured when maximum and minimum ET_o observed was 3.99 mm day^{-1} and 0.41 mm day^{-1} respectively.

Daily Kt values also followed a typical seasonal trend with much day-to-day variation. Transpiration coefficient values ranged from 0.11 – 1.63 for the 2002 ‘Nadorcott’ mandarin. Average monthly Kt values showed a typical trend like all other citrus orchards (Figure 15).

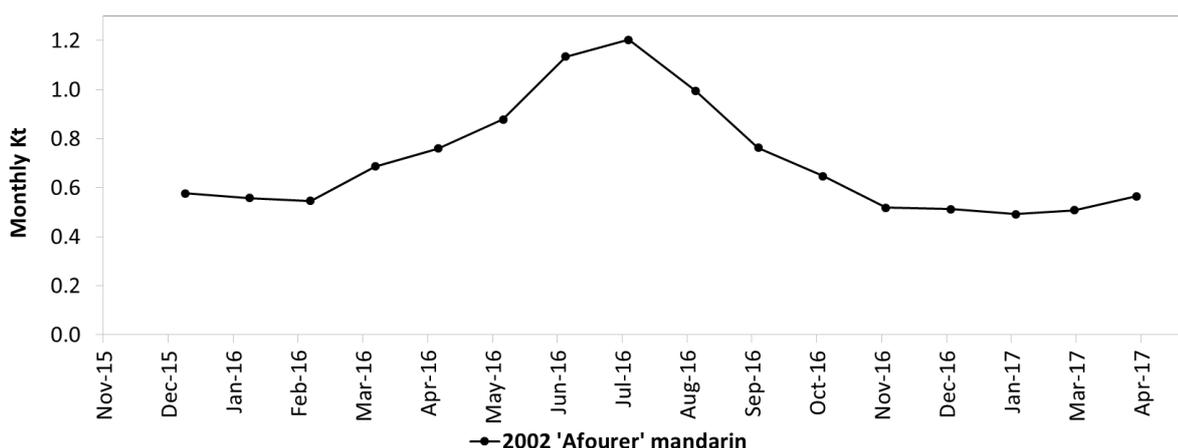


Figure 15. Monthly transpiration coefficient values for the 2002 ‘Nadorcott’ mandarin orchards.

Transpiration was measured for a period of 475 days in 2002 ‘Nadorcott’ mandarin. Total transpiration for the measurement periods were 1 324.5 mm. On average 2.8 mm day^{-1} of water was transpired in 2002 ‘Nadorcott’ mandarin (Table 12).

Table 12. Average tree water use for the four measuring trees in 2002 ‘Nadorcott’ mandarin.

	2002 ‘Nadorcott’ mandarin	
	mm	L
Transpiration		
Total	1 324.8	13 247.5
Maximum per day	4.5	44.9
Minimum per day	0.4	38.1
Average per day	2.8	27.8
Measurement period (days)	475	

The response of all six different citrus orchards in the winter rainfall region (i.e. 3 Valencia orchards, 2 navel orchards and 1 mandarin orchard) to weather variables was similar to the summer orchards. The response of transpiration to increases in solar radiation and temperature was linear in all the orchards. Unlike the summer rainfall region, the weather stations were in close proximity to the trial site hence the better relationship than the summer rainfall region. Also an order 2 polynomial relationship was observed for the response of transpiration to ET_0 and VPD. Approximately more than 77 % of the variation in transpiration was explained by changes in solar radiation in the citrus orchards. Transpiration also increased with VPD up to a certain point, with no further increase in transpiration once VPD had exceeded 3.0 kPa. A similar response was observed for ET_0 and no further increase in transpiration was observed when atmospheric demand exceeded approximately 6 mm.

Modelling citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size

Modelling radiation interception

Modelling radiation interception was done for the different species under study. The model used was developed, tested and validated by Oyarzun *et al.* (2007). It estimates the fraction of intercepted radiation (fIPAR) in row crops based on the fraction of the orchard ground that is shaded by the trees at any given time (FGs). Details of the model are provided in Deliverable 12 (Interim report on modelling citrus water use and water use efficiency, according to seasonal growth stages from planting to mature canopy size). The current deliverable present results of the model validation for the species and/ or varieties varying in canopy size. Validation was done using field measurements of hourly radiation interception taken over fourth nightly field visits in both regions over the year 2016/2017. This presented the chance to test the model with varying canopy dimensions and leaf area densities. Graphs showing the distribution of the estimated against measured data points on a one-to-one line, and statistical indices were used to illustrate the performance of the model. Model performance evaluation was carried out considering both the individual behaviour, i.e., for each species separately, and the overall result grouping all species/varieties together.

Model hourly and daily simulation in winter rainfall: The diurnal variation of the modelled and measured fIPAR. Although we had six field visits in 2016, only one data set is presented in a form of trend of estimated and measured fIPAR against time (which generally show a representative of the model diurnal pattern). The rest is presented as graphs showing the estimated against measured data points on a one-to-one line. The model generally simulated diurnal trends of hourly fraction of PAR intercepted by the canopy well (Figure 16). The general patterns were similar for all measurements sets, with minor differences observed. With the characteristic of the orchards, such negligible slope of the terrain and rows oriented nearly N-S; the model follows an expected hourly symmetrical trend with respect to solar noon with a higher fIPAR in the morning and late afternoon and minimum around midday. Discrepancies that were observed between the modelled patterns and measured values (Figure 16 C) were a consequence of a completely variable day. Under variable sky conditions, as shown by Oyarzun *et al.* (2007) the model fail simulate fIPAR when using daily inputs; which eventually affect the predictive capability of the model on hourly fIPAR.

The results obtained in the evaluation of the model performance for each species are presented in Table 13. The statistical indices achieved over the entire season were acceptable to allow for an adequate prediction of the fIPAR on both hourly and daily basis. As expected, the model did not perform well on hourly bases but it simulated daily values better. This in agreement with the dataset presented by Oyarzun *et al.* (2007), with a slightly different the absolute values from the data shown by these authors.

Table 13. Overall model performance on hourly and daily basis for the different canopy size in winter rainfall orchards. Numbers in brackets represent the planting dates.

Site	Orchard		RMSE	MAE (%)	D	r ²
Citrusdal	Maclean Valencia (2010)	Hourly	0.11	9.2	0.86	0.60
		Daily	0.02	1.5	0.98	0.98
	Midnight Valencia(2008)	Hourly	0.19	7.8	0.87	0.61
		Daily	0.03	1.57	0.96	0.91
	Midnight Valencia(2000)	Hourly	0.89	5.4	0.89	0.67
		Daily	0.03	2.14	0.97	0.93
	Nadorcott Mandarin (2013)	Hourly	0.07	5.7	0.92	0.72
		Daily	0.01	0.73	0.99	0.97
	Nadorcott Mandarin (2002)	Hourly	5.4	4.5	0.82	0.49
		Daily	0.15	1.45	0.98	0.96

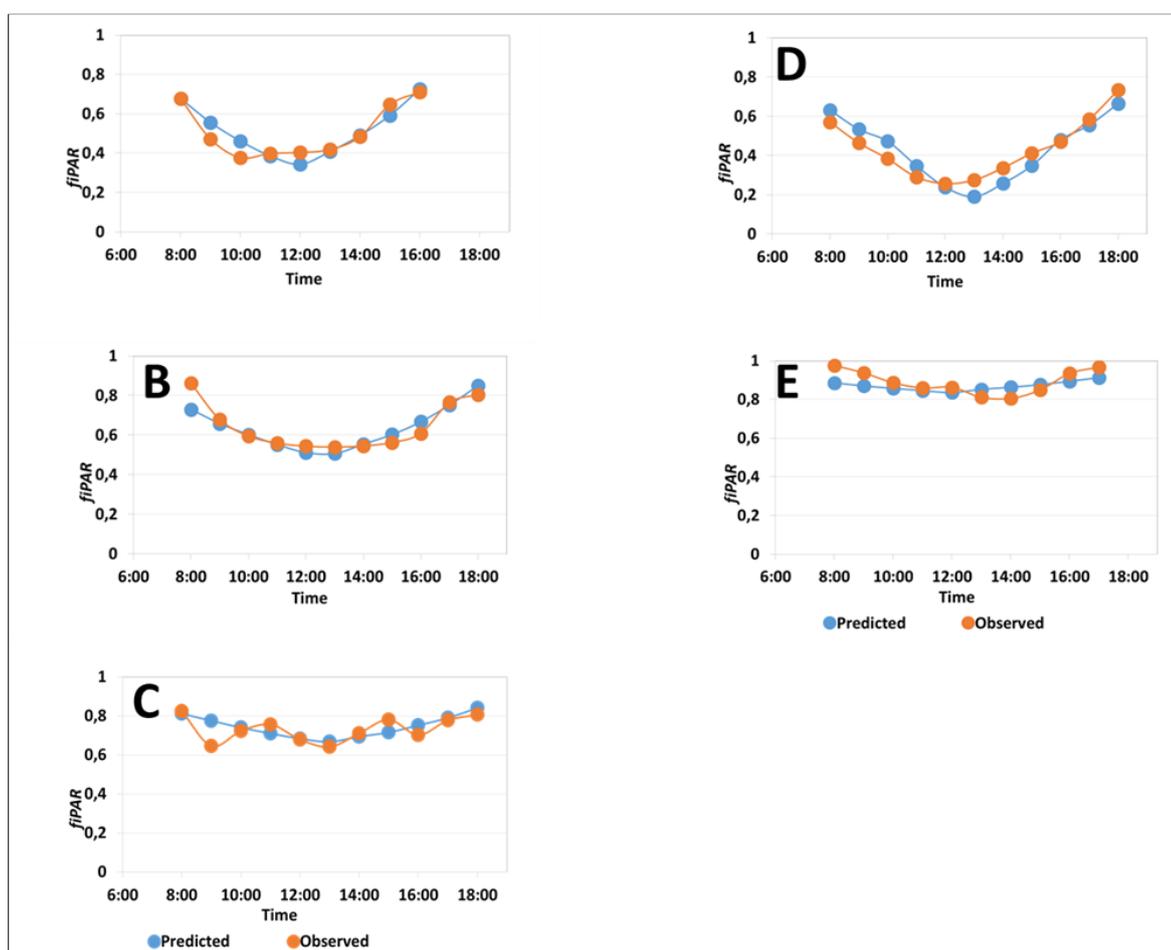


Figure 16. Diurnal variation of the fraction of PAR interception for the four different orchards: ‘Maclean’ Valencia (A), 2008 ‘Midnight’ Valencia (B), 2000 Midnight Valencia (C) 2013 ‘Nadorcott’ Mandarin (D) and 2002 ‘Nadorcott’ Mandarin (E).

In addition, when hourly values for all the different trees and days are combined, the predictive capability of the model on hourly fIPAR is satisfactory. In contrast, daily simulation for the different varieties when considered separately or combined showed a good agreement between the model and measured data (Figure 17).

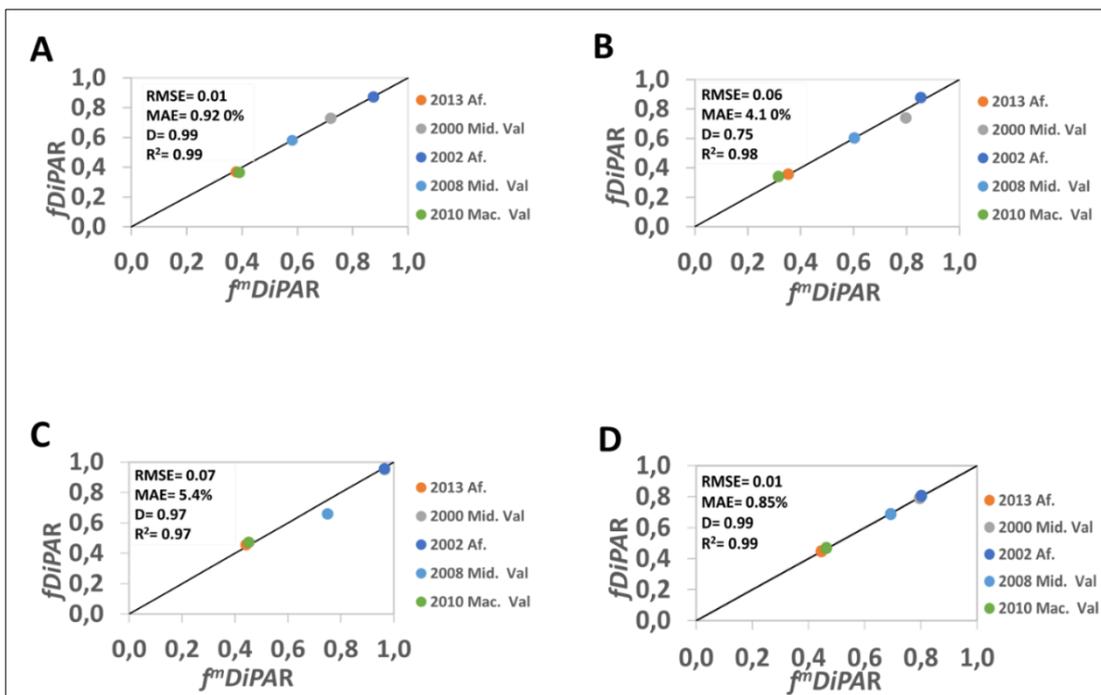


Figure 17. Comparison between field-measured ($f^m DiPAR$) and model-simulated daily fraction of PAR interception ($f DiPAR$) in summer (A), autumn (B), winter (C) and summer (D) for all the different species/varieties considered in the winter rainfall region. The solid line represents the 1:1 situation (perfect agreement).

Model hourly and daily simulation in summer rainfall region: Simulated data sets were consistent with the results observed in the winter rainfall. Hourly simulation followed the same patterns which reflects the effect of row orientation or slope of terrain. Both regions have orchards that have N-S and/or E-W orientation and negligible slope (1.5 to 3°). These parameters greatly influence the shape of hourly patterns of radiation interception. Similarly, differences between modelled and observed in diurnal trends were a result of changing sky condition. In general, hourly and daily (as seen later) predictions were satisfactory. When comparing species individual (Table 14) or grouped together (Figure 18) the statistics indices indicated an agreement between predicted and observed data. However, the model's failure to simulate well both hourly and daily $f iPAR$ in soft citrus was also observed in the summer rainfall (Table 14), possibly a result of the traits of the canopies and differences in canopy porosity measurements.

Table 14. Overall model performance on hourly and daily basis for the different canopy size in summer rainfall orchards. Numbers in brackets represent the planting dates.

Site	Orchard		RMSE	MAE (%)	D	r^2
Letsitele	Star Ruby (2011)	Hourly	0.18	14	0.45	0.4
		Daily	0.02	8.1	0.59	0.4
	Star Ruby (2010)	Hourly	0.11	8.3	0.76	0.50
		Daily	0.04	3.2	0.88	0.69
	Star Ruby (2006)	Hourly	0.06	5.1	0.98	0.56
		Daily	0.1	0.9	0.81	0.87
	Midnight Valencia(2014)	Hourly	0.08	5	0.92	0.72
		Daily	0.07	0.5	0.99	0.98
	Midnight Valencia(2008)	Hourly	0.15	12	0.60	0.20
		Daily	0.6	5	0.60	0.32
	Midnight Valencia(1995)	Hourly	0.06	5.1	0.70	0.50
		Daily	0.02	3.6	0.68	0.59
	Valley Gold (2015)	Hourly	0.05	1.1	0.50	0.61
		Daily	0.12	9.3	0.87	0.68
	Valley Gold (2013)	Hourly	0.2	15	0.30	0.15
		Daily	0.13	11	0.35	0.38

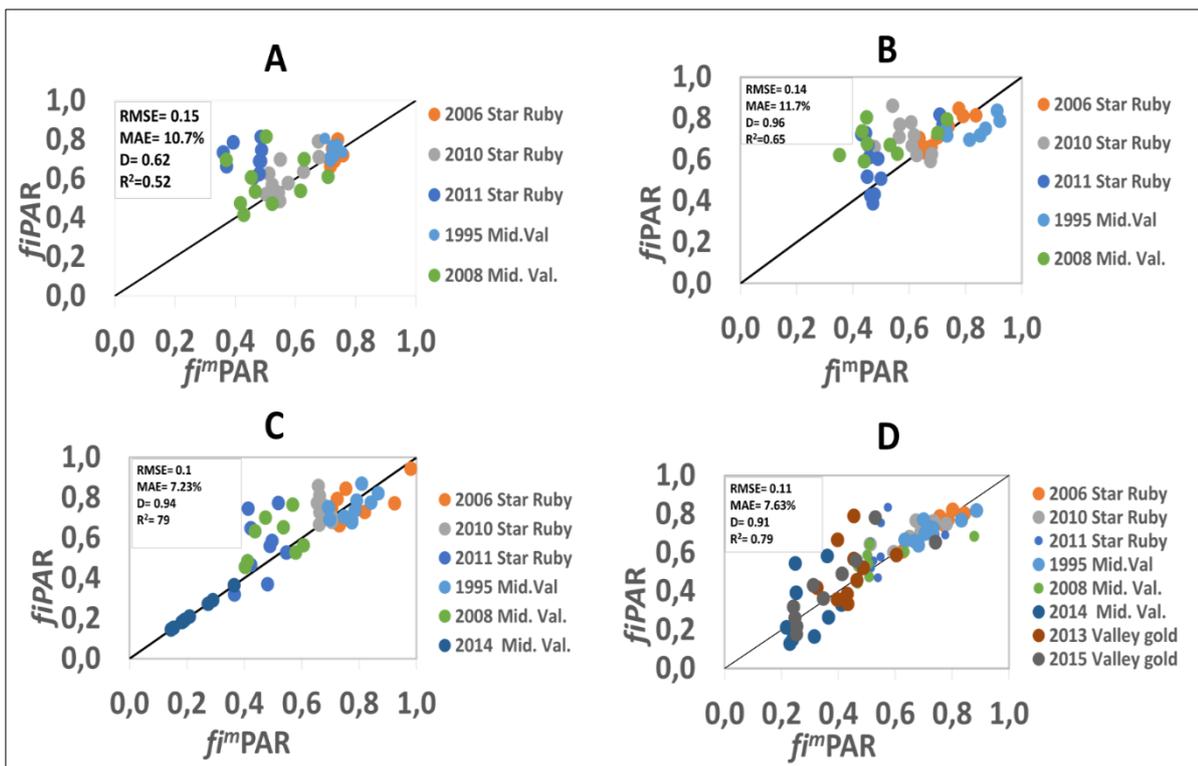


Figure 18. Comparison between field-measured (fi^mPAR) and model-simulated hourly fraction of PAR interception ($fiPAR$) in 2016: Autumn (A), winter(C) Spring (A), and Summer (D) 2017 for all the different species considered in this study. The solid line represents the 1:1 situation (perfect agreement).

Conclusion

The progress in the summer rainfall region has been steady. Full season water use data of the 1995 and 2008 ‘Midnight’ Valencia, all ‘Star Ruby’ orchards has been collected. For the ‘Valley Gold’ mandarins and 2014 ‘Midnight’ Valencia full season data will be met by mid-February 2018. Preliminary results have shown that, for a single season i.e. 2016/17 1995 and 2008 ‘Midnight’ Valencia transpired an estimate of 556 mm and 237 mm respectively. While the 2006, 2010 and 2011 ‘Star Ruby’ orchards transpired an estimate of 424 mm, 319 mm and 152 mm of water. Other parameters which will be required for better estimates of water use such as wound width, wood moisture content, and sapwood and heartwood radius will be determined upon trial termination. Evaporation and evapotranspiration measurements were also conducted which will be used to calibrate the heat ratio method. Other physiological measurements such as leaf stomatal conductance and leaf water potentials were conducted to try and explain water use dynamics of citrus trees.

For the winter rainfall region full season water use data of the 2000, 2008 ‘Midnight’ Valencia, ‘Mclean’ Valencia and 2002 ‘Nadorcott’ orchard has been collected. Due to initial challenges in equipment installations water use data were collected for some months in the navel orchards. For a single season i.e. 2016/17 ‘Nadorcott’ mandarin and ‘Mclean’ Valencia orchard transpired an estimate of 938 mm and 163 mm of water respectively. While the 2000, and 2008 ‘Midnight’ Valencia orchards transpired an estimate of 655 mm and 378 mm of water in 2015/16 season.

Measurement of citrus water use in the winter rainfall region was completed in March 2017. While full season data for all the 8 orchards in Letsitele will be obtained by mid-February 2018. In general, the water use patterns of citrus trees are similar for the summer and winter rainfall regions. Correct figures for water use in the summer rainfall region will be obtained once stem staining and wound width determination has been conducted. Other physiological measurements such as stomatal conductance, leaf water potential will be presented in the final report. Progress has also been made in the modelling of citrus tree transpiration, using a canopy conductance model (Villalobos *et al.* 2013) which requires radiation interception by the canopy and vapour pressure deficit as main input variables.

Future research

Recommendation for future research needed will be provided in the final WRC report, due August 2018.

Technology transfer

No information on grower talks and presentations at conferences, where data from this research was provided. However, it is known that the following presentation was made at the CRI Drought Management Workshop, October, 2017:

Vahrmeijer, T. 2017. Citrus water use and management. CRI Drought Management Workshop, October, Simondium.

References cited

As cited in the interim WRC report.

4.5.2 PROGRESS REPORT: Nitrogen and Potassium release from organic soil amendments over time

Project 1113 (2015/6 – 2016/7) by J.T. Vahrmeijer (CRI), CM van Heerden (UP) and E Tesfamariam (UP)

Summary

In citrus production the use of compost to improve the nutrient content and physical properties of soils is increasing. Concerns exist, however, that citrus fruit colour and quality could be affected by delayed nitrogen (N) release from the soil organic matter. Incubation studies and field trials were conducted to determine the release rates of specific nutrients from different composts (vermicompost, cattle manure, citrus waste compost, wood based compost) and its effect on fruit quality. Results from a 240 day incubation study indicated that potassium (K) is readily released from the different composts, with vermicompost (VC) having the most K released (2.67% decrease in VC K content). Compost analyses indicated that the N content of cattle manure (CM) increased from 2.19% to 2.50% and for wood based compost (WBC) from 0.25% – 0.41%, over a period of 240 days. VC and citrus waste compost (CWC) had no significant change in the N content during the 240 day incubation studies.

Field trials were conducted with different compost treatments applied in a randomised block design, during September of 2015 and 2016. At regular intervals leaf, soil and compost samples as well as leaching water from wetting front detectors were collected from the different treatment plots and analysed. Yield data from the field trial was collected in September 2017. Each treatment was harvested separately and the total yield (kg tree⁻¹) and fruit size distribution were recorded after culling. CWC yielded the highest amount of marketable fruit with a mean of 185.4 kg tree⁻¹, CM 181.4 kg tree⁻¹, VC 178.7 kg tree⁻¹, WBC 158.9 kg tree⁻¹ and the control 148.5 kg per tree. All field and incubation trials were completed and the data collected.

Opsomming

Die gebruik van kompos om die voedingstofinhoud en fisiese eienskappe van grond te verbeter is aan die toeneem in die sitrusbedryf. Daar bestaan egter die moontlikheid dat vertraagde vrystelling van stikstof (N) uit organiese materiaal vrugkleur en kwaliteit mag benadeel. Gevolglik is inkubasiestudies en 'n veldproef geloods om die vrystellingstempo van spesifieke elemente uit verskillende tipes kompos (erdwurmkompos, gekomposteerde beesmis, -sitrusafval, houtsaagsels) te bepaal asook die effek daarvan op vrugkwaliteit. Die resultate van die 240 dae lange inkubasieproef het getoon dat kalium (K) geredelik vanuit die onderskeie kompostipes vrygestel word, met erdwurmkompos (EK) wat die meeste K vrygestel het ('n daling van 2.67% in die EK se K-inhoud). Analise van die kompos het ook getoon dat die N-inhoud van beemiskompos (BM) gedurende bogenoemde inkubasieperiode toeneem van 2.19% tot 2.50%, en vir houtsaagselkompos (HS) van

0.25% tot 0.41%. Die EK en sitrusafvalkompos (SK) het geen betekenisvolle verandering in N-inhoud ondergaan nie.

Veldproewe is uitgevoer met verskillende komposbehandelings wat in 'n ewekansige blokontwerp gedurende September 2015 en 2016 toegedien is. Blaar-, grond- en komposmonsters is op gereelde tussenposes geneem, asook water wat uit die "wetting front detectors". Die monsters is reeds ontleed. Verder is oesgroottes vir die verskillende behandelings in September 2017 bepaal. Elke behandeling is apart geoes, en die totale drag (kg boom^{-1}), asook vruggrootheid-verspreiding, is bepaal. Die SK behandeling het die grootste hoeveelheid bemarkbare vrugte gehad, met 'n gemiddeld van 185 kg boom^{-1} , terwyl BM $181.4 \text{ kg boom}^{-1}$, EK $178.7 \text{ kg boom}^{-1}$, HS $158.9 \text{ kg boom}^{-1}$ en die kontrole $148.5 \text{ kg boom}^{-1}$ onderskeidelik gelewer het. Al die veldwerk en inkubasieproewe is afgehandel en die data reeds ingewin.

4.5.3 **PROGRESS REPORT: Foliar uptake of urea and micronutrients in mandarins grown under shade net in different climatic regions**

Project 1167 (2017/8 – 2018/19) by JT Vahrmeijer (CRI) and C Botha (UP)

Summary

Field experiments in mandarin orchards in the winter and summer rainfall areas: The first season's flower intensity data was collected for the summer and winter rainfall area, where the treatment groups consisted of urea only, urea and micronutrients, and a control group. In the summer rainfall area, the control treatment had visibly less flowers than those treated with urea. In the winter rainfall area, the differences between treatment groups were minimal and there was a large variation between replicates. Flower counts were done by placing a rectangular box, with specific dimensions, on the eastern and western side of the tree. Differences in flower intensity between the two sides of the trees were observed in the summer rainfall area, but interestingly not in the winter rainfall area.

Climatic data was collected in order to calculate the chill accumulation prior to the flowering period. There was a difference in chill unit accumulation in the different areas that contributes to the discrepancy in flowering intensity.

Leaf cuticle thickness was successfully measured for the treatment groups: shade net and non-shaded, young and old leaves under a light microscope with imaging software. The leaf cuticle thickness ranged from: $1.591 \mu\text{m}$ to $1.866 \mu\text{m}$ and there were also differences in the overall leaf thickness. Minimal differences were noted between the shaded and non-shaded leaf cuticle thickness. However, there are significant differences between young and old leaves. Unexpectedly, the cuticle was observed to be thicker on the abaxial side of the leaf, contradictory to various literature. To establish this, another batch of leaves were collected recently for microscope analysis. Leaves were taken for both summer and winter seasons.

Opsomming

Veld-eksperimente in mandarynboorde in die somer- en winterreënvalareas: Blomintensiteitsdata vir die eerste seisoen is ingesamel na voorblom ureum bespuitings vir beide die somer- en winterreënvalarea. Daar was drie behandelings naamlik; ureum alleenlik, ureum en mikro-elemente en 'n kontrole groep. In die somerreënvalarea het die kontrole groep sigbaar minder blomme gedra as die behandelings wat met ureum bespuit is. In die winterreënvalarea was daar nie groot verskille tussen behandelings waargeneem nie en was daar ook 'n groot variasie tussen herhalings. Die blomtellings was geneem deur 'n boks, met spesifieke afmetings, in die blaardak van die boom aan beide die ooste en weste kant van die boom te plaas. Interessant genoeg was daar verskille in die somerreënvalarea in terme van die aantal blomme, maar nie in die winterreënvalarea nie.

Klimaatdata was ook ingesamel om die akkumulering van koue-eenhede voor die blomperiode te bepaal. Die kumulatiewe koue-eenhede vir die verskillende areas verskil, wat ook bydra tot die verskille in blomintensiteite.

Die kutikula dikte van blare van die mandarynbome onder skadunette en sonder skadunette, ou en jong blare is met behulp van ligmikroskopie suksesvol gemeet met spesiale sagteware. Die kutikula dikte varieër tussen 1.591 µm en 1.866 µm. Daar was minimale verskille in die blaar kutikula dikte van bome onder skadunet en die sonder. Groot verskille is tussen die van ou en jong blare waargeneem. Interessant genoeg was die abaksiale kutikula dikker as die adaksiale kant, wat teenstrydig met sommige literatuur is. Om dit te bevestig, was nog meer blare van beide die somer en winter seisone ingesamel vir mikroskopie analise. Daar was ook verskille in die algehele blaardikte, maar daar sal meer in diepte daarna gekyk moet word met meer herhalings.

4.6 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator: Johan Joubert (CRI)

4.6.1 PROGRAMME SUMMARY

Due to high volumes of lemons in the export markets, prices have dropped considerably. We have found that fruit volumes will continue to increase with new orchards coming into production. Lemons (Eureka planted mainly) remain the mainstream competitor in the citrus industry with regard to consumer demands (including health benefits, etc.). Lemons are suitable for most citrus production areas, including the cold areas (4.6.11, 4.6.12); they perform the best compared to the other citrus varieties. The demand for lemons has decreased and as a result there are lower numbers of new plantings. The typical fruit shape and seedlessness of the lemons were crucial in the past, but good fruit quality with some seeds (seed content not a major issue) meets the consumer requirements now.

There is an increase in new mandarin development in the hotter areas with limited suitable cultivars, but the cool and intermediate citrus areas remain the best mandarin producing options (4.6.4, 4.6.6, 4.6.7, 4.6.8, 4.6.16, 4.6.17, 4.6.18, 4.6.19, 4.6.20) due to specific climatic requirements (better colour and acids). Mandarins compete with lemons with high consumer demand; the specific focus should be on low seed numbers, or completely seedless fruit, that peels easily, has good colour development and excellent flavour. The mandarin selection range varies from early to mid and late maturing with continuous new experimental selections for the future. There were good results with the mandarin selections in some hot production areas (semi-desert etc.) and future development potential is promising.

Grapefruit prices remained high (similar to 2015) due to better export quantity control that prevented the international markets from being flooded (4.6.13). Marsh performed well in niche markets, resulting in some growers still planting Marsh trees in limited numbers. The new Valencia selections performed well (4.6.2, 4.6.3, 4.6.5, 4.6.24, 4.6.25) 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 the late Valencia selections required additional research to determine the trigger as well as development of additional options (Jassie etc.).

Navel production stabilised in cooler and intermediate areas with interest towards the mid- and later maturing selections for the hotter areas to optimise the later colour development (4.6.9, 4.6.10, 4.6.21, 4.6.22, 4.6.23). Producers located in the hot production areas which are less suitable for mandarin farming, are still investing in the cooler production areas (Burgersfort and Orighstad) for optimal soft citrus production. Future evaluation sites will be located in main citrus production areas with a range of cultivars on suitable rootstocks, to offer the grower the best possible opportunity to determine what they should plant, with the lowest possible risk. Rootstock research is expanding and the importance of optimal rootstock choices for specific scion, climate and soil type as well as water quality are crucial (4.6.13). There is a range of new rootstocks (Argentinian and from USA) in the pipeline, to address the need for lemon compatibility as well as specific conditions and smaller tree volumes.

Molecular research to identify non-bearing citrus plants has concluded. This research has shown that some cultivars within a citrus type cannot be separated if they are very closely related but usually different groups of cultivars can be distinguished and certainly the different citrus types or species (4.6.31 and 4.6.32).

PROGRAMOPSOMMING

Suurlemoen volumes was baie hoog op die uitvoer markte en pryse het gevolglik drasties gedaal, uitvoer volumes gaan steeds styg met die nuwe aanplantings wat in produksie moet kom. Suurlemoene (Eureka hoofsaaklik aangeplant) bly die een hoofstroom mededinger in die sitrus bedryf wat verbruikers voorkeure

aanbetref vir verskeie redes, insluitend gesondheids voordele ens. Suurlemoene is geskik vir meeste sitrus produserende areas, ingesluit die koue areas (4.6.11, 4.6.12); presteer die beste in vergelyking met die ander sitrus varieteite. Die suurlemoen prys verlaging het tot gevolg dat nuwe aanplantings afgeneem. Die tipiese vrugvorm en saadloosheid van suurlemoene was krities in die verlede, maar goeie kwaliteit vrugte met lae saadinhoud (saadinhoud nie meer so krities nie) voldoen aan die verbruikers se voorkeure.

Daar is 'n toename in nuwe mandarin ontwikkeling in die warm produksie areas met geskikte cultivars (meer beperk en onder nette), maar die koel en intermediere sitrus areas is steeds die beste opsie vir mandaryn produksie (4.6.4, 4.6.6, 4.6.7, 4.6.8, 4.6.16, 4.6.17, 4.6.18, 4.6.19, 4.6.20) wat meer beperk word deur spesifieke klimaats vereistes. Mandaryne kompeteer nou met suurlemoene; hoë verbruikers aanvraag en die spesifieke fokus moet wees op lae saadinhoud, of total saadlose vrugte wat maklik skil met goeie kleurontwikkeling en uitstekende smaak. Die reeks mandaryn seleksies varieer van vroeg tot middel en laat rypwordend, met voortdurende nuwe seleksies vir die toekoms. Die mandaryn seleksies presteer verbasend goed in sekere warmer produksie areas (semi-woestyn ens.) met goeie uitbreidings potensiaal vir die toekoms.

Pomelo pryse was hoog (soos 2015 seisoen) wat grootliks toegekryf kan word aan beter volume beheer om te voorkom dat internasionale markte gevloed word (4.6.13). Marsh het goed presteer in nis markte wat tot gevolg gehad het dat sekere produsente weer Marsh bome aanplant (beperkte volumes). Die nuwe Valencia seleksies presteer goed (4.6.2, 4.6.3, 4.6.5, 4.6.24, 4.6.25) in die geskikte sitrus produksie areas (Letsitele), waar aanvraag vir lae saadinhoud of saadlose Valencia's met goeie opbrengste toeneem. Die probleem met hoe chimera voorkoms by die laat Valencia seleksies vereis addisionele navorsing om die oorsaak vas te stel en ook addisionele opsies te ontwikkel (Jassie ens.).

Nawel produksie stabiliseer in die koeler en intermediere areas met 'n toename in belangstelling vir die mid- en later rypwordende seleksies, gemik op die warmer produksie areas vir optimum kleurontwikkeling later in die seisoen (4.6.9, 4.6.10, 4.6.21, 4.6.22, 4.6.23). Produsente in die warm sitrus produksie streke wat minder geskik is vir mandaryn verbouing, investeer steeds in die koeler areas (Burgersfort en Orighstad) waar sagtesitrus optimaal geproduseer kan word. Toekomstige evaluasie persele sal in die belangrikste sitrus produksie areas gevestig word met die grootste variasie kultivars moontlik op geskikte onderstamme, om vir die sitrus produsent die beste moontlike geleentheid te skep om goed ingeligte besluite te kan neem oor nuwe aanplantings met laagste moontlike risiko. Onderstam navorsing word uitgebrei, die noodsaaklikheid om die optimum onderstam keuse vir 'n spesifieke bostam, klimaat, grond tipe asook waterkwaliteit te bepaal is krities (4.6.13). Daar is 'n reeks nuwe onderstamme (Argentinië en VSA) in die pyplyn om die suurlemoen verenigbaarheid aan te spreek, asook spesifieke toestande en kleiner boomvolumes.

Molekulêre navorsing om nie-draende sitrus plante te identifiseer is voltooi. Hierdie navorsing het aangedui dat sekere kultivars binne 'n sitrus tipe nie onderskei kan word wanneer hulle baie naby verwant is nie, maar gewoonlik kan verskillende kultivar groepe onderskei word en beslis verskillende sitrus tipes of spesies (4.6.31 en 4.6.32).

4.6.2 **PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)** Project 75A by J. Joubert (CRI)

Opsomming

Seleksies wat hierdie seisoen, volgens optimum rypheid van vroeg tot laat goed presteer het vir hierdie vrogte warm produksie area, is soos volg. Valearly is een van die nuwe vroeë Valencia opsies (vroeg intern ryp) wat voor Turkey inpas. Daar was 'n vertraagde kleurontwikkeling op die vrugte gewees met optimum rypheid by Valearly, maar wel dieper orange kleur wanneer opgekleur in vergelyking met meer geel by Turkey. Turkey sal dan volg, wees net versigtig om nie die seleksie te lank te hang nie. Baie skil probleme kan ontwikkel, want die optimum oes tydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees.

Delta kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer, asook McClean SL wat dan die middel van die Valencia seisoen vir hierdie area verteenwoordig. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Skilderkrans, Kobus du Toit Laat en Jassie (optimum vruggrootte verspreiding) gevolg deur Moosrivier Late 1, wat 1.0 sade per vrug produseer.

Valearly en Jassie is steeds eksperimentele/semi-kommersiele seleksies wat goed presteer. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

Summary

Selections that performed well in this season, in this hot, humid production area, according to optimal maturity from early to late, are as follows: Valearly, one of the new early maturing (internal quality) varieties matures before Turkey. There was a delayed colour development on the fruit by the time of optimum maturity with Valearly, but deeper orange colour compared to the more yellow of Turkey when fully coloured. Turkey will follow, but bear in mind that this selection has a sensitive rind. Do not allow the the fruit to hang for too long because the optimal picking period is no longer than 4-6 weeks.

Delta would follow, with good internal quality, production and fruit size, as well as McClean SL representing the middle of the Valencia season for this area. The later selections can broaden the list of choices to extend the season, commencing with Skilderkrans, Kobus du Toit Late and Jassie (optimum fruit size distribution) and followed by Moosrivier Late 1, producing 1.0 seeds per fruit.

Valearly and Jassie remain experimental/semi-commercial selections that performed well. These selections could be included in future plantings when more conclusive information becomes available.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Delta (control), Jassie, Kobus du Toit Late, McClean SL, Moosrivier Late 1, Skilderkrans, Turkey (control) and Valearly at Riverside in Malelane, Mpumalanga.

Table 4.6.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 4.6.2.2. List of Valencia selections evaluated at Riverside (Malelane) during 2017.

Selection	Rootstock	Year Planted	No. of trees
Delta	C35/CC/SC	2012	5/5/5
Jassie	C35/CC/SC	2012	5/5/5
Kobus du Toit Late	C35/CC/SC	2012	5/5/5
McClean SL	C35/CC/SC	2012	5/5/5
Moosrivier Late 1	C35/CC/SC	2012	5/5/5
Skilderkrans	C35/CC/SC	2012	5/5/5
Turkey	C35/CC/SC	2012	5/5/5
Valearly	C35/CC/SC	2012	5/5/5

Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Riverside (Malelane) during the 2017 season.

Delta (control)

Delta produced a fair to good crop on the trees, bearing fruit for the third time this season. Fruit size decreased slightly and ranged from medium to large (count 88 to 64) with good internal quality values, juice levels above 51, Brix of up to 11.2 and acids above 0.8%. Colour development on C35 and Carrizo rootstock was better compared to Swingle (delayed by two colour plates). Delta remained completely seedless and complied with the export requirements. Based on the internal quality results in Table 4.6.2.3, maturity will be from the beginning to the middle of July.

Jassie

The trees were bearing their third crop this year on all three rootstock combinations, averaging between 60 and 80 kg per tree. The fruit size varied from medium to large, count 88 to 64 (avg). The rind texture improved this season becoming smoother with time. Seed count per fruit was lower this season and varied from 1.0 to 4.6 seeds per fruit. Internal quality improved with tree age and produced better juice levels (above 52), good Brix (above 10 at maturity) and fairly high acids (above 1.1). External colour development improved and peaked at T2 with the final evaluations. Maturity seems to be end of July to middle of August based on the results in Table 4.6.2.3.

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 64) on the trees, with 1.8 seeds average. The colour development was better on C35 and Swingle compared to Carrizo. The internal quality was good, juice levels above 51%, Brix up to 11, and higher acids (above 1) for the later maturing selection. External colour peaked from T2 to 3. Maturity seems to be middle to end of July according to Table 4.6.2.3.

McClellan SL

The standard McClellan will be included in future trials as a control to compare the SL selection's performance, although McClellan developed high chimera incidences on the fruit (up to 40%) in commercial plantings. McClellan SL produced fairly round fruit with soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. Many totally seedless selections have fruit set problems and bear less fruit, but this does not appear to be the case with this cultivar. The fruit size peaked at medium-large to large (count 88-56). The internal quality was average to good with good juice levels for the trial site from 51%, Brix up to 11.4 and acceptable acid levels (0.94%). There was a slight delay in external colour ranging from T2-3. Based on the internal quality results in Table 4.6.2.3, maturity will be mid to end of July.

Moosrivier Late 1

Moos Late 1 developed a high acid level (1.1%) when the juice (above 50%) and Brix (above 10 except on C35) content were ready for harvesting at Riverside (third crop), and the external colour peaked at T2 to 3. Moos Late 1 developed 1.0 seeds (cross pollination) per fruit compared to last season's 1.8. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag. Based on the internal quality results in Table 4.6.2.3 estimated maturity for Moos Late 1 will be from the middle to the end of August.

Skilderkrans

Skilderkrans bore fruit on C35, Carrizo and Swingle at the Riverside trial site. Fruit size improved and varied from medium to large (count 88-64). Internally the Brix content was low (above 7) and the acid level of 1.1 to 1.3% indicated a later maturing Valencia selection. Juice level increased to average 51%; above the minimum required export figure. There was no delay in external colour (T2-3) on any of the rootstocks evaluated. The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season due to the low Brix and higher acid levels, delaying peak maturity to end of July and mid-August on all three rootstocks (Table 4.6.2.3).

Turkey (Control)

Fruit size decreased this season from count 88 to 56, with medium to large fruit size. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, easy fruit peeling,

and higher seed count per fruit ranging from 0.9 to 3.8 seeds per fruit. The internal colour was light yellow, and externally the fruit remained yellow up to over-matured fruit. It should be borne in mind that this selection is not a true Valencia and actually has the qualities of a mid-season orange; for instance, the exceptionally soft rag of the fruit, and the soft rind that can result in rind problems if managed incorrectly. The Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to the development of rind disorders. Based on the internal quality results in Table 4.6.2.3, estimated maturity will be middle to end of May.

Valearly

Valearly, bearing a good crop (60-80kg/tree) for the third time 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%), Brix above 10 (accept for Swingle with avg 8.0) and acid above 0.85. In comparison with the other early maturing selections, Valearly seems to be at least two weeks earlier, similar to Weipe, with good internal quality but delayed external colour ranging from T3-4. Estimated maturity according to Table 4.6.2.3, seems to be the end of April.

Conclusions

The internal quality for this season for all the selections evaluated, complied with the export standards. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selection for example Jassie. Jassie also indicated very low chimera fruit numbers on the trees, providing another good late maturing Valencia option to be included in future plantings. There was no Brix: acid ratio below 7.5:1 at peak maturity this season, which is often associated with later maturing selections having higher acid levels. When the acid levels decrease, the ratio increases. There was a better colour development with most of the selections towards peak maturity time, except in the case of Valearly, where the colour development was delayed even after peak quality. The average seed count for this season was fairly low, including Jassie and Turkey (average 3.3 and 2.3 seeds per fruit), indicating lower cross pollination in the mixed trial block. McClean SL remained completely seedless. Jassie will be one of the future possibilities to include in new Valencia plantings (optimum Valencia fruit size distribution, high juice levels, low seed counts and late maturing). Fruit size increased on the trees, between count 88 and up to count 48 on selections with lighter yields.

Table 4.6.2.3. Internal fruit quality data for Valencia and late orange selections at Riverside (Malelane) during the 2017 season.

Cultivar	Roots-tock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Delta	CC	06/06/2017	74-80	72-64	53.6	6.5	1.12	5.8	0.0	T3-4
Delta	CC	30/06/2017	76-81	72-64	52.1	8.2	1.05	7.8	0.0	T2
Delta	CC	21/07/2017	70-80	88-64	55.6	10.1	0.97	10.4	0.0	T2
Delta	C35	16/05/2017	70-76	88-72	54.0	7.6	1.04	7.3	0.0	T4
Delta	C35	30/06/2017	69-75	88-72	51.9	9.4	0.95	9.9	0.0	T2-3
Delta	C35	21/07/2017	75-81	72-64	55.8	11.2	0.84	13.3	0.0	T2
Delta	SC	16/05/2017	72-76	88-72	53.1	7.3	1.26	5.8	0.0	T3
Delta	SC	06/06/2017	75-80	72-64	53.8	7.5	1.14	6.6	0.0	T2-3
Delta	SC	30/06/2017	70-75	88-72	52.8	10.0	1.11	9.0	0.0	T2-3
Delta	SC	21/07/2017	75-80	72-64	57.4	11.1	0.88	12.6	0.0	T2-3
Jassie	CC	16/05/2017	74-81	72-64	55.6	7.4	1.25	5.9	4.1	T3
Jassie	CC	06/06/2017	70-76	88-72	52.5	11.2	1.37	8.2	3.5	T2-3
Jassie	CC	30/06/2017	77-88	72-48	54.3	7.9	1.20	6.6	2.2	T2
Jassie	CC	21/07/2017	75-80	72-64	56.3	10.7	1.15	9.3	4.0	T2
Jassie	C35	16/05/2017	71-76	88-72	56.3	10.2	1.34	7.6	1.0	T3
Jassie	C35	06/06/2017	71-77	88-72	52.7	12.6	1.32	9.5	4.6	T2-3
Jassie	C35	30/06/2017	71-80	88-64	54.3	10.8	1.16	9.4	4.3	T2

Jassie	C35	21/07/2017	76-81	72-64	57.9	11.6	1.13	10.3	3.1	T2
Jassie	SC	16/05/2017	75-80	72-64	55.2	8.6	1.50	5.7	3.5	T3
Jassie	SC	06/06/2017	74-78	72-64	54.3	12.6	1.44	8.8	4.3	T2-3
Jassie	SC	30/06/2017	75-80	72-64	52.6	10.8	1.35	8.0	2.1	T2
Jassie	SC	21/07/2017	75-80	72-64	59.1	10.4	1.25	8.3	3.1	T2
Kobus Late	CC	30/06/2017	68-74	88-72	51.2	9.6	1.04	9.3	1.2	T2-3
Kobus Late	CC	21/07/2017	75-81	72-64	53.0	10.1	0.96	10.5	2.6	T2-3
Kobus Late	C35	30/06/2017	77-81	72-64	51.2	11.2	1.15	9.7	1.5	T2
Kobus Late	C35	21/07/2017	68-71	88	56.6	10.2	0.90	11.3	2.2	T2
Kobus Late	SC	30/06/2017	70-75	88-72	53.3	9.2	1.15	8.0	2.0	T2
Kobus Late	SC	21/07/2017	76-80	72-64	53.0	10.2	0.96	10.6	1.0	T2
McClellan SL	CC	30/06/2017	71-78	88-64	51.8	7.3	1.23	6.0	0.0	T2
McClellan SL	CC	21/07/2017	71-80	88-64	57.4	7.4	0.94	7.9	0.0	T2
McClellan SL	C35	30/06/2017	74-77	72	55.5	10.4	1.15	9.0	0.0	T2-3
McClellan SL	C35	21/07/2017	71-75	88-72	58.7	11.4	1.10	10.4	0.0	T2
McClellan SL	SC	06/06/2017	77-79	72-64	50.8	8.3	1.06	7.8	0.0	T2-3
McClellan SL	SC	30/06/2017	75-79	72-64	51.6	9.6	1.10	8.7	0.0	T2
McClellan SL	SC	21/07/2017	80-85	64-56	54.1	10.8	1.05	10.3	0.0	T2-3
Moos Late 1	CC	06/06/2017	78-80	64	49.5	11.9	1.15	10.3	0.9	T3-4
Moos Late 1	CC	30/06/2017	69-75	88-72	52.9	10.0	1.40	7.1	1.5	T3
Moos Late 1	CC	21/07/2017	75-80	72-64	57.5	11.5	1.11	10.4	0.6	T2
Moos Late 1	C35	06/06/2017	78-79	64	51.4	9.9	1.35	7.3	1.4	T2-3
Moos Late 1	C35	30/06/2017	67-76	105-72	55.8	7.3	1.35	5.4	1.3	T2-3
Moos Late 1	C35	21/07/2017	74-79	72-64	59.7	7.3	1.40	5.2	0.0	T2-3
Moos Late 1	SC	06/06/2017	72-75	88-72	50.0	10.2	1.36	7.5	0.0	T3-4
Moos Late 1	SC	30/06/2017	67-75	105-72	56.7	7.3	1.36	5.4	3.1	T3-4
Moos Late 1	SC	21/07/2017	70-75	88-72	55.4	11.4	1.20	9.5	0.0	T2-3
Skilderkrans	CC	30/06/2017	71-80	88-64	42.3	7.4	1.05	7.0	0.0	T2
Skilderkrans	CC	30/06/2017	77-79	72-64	48.2	7.7	1.28	6.0	0.0	T2
Skilderkrans	CC	21/07/2017	75-81	72-64	50.6	7.7	1.01	7.6	0.0	T2
Skilderkrans	C35	06/06/2017	71-80	88-64	48.4	10.0	1.33	7.5	0.0	T2-3
Skilderkrans	C35	30/06/2017	72-79	88-64	53.3	7.4	1.15	6.4	0.0	T2-3
Skilderkrans	C35	21/07/2017	73-80	72-64	57.5	7.3	1.20	6.1	0.0	T2-3
Skilderkrans	SC	06/06/2017	76-81	72-64	51.0	9.8	1.25	7.8	0.0	T2-3
Skilderkrans	SC	21/07/2017	68-75	88-72	55.1	7.5	1.05	7.2	0.0	T2-3
Turkey	CC	28/03/2017	70-75	88-72	54.3	7.4	1.19	6.2	3.4	T6-7
Turkey	CC	20/04/2017	75-84	72-56	57.2	10.6	1.28	8.3	3.3	T5
Turkey	CC	16/05/2017	75-80	72-64	50.7	10.0	1.00	10.0	2.7	T2-3
Turkey	CC	06/06/2017	75-81	72-64	52.7	10.3	0.94	11.0	3.8	T2-3
Turkey	C35	20/04/2017	76-76	72	57.4	8.2	1.07	7.7	2.3	T5
Turkey	C35	06/06/2017	77-80	72-64	54.7	10.2	0.88	11.6	1.2	T2-3
Turkey	SC	20/04/2017	72-78	88-64	58.2	9.8	1.18	8.3	0.9	T5
Turkey	SC	16/05/2017	73-78	72-64	54.3	8.1	0.95	8.5	1.0	T3
Valearly	CC	28/03/2017	75-82	72-56	52.2	7.9	0.96	8.2	0.0	T6-7
Valearly	CC	20/04/2017	76-81	72-64	54.7	10.1	0.92	11.0	3.0	T5
Valearly	CC	16/05/2017	73-80	72-64	51.8	7.2	0.85	8.5	1.0	T3
Valearly	CC	06/06/2017	76-80	72-64	53.3	7.1	0.95	7.5	0.0	T3-4
Valearly	C35	20/04/2017	75-80	72-64	55.0	10.7	0.92	11.6	0.2	T5
Valearly	C35	16/05/2017	75-78	72-64	52.6	10.4	0.88	11.8	0.7	T2
Valearly	C35	06/06/2017	73-81	72-64	57.6	6.6	0.95	6.9	0.0	T3-4
Valearly	SC	16/05/2017	72-78	88-64	46.1	8.4	1.30	6.5	0.0	T3-4
Valearly	SC	06/06/2017	71-80	88-64	52.6	7.8	1.05	7.5	0.0	T3-4

4.6.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele & Hoedspruit)

Project 75B by J. Joubert (CRI)

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, hierdie seleksie vul Weipe aan as 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 vuggroote. Delta as kontrole pas in voor Gusocora. Gusocora en McClean SL volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings. Midnight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vuggroote, gladde skille en lae saadtellings per vrug. Du Roi is volgende met uitstekende oeste op die bome en medium tot medium/groot vrugte (telling 72 tot 56). Lavalley is huidiglik die laatste rypwordende Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vuggroote en produksie.

Daar is 'n reeks eksperimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit is. Hier volg die seleksies van vroeg, middle, tot laat rypwordend. Die seisoen kan begin word met Valearly wat meeding met Turkey as die vroegste seleksie. Du Toit Laat word meer aan die einde van die Valencia seisoen ryp met medium tot groot vuggroote, gevolg deur Skilderkrans. Laat in die seisoen kan aangevul word met Jassie en Moosrivier Late 1, soos meer inligting beskikbaar word uit verdere evaluasies.

Summary

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production areas. Recommendations have therefore been made accordingly. Valearly will start the season, an addition to Weipe as an early maturing Valencia. Turkey will follow, producing large fruit size with good internal quality and soft fibre. The optimal picking window will be within the first four weeks of peak maturity. Bennie 1 and 2 follow after Turkey with good production and medium to large fruit size. Delta as a control fits in before Gusocora. Gusocora and McClean SL follows next with completely seedless fruit and very good Brix: acid ratios. Midnight 1 and 2 cover the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Du Roi follows with excellent crop on the trees and medium to medium-large fruit size (count 72 to 56). Valencia Late and Lavalley are currently the latest maturing Valencia selections that are being planted commercially, developing excellent fruit size and yield.

A series of experimental/semi-commercial selections has also been included in the hot production areas. The selection range follows from early, mid-, to late-maturing options. The season starts with Valearly, competing with Turkey to be the earliest maturing Valencia. Kobus Du Toit late mature more towards the end of the Valencia season with medium to large fruit size; followed by Skilderkrans. Late in the season; Jassie with optimum fruit size and good internal quality as well as Moosrivier Late 1 could possibly be added to the options, when more information becomes available from future evaluations.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 1&2, Delta, Du Roi, Kobus Du Toit Late, Gusocora, Jassie, Lavalley 1, McClean, McClean SL, Midnight 1, 2, Moosrivier Late 1, Skilderkrans, Turkey, Valearly and Val Late at Bosveld Citrus (Letsitele), Groep 91 (Letsitele) and Moriah Citrus (Hoedspruit).

Table 4.6.3.1. List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2017 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
McClellan SL	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	2011
Val Late	SC	2009

Table 4.6.3.2. List of Valencia selections evaluated at Groep 91 (Letsitele) during the 2017 season.

Selection	Rootstock	Planted
Bennie 1	CC/SC	2006
Benny 2	CC	2006
McClellan	SC/Sunki 812	2013
McClellan SL	SC/Sunki 812/X639	2013
Turkey	C35	2013

Table 4.6.3.4. List of Valencia selections evaluated at Moriah Citrus (Hoedspruit) during the 2017 season.

Selection	Rootstock	Top-worked
Bennie 2	MxT	2011
Gusocora	MxT	2011
Lavalle 1	MxT	2011
Midknight 1 (I15)	MxT	2011
Midknight 2 (F17)	MxT	2011

Results and discussion

Alpha

Fruit production on the Alpha trees improved this season to 80 kg per tree. Alpha was planted on C35 and Swingle at the Bosveld trial site to compare tree development (vigour) and yield production (trees on C35 almost a third smaller). The internal quality was very good, juice levels peaked at 56%, Brix was above 10 and acids were fairly high (between 1.2 and 1.4%). Fruit size decreased slightly and varied from count 72 to 64, still excellent for Valencia production and export. External colour peaked from T2 to T4. Maturity seems to be end of June to middle of July (Table 4.6.3.5).

Bennie 1 and 2

Bennie was evaluated at all three trial sites this season: Bosveld, Groep 91 and Moriah. There was a good crop on the Benny trees at Group 91; with the remaining drought conditions and severe high temperatures. There was a good crop on both selections and fruit size peaked between count 88 and 56 (very good for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Benny 1 and 2 internally produced similar juice levels

(average 50%), Brix (average 11.0), acid (1.5%) and seed counts (average 4.5 seeds per fruit). External colour on both selections by the time of harvest varied between T2 and T3 (better colour on SC this season). Based on ratios, Benny 1 and 2 mature end of June to beginning of July (Table 4.6.3.5).

Delta (control)

Delta on all three rootstocks (C35, CC, SC), as control variety, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 88 and 56 with good internal quality, reaching juice levels of 57%, Brix of 12.9 and acid content of 1.0%. The external colour of the fruit was between T2 and T4. Maturity is middle to the end of June (Table 4.6.3.5).

Du Roi

Du Roi was planted on two rootstocks, C35 and Swingle at the Bosveld trial, and for this season the Swingle combination was evaluated as a control selection due to C35's severe susceptibility to Blight in the Letsitele production area. There was a good yield on both combinations and fruit size peaked between count 72 and 56. The external colour peaked between T2 and T3 and the average seed count was 2.2 seeds per fruit. Swingle developed a juice content of 55%, Brix of 11.8 and acids of 1.3%. Maturity is end of July to middle August (Table 4.6.3.5).

Kobus Du Toit Late

Kobus Du Toit Late was evaluated at the Bosveld trial site on three rootstocks (C35, CC, SC) and produced small, medium and large fruit size (count 105 to 56) on the trees, with 2.9 seeds average. The colour development was better on C35 and Carrizo compared to Swingle. The internal quality was good, juice levels above 50%, Brix up to 12.9, and higher acids (above 1) for the later maturing selection. External colour peaked from T2 to 4. Maturity seems to be middle to end of July to middle August according to Table 4.6.3.5.

Gusocora

Gusocora was evaluated at Moriah and Bosveld Citrus this year on MxT and Swingle rootstocks. The fruit was completely seedless and developed a good internal quality where juice (56%), Brix (12) and acid (1.1) complied with export requirements. The external colour varied from T2 to T4, correlating with the internal quality and Brix:acid ratio of 10. Fruit size was smaller and peaked between counts 88 and 64, optimal fruit size for export Valencias (medium to large). There was a good crop on the trees, bearing in mind that Swingle as well as MxT rootstocks induce good yields and internal quality. It is apparent that Gusocora's maturity is middle to end of July (Table 4.6.3.5).

Jassie

Fruit size at Bosveld on Carrizo, C35 and Swingle was bigger (peaked between count 88 and 56) compared to Mahela (semi-commercial block) with count 88 and 64. Production was good on all the rootstock combinations. Internal quality was good with juice levels of 60%, Brix up to 13 and average acid levels of 1.2% (average). Seed count was lower this year and varied from 1.0 to 5.9 (avg. 3.3) seeds per fruit. Fruit shape was round, with a smooth rind texture, internal colour was light yellow, and juice flavour was good. Fibre strength was fairly soft, rind thickness was medium and the fruit peeled easily. Jassie bore high numbers of fruit inside the tree (good quality and colour). Maturity is middle of July to beginning of August in this area (Table 4.6.3.5).

Lavalle 1

Lavalle was evaluated on both Swingle and MxT rootstock this season. There was a decrease in seed production this season and Lavalle produced 0.4 seeds per fruit compared to last year's seedless fruit (cross pollination). The internal quality complied with export requirements and acid level was above 1.2% at the second evaluation at the middle of July. Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season (seen only 2013). From the ratio on this date it is apparent that Lavalle 1 maturity is end of August to middle of September (Table 4.6.3.5).

McClellan and McClellan SL

McClellan was planted and evaluated this season on MxT and Sunki 812 to compare with McClellan SL. McClellan SL was planted on Carrizo and Swingle at the Bosveld trial site and Swingle, X639 and Sunki 812 at Groep 91, with a good crop production and remained completely seedless similar to all the other trial sites where the selection was included. Fruit quality was poor to average at the Groep 91 site due to trees bearing their first crop; this will improve next season. Fruit size peaked from count 88 to 56 (excellent for Valencia production). External colour varied from T2-4. At the Bosveld site; juice was above 50%, Brix improved to as high as 13.5 (over mature) and acids was above 1.0% (peak maturity) towards the end of the season, resulting in good Brix:acid ratios (above 12:1). Maturity seems to be end of June to middle of July (Table 4.6.3.5).

Midnight 1 & 2

Midnight 1 and 2 bore an average to good yield of between 80 and 100 kg per tree on the two rootstock combinations where MxT was the smallest. The fruit size varied between count 88 and 56, juice content was around 50%, Brix levels lower around 12 (peak maturity) and acids around 1.1%. Midnight 1 outperformed Midnight 2 with a better Brix level. Midnight 1 and 2 developed low seed numbers in the fruit, ranging from 0.5 up to 1.6 seeds per fruit. The characteristic Midnight die-back was more visible on Midnight 1 compared to Midnight 2. Fruit shape was round, rind texture was fairly smooth, and fruit was raggy with a medium rind thickness which peeled moderately. Maturity seems to be middle of July to the end of July (Table 4.6.3.5).

Moosrivier Late 1

This season Moos Late 1 developed a wider fruit size distribution in general (small, medium to large size count 125-64) on Carrizo and C35, with medium to large fruit on Swingle (count 88 to 64). Crop production for Moos Late 1 was good. Moos Late 1 performed well on Swingle, developing internal qualities that met export standards and high acids (up to 1.22% towards peak maturity) indicating a late maturing Valencia selection. The fruit quality on Carrizo and C35 average with lower juice content but excellent Brix (up to 13.1). The seed count per fruit varied from 1.6 to 3.3 (similar to 2016). When internal quality was taken into consideration; estimated maturity end of July to middle August. (Table 4.6.3.5).

Skilderkrans

Skilderkrans at Group 91 will be the back-up site (not evaluated) and the trial block at Bosveld bore fruit on C35, Carrizo and Swingle. Fruit size varied from medium to large-extra large (count 72-40). Internally the Brix content was good (up to 12.6) and the acid level of 1.0 to 1.3% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to average 54.1% later in the season; above the minimum required export figure. There was no delay in external colour 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 higher acid levels except on Carrizo, delaying peak maturity to end of July and mid-August on Swingle and C35 (Table 4.6.3.5).

Turkey

Turkey was planted on three rootstocks: Carrizo, Swingle and C35, to determine the compatibility status. All three combinations performed well; yield was the best in combination with Carrizo relative to tree size. Fruit size distribution ranged from medium and large (count 88-64), good Brix content (average 10.6), lower acid levels and higher Brix:acid ratio. The average seed count per fruit increased with 0.3 and peaked at 3.0 seeds. The external colour (between T2 and T4) at the end of May was similar on all three rootstocks. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 developed the smallest tree size (2.5 m) in combination with Turkey. The Swingle trees were declining and die-back was visible. Based on the ratios, maturity will be end of May to middle June (Table 4.6.3.5).

Valearly

Valearly, bearing an average to good crop for the third time on the trees, was completely seedless this season. The internal quality of the fruit was good with juice levels above 52% later in the season, lower Brix on Carrizo (avg. 9) compared to C35 (11.4) and acid of 0.84. In comparison with the other early maturing selections (Turkey and Weipe), Valearly seems to be at least two weeks earlier, similar to Weipe, with slightly delayed external colour development. Estimated maturity according to Table 4.6.3.5 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 (60 to 80 kg per tree) and fruit size peaked from medium to large (count 88 to 64), optimal Valencia export quality. Acid levels were above 1.1% when the second evaluation was completed, indicating the late maturity qualities of the selection. The juice content improved this season to 56% and Brix 11 with last evaluation. Seed count went down from 3.1 seeds per fruit to 1.8. Maturity will be late in the season and according to Table 4.6.3.5, peak middle to end of August.

Conclusion

Alpha performed similarly to the 2017 evaluation, developing a good crop on the trees. The internal quality was good (juice levels lower) and fruit size peaked between counts 72 to 64 (dropped one count size).

Bennie 1 and 2 produced similar fruit qualities this season, as well as yield production and small to large fruit size (peaked from count 88 to 56). Delta was the control variety for the trial; fruit size peaked between count 88 and 56 with good internal quality, reaching juice levels of 57%, Brix of 12.9 and acid content of 1.0%.

Du Roi was evaluated on Swingle this season with bigger fruit size ranging from count 72 to 56 (similar to 2015 season). Kobus Du Toit Late performed well with good fruit size and promising juice and Brix levels. Gusocora performed well on Swingle and MxT, meeting the export standards (acid levels improved compared to 2016).

Jassie produced an excellent internal quality (high Brix and acid) on Carrizo and Swingle, with small to large fruit size (count 88-64) due to the cropload on the trees. Lavalley 1 was ultra-late, maturing in August/September (acid above 1.7%) on MxT rootstock.

McClellan SL remained completely seedless at the trial site with good internal quality and optimum fruit size (count 88-56).

Fruit quality on Midnight 1 was better, with higher Brix than Midnight 2. External colour was delayed on Midnight 2 this season. Normally, Swingle produces higher acids and develops delayed external colour compared to MxT, but with the Midnight 1 selection the opposite seems to be true.

Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered. Valearly produced a light crop on the trees and matures two weeks earlier compared to Turkey. Future evaluations will determine the value of this cultivar for the citrus industry.

Table 4.6.3.5. Internal fruit quality data for Valencia orange selections at Bosveld Citrus (Letsitele), Groep 91 (Letsitele) and Mahela (Letsitele) and Moriah Citrus (Hoedspruit) during the 2017 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	SC	06/06/2017	Bosveld Citrus	73-80	72-64	56.0	7.7	1.40	5.5	0.7	T3-4
Alpha	SC	05/07/2017	Bosveld Citrus	77-80	72-64	50.5	10.9	1.20	9.1	3.3	T2-3
Benny	SC	05/07/2017	Bosveld Citrus	79-85	64-56	58.3	10.8	1.08	10.0	4.0	T2
Bennie 1	CC	04/05/2017	Group 91	75-83	72-56	53.0	10.8	1.35	8.0	5.2	T3
Bennie 1	SC	04/05/2017	Group 91	73-79	72-64	52.8	10.4	1.55	6.7	6.0	T3
Bennie 1	MxT	14/07/2017	Moriah	77-80	72-64	54.2	11.8	1.79	6.6	1.3	T2

Bennie 2	CC	04/05/2017	Group 91	70-75	88-72	50.2	10.9	1.50	7.3	4.9	T3
Bennie 2	MxT	06/06/2017	Moriah	77-80	72-64	55.2	11.2	1.57	7.1	4.0	T2-3
Bennie 2	MxT	23/06/2017	Moriah	70-74	88-72	51.9	11.1	1.50	7.4	6.0	T2
Delta	CC	26/05/2017	Bosveld Citrus	71-77	88-72	49.8	11.0	1.28	8.6	0.0	T2
Delta	CC	22/06/2017	Bosveld Citrus	74-82	72-56	50.7	11.7	1.10	10.6	0.0	T2
Delta	CC	13/07/2017	Bosveld Citrus	80-85	64-56	55.7	11.1	0.95	11.7	0.0	T2-3
Delta	CC	03/08/2017	Bosveld Citrus	68-70	88	57.1	12.1	0.99	12.2	0.0	T2-3
Delta	CC	22/08/2017	Bosveld Citrus	70-75	88-72	55.6	12.5	0.83	15.2	0.0	T2
Delta	C35	26/05/2017	Bosveld Citrus	73-80	72-64	49.8	9.9	1.22	8.1	0.0	T3
Delta	C35	22/06/2017	Bosveld Citrus	82-85	56	50.8	11.1	0.99	11.2	0.0	T2-3
Delta	C35	13/07/2017	Bosveld Citrus	72-75	88-72	40.7	12.5	1.90	6.6	0.0	T2-3
Delta	C35	03/08/2017	Bosveld Citrus	82-85	56	45.7	12.6	1.45	8.7	0.0	T2-3
Delta	C35	22/08/2017	Bosveld Citrus	76-81	72-64	48.1	12.9	1.60	8.1	0.0	T2
Delta	SC	04/05/2017	Bosveld Citrus	68-75	88-72	52.2	8.2	1.20	6.8	0.0	T4
Delta	SC	06/06/2017	Bosveld Citrus	72-75	88-72	55.2	7.5	1.12	6.7	0.0	T3-4
Delta	SC	22/06/2017	Bosveld Citrus	74-80	72-64	53.0	9.9	1.05	9.5	0.0	T2-3
Delta	SC	05/07/2017	Bosveld Citrus	73-75	72	47.3	11.2	1.03	10.9	0.0	T3-4
Delta	SC	13/07/2017	Bosveld Citrus	74-85	72-56	50.3	11.6	1.68	6.9	0.0	T3-4
Delta	SC	03/08/2017	Bosveld Citrus	71-78	88-64	55.1	12.6	1.18	10.7	0.0	T2
Delta	SC	22/08/2017	Bosveld Citrus	83-85	56	50.3	12.0	1.10	11.0	0.0	T2
Du Roi	SC	06/06/2017	Bosveld Citrus	79-83	64-56	57.9	8.3	1.40	5.9	2.7	T2-3
Du Roi	SC	05/07/2017	Bosveld Citrus	73-80	72-64	55.7	11.8	1.24	9.5	1.7	T2
Kobus Late	CC	04/05/2017	Bosveld Citrus	65-75	105-72	51.5	8.4	1.37	6.1	3.3	T3
Kobus Late	CC	26/05/2017	Bosveld Citrus	70-75	88-72	51.5	10.3	1.15	9.0	4.3	T3
Kobus Late	CC	13/07/2017	Bosveld Citrus	70-75	88-72	51.7	12.4	1.23	10.1	4.8	T2
Kobus Late	CC	03/08/2017	Bosveld Citrus	70-75	88-72	60.3	12.9	1.18	10.9	1.4	T2
Kobus Late	CC	22/08/2017	Bosveld Citrus	70-74	88-72	51.3	12.8	1.07	12.0	1.8	T2

Kobus Late	C35	26/05/2017	Bosveld Citrus	57-72	88	50.0	11.1	1.53	7.3	3.5	T2
Kobus Late	C35	22/06/2017	Bosveld Citrus	65-67	105	52.0	11.7	1.54	7.6	3.8	T3-4
Kobus Late	C35	03/08/2017	Bosveld Citrus	67-74	105-72	50.1	12.7	1.42	8.9	2.6	T2-3
Kobus Late	C35	22/08/2017	Bosveld Citrus	67-73	105-72	52.6	12.7	1.13	11.3	1.3	T2-3
Kobus Late	SC	26/05/2017	Bosveld Citrus	68-71	88	52.1	7.4	1.44	5.1	2.0	T3
Kobus Late	SC	22/06/2017	Bosveld Citrus	66-72	105-88	55.1	11.4	1.46	7.8	4.7	T2-3
Kobus Late	SC	13/07/2017	Bosveld Citrus	69-79	88-64	50.7	11.6	1.53	7.6	4.0	T3-4
Kobus Late	SC	03/08/2017	Bosveld Citrus	71-76	88-72	59.6	11.7	1.02	11.5	2.7	T2-3
Kobus Late	SC	22/08/2017	Bosveld Citrus	71-79	88-64	51.7	12.9	1.14	11.3	0.0	T2
Gusocora	SC	06/06/2017	Bosveld Citrus	70-77	88-72	56.8	8.5	1.09	7.8	0.0	T3-4
Gusocora	SC	05/07/2017	Bosveld Citrus	69-72	88	50.9	12.0	0.90	13.3	0.0	T3-4
Gusocora	MxT	06/06/2017	Moriah	73-76	72	50.1	10.0	1.23	8.1	0.0	T2-3
Gusocora	MxT	14/07/2017	Moriah	70-74	88-72	53.7	10.3	1.26	8.2	0.0	T2
Gusocora	MxT	04/08/2017	Moriah	72-79	88-64	54.6	11.7	1.10	10.6	0.0	T2
Jassie	CC	26/05/2017	Bosveld Citrus	72-76	88-72	50.4	11.1	1.32	8.4	5.9	T2
Jassie	CC	22/06/2017	Bosveld Citrus	76-80	72-64	57.4	11.3	1.10	10.3	3.8	T3
Jassie	CC	13/07/2017	Bosveld Citrus	74-77	72	57.3	13.0	1.02	12.7	0.0	T2
Jassie	CC	03/08/2017	Bosveld Citrus	75-80	72-64	55.8	12.4	0.93	13.3	2.3	T2-3
Jassie	CC	22/08/2017	Bosveld Citrus	74-79	72-64	57.8	12.8	0.87	14.7	0.0	T2
Jassie	C35	26/05/2017	Bosveld Citrus	68-71	88	51.4	12.3	1.73	7.1	5.6	T4
Jassie	C35	22/06/2017	Bosveld Citrus	71-74	88-72	57.2	12.6	1.50	8.4	5.6	T2
Jassie	C35	13/07/2017	Bosveld Citrus	73-75	72	48.3	12.8	1.32	9.7	3.7	T2
Jassie	C35	03/08/2017	Bosveld Citrus	69-76	88-72	60.5	13.3	1.17	11.4	2.6	T2
Jassie	C35	22/08/2017	Bosveld Citrus	74-78	72-64	54.0	12.9	1.08	12.0	5.2	T2
Jassie	SC	26/05/2017	Bosveld Citrus	70-75	88-72	53.3	10.0	1.27	7.9	2.5	T3
Jassie	SC	22/06/2017	Bosveld Citrus	68-76	88-72	56.4	11.3	1.15	9.8	3.2	T2
Jassie	SC	13/07/2017	Bosveld Citrus	70-75	88-72	49.3	12.3	1.19	10.4	2.3	T2

Jassie	SC	03/08/2017	Bosveld Citrus	74-79	72-64	60.5	12.7	1.16	10.9	4.3	T2-3
Jassie	SC	22/08/2017	Bosveld Citrus	77-82	72-56	58.2	12.7	1.05	12.2	0.0	T2
Lavalle	SC	06/06/2017	Bosveld Citrus	82-86	56-48	56.2	8.4	1.36	6.2	0.7	T3-4
Lavalle	SC	05/07/2017	Bosveld Citrus	74-82	72-56	54.5	10.8	1.20	9.0	0.0	T2
Lavalle	MxT	06/06/2017	Moriah	77-82	72-56	57.3	10.3	1.69	6.1	0.0	T2-3
Lavalle	MxT	23/06/2017	Moriah	71-80	88-64	45.7	11.7	1.96	6.0	0.0	T2-3
Lavalle	MxT	14/07/2017	Moriah	74-80	72-64	58.1	11.5	1.73	6.6	0.8	T2
Lavalle	MxT	04/08/2017	Moriah	71-78	88-64	64.4	11.8	1.79	6.6	0.9	T2
McClea n	Sunki 812	05/07/2017	Group 91	72-75	88-72	58.5	10.0	1.38	7.2	1.5	T2
McClea n	SC	05/07/2017	Group 91	71-73	88-72	57.1	11.3	1.15	9.8	2.3	T2
McClea n SL	CC	22/06/2017	Bosveld Citrus	80-87	64-48	51.3	9.8	1.10	8.9	0.0	T2-3
McClea n SL	CC	13/07/2017	Bosveld Citrus	70-79	88-64	50.7	11.9	1.25	9.5	0.0	T2-3
McClea n SL	CC	03/08/2017	Bosveld Citrus	76-83	72-56	54.7	12.7	0.82	13.5	0.0	T2-3
McClea n SL	CC	22/08/2017	Bosveld Citrus	80-85	64-56	51.5	12.6	0.96	13.1	0.0	T2
McClea n SL	SC	26/05/2017	Bosveld Citrus	72-77	88-72	50.0	10.4	1.37	7.6	0.0	T2
McClea n SL	SC	06/06/2017	Bosveld Citrus	75-77	72	57.4	7.8	1.24	6.3	0.0	T3-4
McClea n SL	SC	22/06/2017	Bosveld Citrus	74-80	72-64	57.3	9.3	1.13	8.2	0.0	T2
McClea n SL	SC	13/07/2017	Bosveld Citrus	71-76	88-72	50.5	13.0	1.35	9.6	0.0	T2-3
McClea n SL	SC	03/08/2017	Bosveld Citrus	74-82	72-56	61.5	12.6	1.16	10.9	0.0	T2
McClea n SL	SC	22/08/2017	Bosveld Citrus	74-80	72-64	56.1	12.3	0.91	13.5	0.0	T2
McClea n SL	SC	05/07/2017	Group 91	79-80	64	43.6	8.7	0.90	9.7	0.0	T2-3
McClea n SL	Sunki 812	05/07/2017	Group 91	73-80	72-64	54.3	9.5	1.05	9.0	0.0	T2
McClea n SL	X639	05/07/2017	Group 91	70-77	88-72	52.3	9.6	1.03	9.4	0.0	T2
Midknig ht 1	SC	06/06/2017	Bosveld Citrus	73-80	72-64	53.7	8.2	1.06	7.7	0.0	T3-4
Midknig ht 1	SC	05/07/2017	Bosveld Citrus	75-80	72-64	52.9	12.2	1.05	11.6	0.5	T2
Midknig ht 1 (I15)	MxT	06/06/2017	Moriah	76-78	72-64	50.6	10.1	1.24	8.1	0.0	T3
Midknig ht 1 (I15)	MxT	23/06/2017	Moriah	75-80	72-64	51.0	12.0	1.12	10.8	0.0	T2

Midknig ht 1 (I15)	MxT	14/07/2017	Moriah	74-81	72-64	53.9	11.6	0.95	12.2	1.6	T2
Midknig ht 1 (I15)	MxT	04/08/2017	Moriah	76-80	72-64	51.0	12.6	1.17	10.8	0.0	T2-3
Midknig ht 2	SC	06/06/2017	Bosveld Citrus	75-85	72-56	53.6	8.7	1.14	7.6	0.0	T3-4
Midknig ht 2	SC	05/07/2017	Bosveld Citrus	75-80	72-64	50.8	9.0	1.17	7.7	0.0	T2
Midknig ht (F17)	MxT	23/06/2017	Moriah	68-70	88	52.5	8.1	1.32	6.1	1.2	T2-3
Midknig ht (F17)	MxT	14/07/2017	Moriah	75-80	72-64	53.4	10.2	1.39	7.3	0.0	T2-3
Moos Late 1	CC	22/06/2017	Bosveld Citrus	67	105	53.0	12.7	1.77	7.2	2.3	T3
Moos Late 1	CC	13/07/2017	Bosveld Citrus	65-70	105-88	50.1	13.0	1.64	7.9	2.3	T2
Moos Late 1	CC	03/08/2017	Bosveld Citrus	73-79	72-64	50.6	13.1	1.61	8.1	1.6	T2
Moos Late 1	CC	22/08/2017	Bosveld Citrus	71-78	88-64	50.0	12.6	1.29	9.8	3.5	T2-3
Moos Late 1	C35	26/05/2017	Bosveld Citrus	55-70	125-88	51.7	11.2	1.83	6.1	2.5	T2
Moos Late 1	C35	22/06/2017	Bosveld Citrus	67-75	105-72	52.2	12.5	1.66	7.5	3.1	T2
Moos Late 1	C35	13/07/2017	Bosveld Citrus	73-79	72-64	53.1	12.5	1.55	8.1	2.6	T2
Moos Late 1	C35	03/08/2017	Bosveld Citrus	70-80	88-64	55.6	12.8	1.40	9.1	2.5	T2
Moos Late 1	SC	22/06/2017	Bosveld Citrus	70-75	88-72	51.3	11.8	1.65	7.2	2.2	T3-4
Moos Late 1	SC	13/07/2017	Bosveld Citrus	75-80	72-64	50.7	11.9	1.23	9.7	1.0	T2-3
Moos Late 1	SC	03/08/2017	Bosveld Citrus	70-80	88-64	59.9	12.5	1.22	10.2	2.2	T2
Skilderkr ans	CC	13/07/2017	Bosveld Citrus	85-90	56-40	45.5	10.7	1.00	10.7	0.0	T2
Skilderkr ans	CC	03/08/2017	Bosveld Citrus	80-89	64-48	60.7	11.4	0.93	12.3	0.0	T2
Skilderkr ans	CC	22/08/2017	Bosveld Citrus	80-85	64-56	54.6	11.7	1.00	11.7	0.0	T2-3
Skilderkr ans	C35	22/06/2017	Bosveld Citrus	70-72	88	45.6	11.5	1.70	6.8	1.8	T2-3
Skilderkr ans	C35	13/07/2017	Bosveld Citrus	76-83	72-56	57.3	12.2	1.44	8.5	3.0	T2
Skilderkr ans	C35	03/08/2017	Bosveld Citrus	80-85	64-56	59.9	12.3	1.51	8.1	0.0	T2-3
Skilderkr ans	C35	22/08/2017	Bosveld Citrus	75-80	72-64	54.4	12.6	1.40	9.0	1.3	T2-3
Skilderkr ans	SC	13/07/2017	Bosveld Citrus	85-90	56-40	45.3	10.5	1.18	8.9	0.0	T2
Skilderkr ans	SC	03/08/2017	Bosveld Citrus	75-80	72-64	63.6	11.8	1.30	9.1	0.0	T2

Turkey	CC	06/04/2017	Bosveld Citrus	75-80	72-64	51.8	8.0	1.05	7.6	0.0	T6
Turkey	CC	04/05/2017	Bosveld Citrus	74-75	72	53.0	10.7	1.05	10.2	3.1	T3
Turkey	CC	26/05/2017	Bosveld Citrus	71-73	88-72	52.5	11.8	1.00	11.8	3.2	T2
Turkey	C35	06/04/2017	Bosveld Citrus	70-78	88-64	41.2	10.3	1.25	8.2	3.4	T7
Turkey	C35	04/05/2017	Bosveld Citrus	69-73	88-72	57.2	11.9	1.05	11.3	1.8	T2
Turkey	C35	26/05/2017	Bosveld Citrus	75-77	72	54.4	12.1	0.88	13.8	2.6	T3
Turkey	SC	06/04/2017	Bosveld Citrus	72-75	88-72	45.9	8.1	1.14	7.1	2.6	T6
Turkey	C35	04/05/2017	Group 91	74-76	72	53.7	12.1	1.40	8.6	7.4	T2-3
Valearly	CC	06/04/2017	Bosveld Citrus	71-75	88-72	41.1	8.0	0.91	8.8	0.0	T6
Valearly	CC	04/05/2017	Bosveld Citrus	75-80	72-64	52.4	9.3	0.75	12.4	0.0	T2
Valearly	C35	04/05/2017	Bosveld Citrus	72-75	88-72	55.3	11.4	0.86	13.3	0.0	T2
Val Late	SC	06/06/2017	Bosveld Citrus	74-81	72-64	55.6	8.0	1.11	7.2	1.2	T3-5
Val Late	SC	05/07/2017	Bosveld Citrus	72-75	88-72	57.7	10.9	1.13	9.6	2.4	T2-3

4.6.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele & Malelane)

Project 75C by J. Joubert (CRI)

Opsomming

Etna, Sirio en Tango word die vroegste ryp volgens resultate van die 2017 seisoen vir hierdie warm produksie area, met Tango die kleinste vruggrootte en goeie interne kwaliteit. Furr volg daarna met die hoogste saadtelling per vrug vir hierdie proef. Volgende is African Sunset en Orah, met die tweede hoogste saad telling per vrug. Die middel van die mandaryne word verteenwoordig deur Winola, gevolg deur Valley Gold en Mor 26 met die hoogste Brix vlakke in vergelyking met die ander seleksies (tot Brix van 12). Yosemite Gold en Gold Nugget was volgende om ryp te word en verteenwoordig die mid-laat van die Mandaryn Hibried reeks, ge-evalueer met 'n goeie interne kwaliteit vrug (Brix:suur verhouding van 12.5:1), asook goeie eksterne kleur ontwikkeling (T1-2). Tahoe Gold volg, met van die hoogste sap inhoud van 64% vir hierdie seisoen. Shasta Gold was die tweede laaste seleskie gereed vir oes teen middle tot einde Julie, en was totaal saadloos. Tambor 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.

Summary

Etna, Sirio and Tango matures first according to the results of the 2017 season for the warm production areas, and Tango developed the smallest fruit size and good internal quality. Furr follows, developing the highest seed count per fruit for this trial. Next will be African Sunset and Orah, developing the second highest seed count per fruit. The mid-maturing mandarins are represented by Winola, followed by Valley Gold and Mor 26, which developed the highest Brix levels compared to the other selections (up to Brix of 12). Yosemite Gold and Gold Nugget matured next, towards the mid-late period of the Mandarin Hybrid range evaluated at this trial site, with good internal quality (Brix: acid ratio of 12.5:1) as well as good external colour (T1-2). Tahoe

Gold followed, with the highest juice level of 64% for this season. Shasta Gold was the second last selection to mature at the middle to end of July and was completely seedless. Tambor was the last selection to mature at these trial sites, ending the Mandarin Hybrid season. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Bosveld Citrus (Letsitele), Overbrug (Hoedspruit), Mahela Citrus (Letsitele), Moriah Citrus (Hoedspruit) and Riverside (Malelane) from the Limpopo region. The following varieties were evaluated: African Sunset (B24), Furr (Clemcott), Gold Nugget, Hadas, Mor 26, ARC Nadorcott LS, Orah, Shasta Gold, Tahoe Gold, Tambor, Tango, Valley Gold (B17), Winola and Yosemite Gold.

Table 4.6.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2017 season.

Selection	Rootstock	Planted
African Sunset (B24)	SC	2009
Gold Nugget	CC	2010
Mor 26	SC	2009
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Valley Gold (B17)	SC	2009

Table 4.6.4.2. List of Mandarin Hybrid selections evaluated at Overbrug (Hoedspruit) during the 2017 season.

Selection	Rootstock	Planted
African Sunset (B24)	C35	2011
ARC Nadorcott LS	CC	2011

Table 4.6.4.3. List of Mandarin Hybrid selections evaluated at Mahela (Letsitele) during the 2017 season.

Selection	Rootstock	Planted
Etna	CC	2014
Furr	CC	2014
Gold Nugget	CC	2013
Samba	CC	2014
Sirio	CC	2014
Tango	CC	2013
Tahoe Gold	CC	2013
Yosemite Gold	CC	2013

Table 4.6.4.3. List of Mandarin Hybrid selections evaluated at Moriah (Hoedspruit) during the 2017 season.

Selection	Rootstock	Topwork
Furr (Clemcott)	MxT	2011
Hadas	MxT	2011
Mor 26	MxT	2011
Orah	MxT	2011
Tambor	MxT	2011
Valley Gold (B17)	MxT	2011
Winola	MxT	2011

Table 4.6.4.4. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2017 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

All the UCR 5 selections bore fruit for the fifth time this season. The trees at Bosveld are one year older than the trees at Riverside and this affected the quality and quantity of the fruit. This was the fourth season to evaluate African Sunset, Mor 26 and Valley Gold at the Bosveld trial site. The trees at Moriah Citrus were evaluated for the third time this year; the trial site became part of the CRI evaluation criteria in 2015. The mandarin selections were evaluated for the second time at Overbrug (Hoedspruit) and information was limited, future evaluations will improve recommendations.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over-mature. This process from the start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

African Sunset (B24)

There was enough fruit on the trees to complete two evaluations at the Bosveld and one evaluation at the Overbrug trial site, due to a fairly light crop. The large to very large fruit size (count 1XXX) is also a selection quality, but the light crop contributes to this scenario. African Sunset developed a protruding navel-end on most of the fruit; the bigger the fruit size the more visible the navel-end. The internal quality was better at the Overbrug site (high juice (57%), Brix 11.8, and fairly low acid levels (0.96%) compared to Bosveld with low juice (average 44.4%), Brix 8, and fairly low acid levels (average 0.87%). Fruit was completely seedless at both sites. External colour remained delayed ranging from T3 to T4, bearing in mind the hot production areas. Based on the internal quality results in Table 4.6.4.5, estimated maturity will be middle to the end of May.

Etna

Etna bore a good crop with large to extra-large fruit (count 1X to 1XX) and good internal quality for a hot production area. External colour was delayed at peak maturity (T4). Average seed count was 1.7 seeds per fruit this season due to cross-pollination. Maturity seems to be middle of April for the hot production areas, according to the information in Table 4.6.4.5.

Furr (Clemcott)

Furr developed large to extra-large fruit size (count 1X – 1XXX) on the trees at Mahela and Moriah Estate, one of the characteristics of the cultivar, as well as an excellent crop on the trees (60 to 80 kg/tree). The external colour development on the fruit was good for the Letsitele and Hoedspruit area (T2-3). Internally the fruit quality was very good, developing high juice (up to 53%) and Brix (up to 12) levels with acceptable acids. Another quality of the fruit is the high seed count of between 6 and 23 seeds per fruit (high cross pollination). Maturity

seems to be middle to the end of May to middle of June for the hot production areas, according to the information in Table 4.6.4.5.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower, as well as to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The internal quality of the fruit improved with tree age (still better at Riverside than Bosveld and Mahela) and developed good juice (up to 54%), high Brix (up to 12) and lower acid (above 0.7%) levels and an improved external colour (T2-4). Keep in mind the fairly young tree age (avg 6-7 years old) of this trial. 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 4.6.4.5, estimated maturity will be the end of May to middle of June.

Hadas

Hadas is a very late maturing mandarin selection according to the high acid levels at the middle of July (2.34%), but with good external colour (T2). The external colour of the fruit remains a deep yellow intensity. Seed levels decreased this season, ranging from 0 to 1 seeds per fruit and the fruit size varied from medium to large (count 2 to 1). Based on the internal quality results in Table 4.6.4.5, estimated maturity will be end of August to middle of September.

Mor 26

Mor 26 produced a light (Bosveld) to average (Moriah) crop on the trees for the 2017 season. The fruit size was erratic and peaked between count 2 and 1XX, medium to large fruit. The external colour development was yellow and peaked at T2. The internal quality was good with high juice levels of up to 53%, Brix up to 13 and acceptable acid levels (0.9%). There were on average 1.7 seeds in the fruit at Bosveld and 1.9 at Moriah. Based on the internal quality results in Table 4.6.4.5, estimated maturity will be the end of May to the beginning of June.

Nadorcott ARC & LS

There was very limited fruit on the ARC trees this season to evaluate (management improvement). The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Nadorcott LS produced a fruit size that peaked at count 1 due to the lighter crop load. Nadorcott LS were completely seedless this season. Maturity seems to be two weeks earlier on the LS selection, according to Table 4.6.4.5, but information was limited due to only one evaluation being done (beginning to middle of June).

Orah

Orah was evaluated for the third time this season, producing a good crop on the trees with medium to large (avg. count 1XX) fruit size. The average seed count in the fruit decreased compared to 2016 to 6.0 seeds per fruit, one of the characteristics of the selection. Internal quality was good, the Brix levels were above 12.5 by time of harvest, as well as good juice levels (above 56%) and acceptable acids (0.94%). Early external colour development ranged from T3 to T4 (only one evaluation). Based on the internal quality results in Table 4.6.4.5, estimated maturity will be end of May (degreening) to the middle of June.

Samba

Samba on Carizzo rootstock produced an average first crop with good internal quality on the large fast growing thornless trees at Mahela (Letsitele). 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 block (future evaluations will confirm low seed numbers) and peaked from medium to large fruit size (count 1 to 1XX). Based on the internal quality results in Table 4.6.4.5, estimated maturity will be middle to the end of May.

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. The fruit quality at the Mahela, Riverside and Bosveld was very similar compared to the previous season. The flavour improved with high juice (up to 50%) 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 (except at Bosveld – 0.3 seeds per fruit). The fruit size increased this season and peaked from large to very large (count 1XX-1XXX). The internal quality was good with juice levels up to 50%, Brix above 11 and acceptable acid levels (above 0.9%). Based on the internal quality results in Table 4.6.4.5 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 Bosveld, Mahela and Riverside. The fruit size decreased due to the heavy crop and peaked from large to extra-large (count 1-1XX) and the fruit shape was similar to that of a Minneola tangelo fruit. The external colour improved between T2-4 when the internal quality was optimum. Tahoe produced fruit with soft fibre strength that peels easily, and there was average 0.3 seeds per fruit evaluated (crosspollination in mixed orchard). The internal quality was good with juice levels of as high as 64%, Brix averaged 11.1 and acid levels were acceptable by the time of harvest. Based on the internal quality results in Table 4.6.4.5, estimated maturity will be middle to the end of June.

Tambor

Tambor is an addition to the late maturing mandarin selections for the hot production areas, producing 1.5 seeds per fruit, fairly low compared to the Furr and Orah selections. The external colour was on the yellow side at peak maturity, but with good internal quality, developing juice levels above 57%, Brix above 11 and acids above 1.5 at the last evaluation. Fruit size peaked from count 1X to count 1XX, very large for mandarin varieties. Based on the internal quality results in Table 4.6.4.5, estimated maturity will be end of July to the middle of August.

Tango

There was a good crop on the trees at all the trial sites this season compared to the average last year. Tango was completely seedless at all sites (in 2016 there was 0.2 seeds at Mahela). The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). The Tango trees were 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 50%), Brix levels improved (average 10) and peaked at 11.9 for the Bosveld trial (lowest Brix at Mahela site – 7.0), acid levels were on the lower side early in the season (indicating a shorter shelf life), and deep orange coloured fibre. Fruit size peaked at count 2 to 1XX (medium to large). Based on the internal quality results in Table 4.6.4.5, estimated maturity will be end of April to the middle of May with delayed external colour development.

Valley Gold (B17)

Valley Gold was evaluated for the fourth time at the Bosveld trial site and for the second time at Moriah. The internal quality was good with Brix averaging 11.9 and acid levels around 1.0% and external colour between T2 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 Bosveld and high fruit split was present on the trees at Moriah resulting in upto 30 to 60% fruit drop. Maturity is estimated to be end of May to middle of June for these hot production areas.

Winola

Winola was completely seedless at Moriah Estate, producing an average to good crop on the young trees. Fruit size peaked at very large (count 1XX to 1XXX). Internal quality improved with high juice levels above 53%, very little granulation problems in the fruit compared to 2015. Brix (above 10) and acids were better (1.4%) and comply with the export standards. Maturity is estimated to be the end of June for these hot production areas.

Yosemite Gold

The fruit set on Yosemite Gold remained very light at the Riverside and Mahela trial sites with no crop on the trees at Bosveld. Additional measures may be necessary to increase the crop on the trees, for example Gibb sprays or girdling. Yosemite Gold developed a very promising soft citrus type fruit shape (similar to Minneola tangelo). The fruit was firm, rind texture was smooth, and the fibre was soft, peeled very easily and completely seedless this season. Yosemite developed the biggest tree size compared to the other UCR 5 selections. This aggressive growth characteristic will be one of the reason for the poor crop on the trees (vegetative growth), and must be redirected into fruit set and crop on the trees (dwarfing rootstocks option). Fruit size decreased and varied from large to very large (count 1-1XX), similar to Tahoe Gold, due to the light crop on the trees. The internal quality improved this season with higher juice (above 53%), average Brix and good acid levels. External colour developed along with the internal quality towards the end of the evaluations (T2). Based on the internal quality results in Table 4.6.4.5, estimated maturity will be mid-June to mid-July.

Additional selection

The internal quality of Tasty was average with lower juice levels (47.2%), higher Brix (above 10) and acids were lower (0.65%), indicating the early maturing characteristics of the selection with completely seedless fruit. The fruit size peaked from count 1 to count 1X.

Sirio produced large fruit (count 1XX) on the trees due to a light crop with average internal quality in the hot production areas. The fruit was completely seedless.

Conclusion

There was an improvement in the external colour delay in the hot areas that were a problem in the past; future evaluations will clarify the situation. Degreening may be an option for the Gold Nugget and TDEs (fruit colour development was yellow with degreening), but ethylene reacted slowly with Tango (W. Murcott selection) and Nadorcott. Furr, Tambor, Orah, Gold Nugget, Winola and Yosemite Gold may be a possibility to consider for the hot areas due to stronger fruit with optimal fruit size, and good internal quality when external colour becomes more intense (T1-2). In the hot areas it will be crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit. There was severe sunburn on the Shasta Gold fruit compared to the cooler production areas.

African Sunset, Tahoe Gold, Shasta Gold, Yosemite Gold, Furr, Gold Nugget, Mor 26 and Winnola and had the larger fruit size, followed by Tambor, Samba and then Orah. The smallest fruit size was produced on Tango and Hadas. 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.

Etna, Sirio and Tasty 1 was evaluated for the first time this season; future evaluations will continue to determine suitability for this production area.

Table 4.6.4.5. Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele), Overbrug (Hoedspruit), Mahela (Letsitele), Moriah (Hoedspruit) and Riverside (Malelane) during the 2017 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
African Sunset (B24)	SC	15/04/2017	Bosveld	80-85	1XXX	48.6	9.7	1.01	9.6	0.0	T7
African Sunset (B24)	SC	06/06/2017	Bosveld	82-86	1XXX	40.2	8.2	0.72	11.4	0.0	T3-4
African Sunset (B24)	CC	05/05/2017	Overbrug	77-82	1XXX	57.4	11.8	0.96	12.3	0.0	T4
Etna	CC	06/04/2017	Mahela	68-75	1X-1XX	51	9.9	0.76	13.0	1.7	T4
Furr	CC	03/05/2017	Mahela	78-81	1XXX	53.8	10.5	0.85	12.4	23.2	T3

Furr	MxT	06/04/2017	Moriah	71-81	1X-1XXX	42.7	11.1	1.20	9.3	15.8	T4
Furr	MxT	04/05/2017	Moriah	74-77	1XX	53.2	12.2	1.04	11.7	14.8	T2
Furr	MxT	06/06/2017	Moriah	75-80	1XX-1XXX	57.0	12.2	0.98	12.4	6.2	T3
Gold Nugget	CC	25/05/2017	Bosveld	62-67	2-1	46.7	10.2	1.07	9.5	0.0	T3-4
Gold Nugget	CC	22/06/2017	Bosveld	66-71	1-1X	50.7	12.8	1.19	10.8	0.0	T2-3
Gold Nugget	CC	25/05/2017	Mahela	72-74	1XX	54.2	11.3	0.65	17.4	0.0	T3
Gold Nugget	CC	22/06/2017	Mahela	71-80	1X-1XXX	40.5	12.3	0.74	16.6	0.0	T2
Gold Nugget	CC	20/04/2017	Riverside	60-66	2-1	54.7	10.8	1.00	10.8	0.0	T4
Gold Nugget	CC	30/06/2017	Riverside	62-69	2-1X	50.3	8.4	0.75	11.2	0.0	T3-4
Gold Nugget	CC	21/07/2017	Riverside	65-70	1-1X	52.7	10.8	0.92	11.8	0.0	T2
Hadas	MxT	14/07/2017	Moriah	59-65	2-1	43.2	12.8	2.34	5.5	1.0	T2
Mor 26	SC	15/04/2017	Bosveld	66-70	1-1X	51.6	10.2	1.65	6.2	1.7	T6
Mor 26	MxT	04/05/2017	Moriah	65-70	1-1X	47.7	11.6	1.33	8.7	1.6	T2
Mor 26	MxT	06/06/2017	Moriah	70-74	1X-1XX	48.1	13.2	0.99	13.3	1.4	T2
Mor 26	MxT	23/06/2017	Moriah	61-73	2-1XX	53.5	12.6	0.91	13.8	1.2	T2
Mor 26	MxT	14/07/2017	Moriah	74-80	1XX-1XX	44.7	13.1	1.02	12.9	3.3	T2
Nadorcott LS	CC	05/05/2017	Overbrug	65-67	1	52.7	12.5	1.23	10.2	0.0	T3
Orah	MxT	06/06/2017	Moriah	72-74	1XX	56.7	12.5	0.94	13.3	6.0	T3-4
Samba	CC	06/04/2017	Mahela	65-75	1-1XX	48.9	9.2	0.78	11.8	0.0	T4-5
Samba	CC	03/05/2017	Mahela	65-70	1-1X	49.7	9.6	0.80	12.0	0.0	T2
Samba	CC	25/05/2017	Mahela	69-71	1X	52.4	10.2	0.81	12.6	0.0	T2
Shasta Gold	CC	22/06/2017	Bosveld	77-80	1XX-1XXX	46.5	12.2	1.35	9.0	0.3	T2
Shasta Gold	CC	17/07/2017	Bosveld	80-95	1XXX	50.9	11.1	0.91	12.2	0.0	T2-3
Shasta Gold	CC	30/06/2017	Riverside	80-85	1XXX	47.9	11.9	1.30	9.2	0.0	T2
Sirio	CC	03/05/2017	Mahela	72-76	1XX	49.2	9.1	0.89	10.2	0.0	T2
Tahoe Gold	CC	25/05/2017	Bosveld	65-69	1-1X	55.6	12.5	1.35	9.3	1.1	T3
Tahoe Gold	CC	22/06/2017	Bosveld	68-75	1X-1XX	58.9	11.6	1.13	10.3	0.0	T2
Tahoe Gold	CC	03/05/2017	Mahela	67-75	1-1XX	63.7	9.8	0.90	10.9	0.0	T2
Tahoe Gold	CC	20/04/2017	Riverside	65-73	1-1XX	62.5	10.5	1.35	7.8	0.1	T4
Tambor	MxT	06/06/2017	Moriah	75-82	1X-1XX	57.4	11.8	1.59	7.4	2.0	T2
Tambor	MxT	23/06/2017	Moriah	75-80	1X-1XX	57.5	11.4	1.92	5.9	1.0	T2-3
Tango	CC	15/04/2017	Bosveld	65-70	1-1X	49.7	11.1	1.39	8.0	0.0	T5
Tango	CC	25/05/2017	Bosveld	62-66	2-1	49.7	11.3	1.10	10.3	0.0	T3
Tango	CC	22/06/2017	Bosveld	66-73	1-1XX	55.2	11.9	1.08	11.1	0.0	T2
Tango	CC	06/04/2017	Mahela	66-67	1	52.2	7.0	0.85	8.2	0.0	T7
Tango	CC	25/05/2017	Mahela	63-67	2-1	55.0	7.1	0.54	13.1	0.0	T3
Tango	CC	28/03/2017	Riverside	64-70	1-1X	59.1	9.5	0.80	11.9	0.0	T3-6
Tango	CC	20/04/2017	Riverside	60-66	2-1	56.7	9.7	0.65	14.9	0.0	T4
Tasty 1	CC	03/05/2017	Mahela	65-70	1-1X	47.2	10.8	0.65	16.6	0.0	T2
Valley Gold (B17)	SC	15/04/2017	Bosveld	65-70	1-1X	51.3	12.5	1.67	7.5	1.3	T6
Valley Gold (B17)	SC	06/06/2017	Bosveld	80-85	1XX	47.7	11.4	1.00	11.4	0.0	T2-3
Valley Gold (B17)	MxT	06/06/2017	Moriah	71-79	1X-1XX	53.6	11.9	1.02	11.7	0.0	T2
Winola	MxT	06/06/2017	Moriah	75-80	1XX-1XXX	52.8	10.8	1.43	7.6	0.0	T2
Yosemite Gold	CC	03/05/2017	Mahela	70-75	1X-1XX	53.8	7.0	1.25	5.6	0.0	T3
Yosemite Gold	CC	16/05/2017	Riverside	65-71	1-1X	55.0	10.1	1.15	8.8	0.0	T3
Yosemite Gold	CC	30/06/2017	Riverside	70-75	1X-1XX	58.9	9.5	1.15	8.3	0.0	T2

4.6.5 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry production areas (Weipe and Tshipise)

Project 899A by J. Joubert (CRI)

Opsomming

Hierdie was die derde seisoen wat die NGB proef ge-evalueer is en die tweede seisoen vir Alicedale as gevolg van voldoende vrugte aan die bome, en betekenisvolle data kon versamel word. Valearly begin die seisoen as die vroegste Valencia met 'n vertraagde vrugkleur op oorryp vrugte, gevolg deur Weipe met lae suurvlaakte en wind skade op die skil. Delta sal volgende in lyn wees, gevolg deur Skilderkrans en McClean SL met later vrugkleur en totaal saadlose vrugte. Volgende om ryp te word sal die gewone McClean wees met goeie kleur en 1.5 sade per vrug. 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 (96.9 kg per boom).

Summary

This was the third season to evaluate the NGB trial site and second season for the Alicedale site due to fruit numbers on the trees, and meaningful data collected. Valearly will start the season as the earliest maturing Valencia with a colour delay on the overmature fruit, followed by Weipe with low acid levels and wind damage on the rind. Delta will be next in line, followed by Skilderkrans and McClean SL with delayed colour and completely seedless fruit. Next to mature will be McClean with advanced colour and 1.5 seeds per fruit. Kobus du Toit Late follows as part of the middle maturing Valencia section, Moos Late 1 and Rhode Red. Gusocora with seedless fruit, as well as Henrietta and Bennie will be next, followed by Louisa, and Jassie, towards the late Valencia section, with excellent internal quality and optimal colour development (96.9 kg per tree).

Objective

- To find suitable Valencia selections with superior characteristics for the hot dry inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Bennie, Delta, Gusocora, Henrietta, Jassie, Kobus du Toit Late, Lavallo, Louisa, McClean, McClean SL, Mooslate 1, Rhode Red, Skilderkrans, Turkey, Valearly and Weipe at Alicedale (Tshipise) and NGB (Weipe).

Table 4.6.5.1. List of Valencia selections evaluated at Alicedale (Tshipise) during 2017.

Selection	Rootstock	Planted
Bennie	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	Sunki 812/RL/X639	2013
Lavallo	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	RL/X639	2013
Turkey	C35/X639	2013
Weipe	C35/Sunki 812/RL/X639	2013

Table 4.6.5.2. List of Valencia selections evaluated at NGB (Weipe) during 2017.

Selection	Rootstock	Topwork
Delta	X639	2012
Kobus du Toit Late	X639	2012
Jassie	X639	2012
McClellan	X639	2012
McClellan SL	X639	2012
Moosrivier Late 1	X639	2012
Rhode Red	X639	2012
Skilderkrans	X639	2012
Valearly	X639	2012

Results and discussion

The Alicedale trial site at Tshipise bore fruit on most of the cultivars on different rootstocks and evaluations were done accordingly. There was a good fruit set on the trees for 2018 (determine yield production) and all cultivar combinations will be evaluated in the next season.

Bennie

There was a good crop on Bennie (best with RL) and the fruit size peaked between count 72 and 56 (very good for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Bennie produced good juice levels (average 50%), Brix (average 11.0) and acid (1.1%) and fairly low seed counts (average 1.8 seeds per fruit). External colour by the time of harvest varied between T2 and T4. Based on ratios, Bennie end of June to beginning of July (Table 4.6.5.3).

Delta

The Delta (control) trees was topworked at NGB in the Weipe area on X639 rootstock because of high pH soils in the area. Fruit size distribution was uniform and range from count 88 to 56, medium to large fruit and optimum Valencia requirements. Internal quality was good with high juice (up to 52%), Brix (11.6) and fairly low acid levels through the season (average 1.0%). Based on the internal quality results in Table 4.6.5.3, estimated maturity will be mid-June to mid-July.

Gusocora

Gusocora was evaluated at Alicedale this year on C35, RL, Sunki 812 and X639 rootstocks. The fruit was completely seedless (0.5 seeds with one evaluation) and developed a good internal quality where juice (up to 53%), Brix (up to 9.6) and acid above 1.0 complied with export requirements. The external colour (delayed) varied from T2 to T4, correlating with the internal quality and Brix:acid ratio (8:1 for maturity). Fruit size was smaller and peaked between counts 88 and 56, optimal fruit size for export Valencias (medium to large). There was a good crop on the trees (best on RL). It is apparent that Gusocora's maturity is middle to end of July (Table 4.6.5.3).

Henrietta

Henrietta was evaluated on all four rootstock combinations at Alicedale, Tshipise this season. Juice levels peaked above 55% average with lower Brix (up to 9) and acids 1.3 with the third evaluation. The external colour development was good and peaked between T2 and T3 for the season. Average seeds per fruit total decreased to 1.8 seeds per fruit (2.9 seeds for 2016). Based on the internal quality results in Table 4.6.5.3, estimated maturity will be mid-July to mid-August.

Jassie

Jassie seems to be one of the most promising new Valencia selections being tested and evaluated in the different citrus production and climatic areas. The best crop production (97 kg/tree) on the new trees was a combination with Jassie (RL) at the Alicedale trial site (precocious bearing). Fruit size distribution was excellent

and slightly smaller due to the high yield on the trees; the counts were from 88 to 64. Fruit quality was good with high juice (average 52.3), Brix of up to 11 (Sunki 812) and fairly high acid levels (above 1.0%) at the final evaluation, indicating the late characteristics of the cultivar. The seed counts varied from 0 up to 6.1 seeds per fruit (average 4.1 seeds per fruit). Based on the internal quality results in Table 4.6.5.3, estimated maturity will be mid-July to mid-August.

Kobus du Toit Late

There was an external colour delay on the fruit during the season, ranging from T2 to T4 up until the last evaluation. Fruit average size varied from medium to large, count 88 to 64. Internal quality was fair to good depending on the age of the trees and the rootstock combinations. Kobus du Toit Late performed the best on Sunki 812 at Alicedale. Seed production increased slightly this season but was still fairly low for a seeded selection (average 3.2 seeds per fruit). Acids levels was above 1.0% the entire season, except for on X639 at NGB. Maturity, based on the internal quality results in Table 4.6.5.3, is estimated to be the end of June to middle of July for these hot production areas.

Lavalle

Lavalle was evaluated on all four rootstocks this season. There was a decrease in seed production this season and Lavalle produced completely seedless fruit. The internal quality complied with export requirements and acid level was above 1.2% at the second evaluation at the middle of July (Alicedale harvested fruit to determine final crop). Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season. From the ratio on this date it is apparent that Lavalle's maturity is end of August to middle of September (Table 4.6.5.3).

Louisa

There was a light crop on all four rootstock combinations at Alicedale with RL cropping 59 kg per tree. The fruit was completely seedless, except for one evaluation (0.3 seeds/fruit). The internal quality was average to good with lower juice (45 to 53%) and higher Brix levels (up to 10.5). The fruit colour was fairly yellow by the time of peak maturity between T2 and T4. Fruit size peaked from medium to large, count 88 to count 56. Based on the internal quality results in Table 4.6.5.3, estimated maturity will be middle to the end of July.

McClellan SL

When compared to all the other Valencia trial sites, McClellan SL remained completely seedless fruit. This year all the combination with McClellan SL was bearing fruit (21 to 46 kg per tree) at Alicedale to evaluate, indicating potential for the future. The fruit size peaked between count 72 and 48/40 (medium to large/very large) with good internal quality (juice up to 52%, Brix 9.3, acid 1.0). Maturity (Table 4.6.5.3) is estimated to be the end of June to middle of July.

Moosrivier Late 1

Moosrivier produced fruit with medium to large fruit size (count 88 to 64) at the NGB trial site on X639 rootstock. Internal quality improved and was very good with high juice (average 65.3%), average Brix (11.5) and improved acids (above 1.0) after completion of the evaluations. Seed count per fruit varied from 1.0 to 3.0 seeds and colour development peaked between T2 and T3. Based on the internal quality results in Table 4.6.5.3, estimated maturity will be middle to the end of July.

Skilderkrans

Skilderkrans was evaluated at Alicedale and NGB in the hot production areas. Fruit size varied from medium to large/extra-large (count 72-48). Internally the Brix content was good (up to 11.3) and the acid level of 1.2 to 1.3% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to average 51.1% later in the season; above the minimum required export figure. There was no delay in external colour 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 higher acid levels, delaying peak maturity to end of July and mid-August on Swingle and C35 (Table 4.6.5.3).

Turkey

Turkey cropped fruit on all four rootstock combinations at Alicedale performing well on RL, Sunki 812 and C35 with high juice (above 52%) and lower Brix (above 7.5) levels, as well as acceptable acids (above 1.0). Colour development was good throughout the season and seed number remained fairly low (between 3.2 and 3.8 seeds per fruit). Maturity (Table 4.6.5.3) is estimated to be the end of May to middle of June for these hot production areas.

Weipe

The Weipe selection was developed to replace the Limpopo SL as an early maturing Valencia. Weipe was evaluated for the second time at the Alicedale trial site and was planted on C35, Sunki 812, RL and X639. There was limited fruit on the trees due to the smaller sized trees and light crop (24.9 to 42.6 kg/tree). Fruit size was medium to large/extra-large (count 72-40), internal quality was fair (juice below 45%, acid 0.89%) with higher Brix level (up to 11.1). Colour development ranged from T2 to T3. Maturity is estimated to be middle to end of May (Table 4.6.5.3).

Additional selections

Valearly was only evaluated once early this season due to a low acid level of 0.7%, juice below 50% and a good Brix (10.5). The fruit size varied from count 88 to 72 (medium) and there were 0.9 seeds per fruit.

Rhode Red developed high numbers of Chimeras on the fruit and future evaluations will determine the potential of the cultivar due to instability.

Conclusions

Bennie matures well on the trees and to reduce rind pitting problems the recommendation will be to harvest the fruit from middle July onwards (stronger rind). Gusocora seems to have optimal colour development at peak maturity and degreening might be an option.

Valearly's colour was delayed at peak maturity (T2) and resulted in low acids (0.7) for the cultivar later in the season as well as poor shelf life. Rhode Red and Skilderkrans developed high numbers of Chimeras on the fruit this season, questioning the stability of the selection.

All the selections evaluated developed seeds in their fruit, except for Delta, Gusocora, Louisa, Lavallo, McClean seedless, Skilderkrans 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).

Jassie outperformed the other Valencia selections with optimum fruit size distribution, good internal quality, highest crop load, good colour development and fairly low seed numbers.

More selections came into production this season; at this stage information was limited due to young tree age and a limited number of fruit on the trees.

Table 4.6.5.3. Internal fruit quality data for Valencia orange selections at Alicedale (Tshipise) and NGB (Weipe) during the 2017 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Bennie	C35	25/05/2017	Alicedale	77-82	72-56	51.0	8.2	1.14	7.2	4.3	T3-5
Bennie	RL	25/05/2017	Alicedale	75-81	72-64	49.4	7.9	1.00	7.9	1.3	T3
Bennie	Sunki 812	25/05/2017	Alicedale	77-81	72-64	49.4	8.8	1.27	6.9	2.1	T3-4
Bennie	X639	25/05/2017	Alicedale	75-80	72-64	54.7	7.6	1.23	6.2	4.0	T3-4
Bennie	C35	22/06/2017	Alicedale	85-90	56-40	45.7	10.0	1.29	7.8	0.0	T2-3
Bennie	RL	22/06/2017	Alicedale	82-85	56-56	52.9	8.4	0.89	9.4	0.0	T3-4
Bennie	Sunki 812	22/06/2017	Alicedale	82-86	56-48	50.4	9.0	1.18	7.6	2.8	T3-4

Bennie	X639	22/06/2017	Alicedale	80-82	64-56	51.9	8.8	1.04	8.5	0.0	T3-4
Delta	X639	24/05/2017	NGB	77-80	72-64	49.5	9.0	1.12	8.0	0.0	T2-3
Delta	X639	21/06/2017	NGB	75-83	72-56	52.4	9.9	1.08	9.2	0.0	T2-3
Delta	X639	02/08/2017	NGB	72-82	88-56	52.9	11.6	0.85	13.6	0.0	T2
Du Toit Late	RL	22/06/2017	Alicedale	72-77	88-72	51.2	8.5	1.06	8.1	4.8	T3-4
Du Toit Late	Sunki 812	22/06/2017	Alicedale	70-75	88-72	58.1	10.0	1.24	8.1	3.8	T2-3
Du Toit Late	Sunki 812	13/07/2017	Alicedale	74-80	72-64	56.0	13.5	1.05	12.9	6.5	T3-4
Du Toit Late	X639	13/07/2017	Alicedale	71-75	88-72	55.3	8.4	1.06	7.9	3.2	T3-4
Du Toit Late	X639	21/06/2017	NGB	85-90	56-40	47.7	11.0	1.02	10.8	3.5	T2
Du Toit Late	X639	12/07/2017	NGB	75-78	72-64	49.7	7.4	0.92	8.0	0.7	T2-3
Du Toit Late	X639	02/08/2017	NGB	82-86	56-48	49.2	11.6	0.84	13.8	0.0	T2
Gusocora	C35	22/06/2017	Alicedale	76-81	72-64	52.1	7.3	0.95	7.7	0.0	T2-3
Gusocora	RL	22/06/2017	Alicedale	73-80	72-64	51.0	7.3	1.17	6.2	0.0	T3-4
Gusocora	Sunki 812	22/06/2017	Alicedale	72-78	88-64	47.2	9.9	0.98	10.2	0.0	T3-4
Gusocora	X639	22/06/2017	Alicedale	78-83	64-56	51.6	9.0	1.20	7.5	0.0	T3-4
Gusocora	C35	13/07/2017	Alicedale	81-83	64-56	49.5	9.5	1.02	9.3	0.0	T2-3
Gusocora	RL	13/07/2017	Alicedale	75-78	72-64	52.6	8.5	0.95	8.9	0.5	T2-3
Gusocora	Sunki 812	13/07/2017	Alicedale	83-90	56-40	42.7	9.6	1.03	9.3	0.0	T3-4
Gusocora	X639	13/07/2017	Alicedale	80-85	64-56	53.7	9.6	0.99	9.7	0.0	T2-3
Henrietta	C35	22/06/2017	Alicedale	70-76	88-72	58.2	7.5	1.23	6.1	1.8	T3-4
Henrietta	RL	22/06/2017	Alicedale	78-84	64-56	54.4	7.0	1.30	5.4	0.7	T3-4
Henrietta	Sunki 812	22/06/2017	Alicedale	79-84	64-56	51.5	7.9	1.22	6.5	2.3	T2-3
Henrietta	X639	22/06/2017	Alicedale	75-83	72-56	53.1	7.2	1.34	5.4	1.3	T3-4
Henrietta	C35	13/07/2017	Alicedale	72-80	88-64	55.5	9.2	1.26	7.3	0.0	T2-3
Henrietta	RL	13/07/2017	Alicedale	80-85	64-56	52.2	8.7	1.32	6.5	0.0	T2-3
Henrietta	Sunki 812	13/07/2017	Alicedale	75-81	72-64	57.6	9.6	1.25	7.7	7.9	T2-3
Henrietta	X639	13/07/2017	Alicedale	74-82	72-56	53.3	9.2	1.22	7.5	0.0	T2-3
Jassie	C35	25/05/2017	Alicedale	67-73	105-72	55.4	7.4	1.43	5.2	5.8	T4
Jassie	RL	25/05/2017	Alicedale	70-76	88-72	52.1	6.1	1.34	4.6	6.1	T3-4
Jassie	Sunki 812	25/05/2017	Alicedale	70-76	88-72	50.8	9.9	1.29	7.7	4.3	T3-4
Jassie	X639	25/05/2017	Alicedale	70-77	88-72	50.0	7.4	1.58	4.7	4.6	T4
Jassie	C35	22/06/2017	Alicedale	76-84	72-56	42.4	9.9	1.24	8.0	4.0	T3-4
Jassie	RL	22/06/2017	Alicedale	69-73	88-72	55.5	7.0	1.16	6.0	5.5	T3-4
Jassie	Sunki 812	22/06/2017	Alicedale	72-78	88-64	54.6	9.9	1.32	7.5	0.0	T3-4
Jassie	X639	22/06/2017	Alicedale	72-74	88-72	47.0	7.3	1.24	5.9	2.7	T2-3
Jassie	C35	13/07/2017	Alicedale	70-80	88-64	56.5	10.2	1.10	9.3	4.5	T2-3
Jassie	RL	13/07/2017	Alicedale	70-75	88-72	54.4	8.4	1.13	7.4	3.5	T2
Jassie	Sunki 812	13/07/2017	Alicedale	70-75	88-72	54.5	11.7	1.20	9.8	4.3	T2-3
Jassie	X639	13/07/2017	Alicedale	69-78	88-64	54.9	9.3	1.20	7.8	3.8	T2-3
Jassie	X639	24/05/2017	NGB	70-76	88-72	54.3	10.0	1.13	8.8	3.5	T3-4
Jassie	X639	12/07/2017	NGB	80-84	64-56	55.2	10.8	1.07	10.1	1.3	T2
Jassie	X639	02/08/2017	NGB	84-90	56-40	53.7	11.8	0.99	12.0	1.5	T2-3
Lavalle	C35	22/06/2017	Alicedale	77-80	72-64	58.4	8.4	1.57	5.4	0.0	T3-4
Lavalle	RL	22/06/2017	Alicedale	77-81	72-64	61.1	7.2	1.38	5.2	0.0	T3-4
Lavalle	Sunki 812	22/06/2017	Alicedale	76-85	72-56	58.1	8.6	1.75	4.9	0.0	T3-4
Lavalle	X639	22/06/2017	Alicedale	80-84	64-56	57.2	9.9	1.49	6.7	0.0	T3-4
Louisa	C35	22/06/2017	Alicedale	81-85	64-56	54.5	6.8	1.18	5.8	0.0	T2-3
Louisa	RL	22/06/2017	Alicedale	74-80	72-64	53.7	7.0	1.08	6.5	0.0	T2-3
Louisa	X639	22/06/2017	Alicedale	74-81	72-64	52.8	7.1	1.12	6.3	0.0	T3-4
Louisa	C35	13/07/2017	Alicedale	81-86	64-48	51.1	9.4	1.06	8.9	0.0	T3-4
Louisa	RL	13/07/2017	Alicedale	80-85	64-56	53.3	8.3	0.99	8.4	0.3	T2-3
Louisa	Sunki 812	13/07/2017	Alicedale	70-80	88-64	48.9	10.5	1.38	7.6	0.0	T2-3
Louisa	X639	13/07/2017	Alicedale	71-80	88-64	45.8	9.4	1.01	9.3	0.0	T2-3

McClellan SL	C35	13/07/2017	Alicedale	76-84	72-56	51.2	9.1	0.90	10.1	0.0	T3-4
McClellan SL	RL	13/07/2017	Alicedale	80-85	64-56	50.0	8.4	0.98	8.6	0.0	T3
McClellan SL	Sunki 812	13/07/2017	Alicedale	85-90	56-40	52.3	9.3	1.06	8.8	0.0	T2-3
McClellan SL	X639	24/05/2017	NGB	73-81	72-64	51.8	9.9	1.07	9.3	0.0	T3
McClellan SL	X639	02/05/2017	NGB	75-80	72-64	50.0	7.7	1.22	6.3	0.0	T4
McClellan SL	X639	02/08/2017	NGB	75-81	72-64	46.8	10.6	0.75	14.1	0.0	T2-3
McClellan	X639	21/06/2017	NGB	68-75	88-72	46.6	11.6	1.32	8.8	1.5	T2
McClellan	X639	02/08/2017	NGB	75-81	72-64	53.4	12.3	1.06	11.6	1.4	T2-3
MoosLate 1	X639	21/06/2017	NGB	73-76	72	54.3	11.6	1.34	8.7	3.0	T3
MoosLate 1	X639	12/07/2017	NGB	72-77	88-72	54.3	11.5	1.18	9.7	1.0	T2-3
MoosLate 1	X639	02/08/2017	NGB	70-74	88-72	60.2	11.4	1.24	9.2	1.0	T2-3
Rhode Red	C35	22/06/2017	Alicedale	76-77	72	54.6	7.2	0.95	7.6	3.1	T3-4
Rhode Red	RL	22/06/2017	Alicedale	72-75	88-72	53.2	7.0	1.11	6.3	1.3	T3-4
Rhode Red	Sunki 812	22/06/2017	Alicedale	71-75	88-72	55.6	7.9	1.10	7.2	0.0	T3-4
Rhode Red	X639	22/06/2017	Alicedale	71-83	88-56	56.1	7.3	1.30	5.6	4.3	T3-4
Rhode Red	C35	13/07/2017	Alicedale	71-80	88-64	50.9	8.8	1.02	8.6	1.3	T3-4
Rhode Red	RL	13/07/2017	Alicedale	70-75	88-72	54.1	7.3	1.11	6.6	0.0	T3-4
Rhode Red	X639	13/07/2017	Alicedale	75-85	72-56	55.7	9.5	1.05	9.0	1.6	T2
Rhode Red	X639	24/05/2017	NGB	69-76	88-72	52.9	8.1	1.32	6.1	0.0	T4
Rhode Red	X639	21/06/2017	NGB	72-78	88-64	54.1	8.9	1.12	7.9	1.6	T2-3
Rhode Red	X639	12/07/2017	NGB	65-74	105-72	55.1	10.7	1.08	9.9	0.0	T2-3
Skilderkrans	RL	22/06/2017	Alicedale	77-80	72-64	52.0	7.7	1.15	6.7	0.0	T3-4
Skilderkrans	X639	22/06/2017	Alicedale	82-86	56-48	52.8	7.9	1.25	7.0	0.0	T2-3
Skilderkrans	X639	06/06/2017	NGB	74-78	72-64	46.7	7.2	1.27	5.7	0.0	T2-3
Skilderkrans	X639	21/06/2017	NGB	80-86	64-48	52.5	7.9	1.25	6.3	0.3	T2-3
Skilderkrans	X639	12/07/2017	NGB	82-86	56-48	51.7	11.3	1.22	9.3	0.0	T2
Turkey	X639	03/05/2017	Alicedale	72-76	88-72	55.9	9.4	1.28	7.3	3.8	T3
Turkey	C35	25/05/2017	Alicedale	72-81	88-64	52.9	8.4	1.10	7.6	3.3	T3
Turkey	X639	25/05/2017	Alicedale	75-80	72-64	53.3	8.8	1.00	8.8	3.2	T3
Valearly	X639	24/05/2017	NGB	71-77	88-72	48.1	10.5	0.68	15.4	0.9	T2
Weipe	C35	25/05/2017	Alicedale	80-88	64-48	47.3	8.2	0.81	10.1	0.0	T2-3
Weipe	RL	25/05/2017	Alicedale	80-84	64-56	43.5	7.6	0.81	9.4	0.0	T2
Weipe	Sunki 812	25/05/2017	Alicedale	85-90	56-40	48.8	8.8	0.95	9.3	0.0	T2
Weipe	X639	25/05/2017	Alicedale	78-81	64-64	47.7	8.6	0.88	9.8	0.0	T2

4.6.6 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall & Groblersdal)
Project 941C by J. Joubert (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die drie proef persele, a.g.v. die klimaatsone (intermediere areas). Die resultate het aangedui dat Tango die vroegste ryp geword het, met die kleinste vruggrootte en gemiddelde tot goeie interne kwaliteit. Tango en Yosemite Gold was total saadloos gewees hierdie seisoen. I22 het ook as een van die vroeer seleksies ingepas met 'n baie ligte oes op die bome. Shasta Gold het 'n goeie oes op die bome gehad soortgelyk aan die 2015 seisoen en alternatiewe drag patrone moet ondersoek word (Gibb bespuitings krities). Edit x Nova en Meirav 63 pas hier in saam met die mid seleksies met diep oranje skilkleur Yosemite Gold was volgende gereed vir oes, gevolg deur Tahoe Gold. Tahoe Gold het groot tot baie groot vrugte vir hierdie seisoen geproduseer. Gold Nugget en Nadorcott ARC, gevolg deur Shasta Gold was die laaste seleksie gereed vir oes, teen einde Junie, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

The quality of the Mandarin Hybrid fruit between the three different trial sites was similar, due to the climatic region (intermediate areas). The results indicated that Tango matures first with the smallest fruit size and fair to good internal quality. Tango and Yosemite Gold were completely seedless this season. I22 also indicated to be a fairly early maturing selection with a very light crop on the trees. The Shasta Gold trees cropped a good yield this season similar to the heavy crop in 2015. Alternate bearing patterns must be investigated (Gibb applications crucial). Edit x Nova and Meirav 63 seem to fit in with the mid-maturing selections with deep orange rind colour. Yosemite Gold was next to mature, followed by Tahoe Gold. Tahoe Gold developed large to extra-large fruit size for this season. Gold Nugget and Nadorcott ARC, followed by Shasta Gold was the last selection to mature, at the end of June, ending off the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

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 Engelbrecht Trust (Marble Hall), Moosrivier Estate (Marble Hall) and Schoonbee Estate (Marble Hall) from the Limpopo region. The following varieties were evaluated: African Sunset, Edit x Nova, Gold Nugget, IRM 1&2, Meirav 63 & 119, Nadorcott ARC & LS, Shani SL, Tahoe Gold, Tango and Yosemite Gold.

Table 4.6.6.1. List of Mandarin Hybrid selections evaluated at Engelbrecht Trust (Marble Hall) during the 2017 season.

Selection	Rootstock	Planted
African Sunset	CC	2011
Edit x Nova	CC	2011
IRM 2	CC	2011
Meirav 63	CC	2011
Nadorcott ARC	CC	2011
Nadorcott LS	CC	2011
Shani SL	CC	2011

Table 4.6.6.2. List of Mandarin Hybrid selections evaluated at Moosrivier Estate (Marble Hall) during the 2017 season.

Selection	Rootstock	Planted
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 2	Sunki 812	2013
Mor 15	Sunki 812	2013
Mort 26	Sunki 812	2013

Shani SL	Sunki 812	2013
Shasta Gold	CC/C35/X639	2013
Tahoe Gold	CC/C35/X639	2013
Tango	CC/C35/X639	2013
Yosemite	C35	2013

Table 4.6.6.3. List of Mandarin Hybrid selections evaluated at Schoonbee Estate (Marble Hall) during the 2017 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2012
Tahoe Gold	CC	2012
Tango	CC	2012
Shasta Gold	CC	2012

Results and discussion

The trees at Schoonbee Estate were top worked in 2012 and at Klipbokspruit in 2011, this having an impact on the quality and quantity of the fruit. Klipbokspruit will be used as a back-up site in the future to supply additional information and were relocated to a new site. 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 second crop for this season with limited fruit and young tree internal quality and fruit size characteristics.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity, with a ratio of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

African Sunset (B24)

There was a better crop on the trees to complete four evaluations at the Engelbrecht site this season. The very large fruit size (count 1XX to 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 average with low juice (49%), Brix above 10 and acceptable acid levels. External colour peaked at T2 to T3 for this intermediate production area.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The fruit on all the trees at Moosrivier and Schoonbee were completely seedless, and fruit size at Moosrivier was large (count 1-1XXX) due to young trees and Schoonbee peaked from count 2 to 1X. The internal quality was average, low juice (from 42 % up to 49%) was captured throughout the season; Brix and acid levels were better at both sites (avg. 11.9 and 1.2%). The external colour improved this season and peaked between T2 and T4. Based on the internal quality results in Table 4.6.6.4, 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 2 produced good to very good internal quality fruit with 51.2% juice, Brix above 12 and acids above 1%. There was a slight colour delay on the IRM 2 fruit at peak maturity. Seed counts very low and peaked at 1.4 compared to IRM 1 with 1.8 seeds per fruit in the combined trial block (cross pollination).

Nadorcott ARC & LS

The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Nadorcott LS produced similar crops on the trees (60 kg/tree) compared to 2016 and the fruit size varied from count 2-1XX (smaller) due to the crop load. Both selections evaluated were completely seedless (0.1 seeds per fruit). Maturity seems to be two weeks earlier on the LS selection, according to Table 4.6.6.4, but information was limited due to only one evaluation being done (beginning to middle of June).

Mor 2, 15 and 26

Mor 2 and 15 was planted as control for the 26 selection at Moosrivier. The fruit size was erratic and peaked between count 2 and 1XX, medium to large fruit. The external colour development was yellow and peaked at T2. The internal quality was fair to good with juice levels of up to 51%, Brix up to 14 and fairly high acid levels (avg. 1.3%). There were on average 1.5 seeds in the fruit at the Moosrivier site. Based on the internal quality results in Table 4.6.6.4, estimated maturity will be the end of May to the beginning of June.

Tahoe Gold

Tahoe developed a small tree size (compact tree) when compared to the other UC5 varieties. The tree bears fruit in bundles in a similar way to grapefruit. The fruit size peaked from large to very large (count 1-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was no delay this season in the external colour development at Moosrivier and Schoonbee trial sites (T2). Tahoe produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated were completely seedless. The internal quality improved from average to good; juice up to 51%, Brix up to 13 and high acids (Table 4.6.6.4). Estimated maturity is end of May to middle June.

Tango

Tango was completely seedless at both trial sites. There was a good to very good crop on the trees at Schoonbee 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 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 56%), Brix was average/good (up to 12) and the acid levels stabilizing below 1.0 towards the end of the season. Fruit size varied on young trees and peaked at count 3 to 1XX (small/medium to large). Based on the internal quality results in Table 4.6.6.4, estimated maturity will be end of April to middle of May (delayed external colour).

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 with improved Brix for the season (above 12). External colour developed along with the internal quality towards the end of the evaluations (T1 to T2). Based on the internal quality results in Table 4.6.6.4, estimated maturity will be the middle to end of June.

Additional selection

The internal quality of Edit x Nova was good with high juice levels above 55% (except for one late evaluation on Sunki 812), no granulation problems in the fruit compared to Nova. Brix (average 12.7) and higher acids (1.2%), indicating the mid maturing characteristics of the selection in the intermediate production areas, with low seeded fruit (average 0.4 seeds per fruit). The fruit size peaked from count 3 to count 1X (smaller compared to 2016).

I22 cropped a very poor yield on the trees and no evaluations was possible, bearing in mind the young tree age. Meirav 63 and 119 developed a deep orange rind colour (T1 with peak maturity). Internal quality improved

and was good with high juice content (above 50%), Brix of 13.4 and acids above 1.1%. The fruit evaluated was low seeded (average 0.1) at the Moosrivier and Engelbrecht trial sites.

Shani SL bore a fair to good crop on the trees with medium to large fruit size (count 2 to count 1X) as well as deep orange rind colour (T2-T3). Internal quality was good early in the season with good juice (up to 58%), Brix (12.2) and acids (average 1.6%) levels. The fruit was low seeded to completely seedless (0 to 0.2 seeds per fruit).

Conclusion

The delay in external colour development improved this season; future evaluation will confirm this. Degreening may be an option for the Gold Nugget and TDEs, but ethylene reacted slowly or not at all with Tango (W. Murcott selection) and Nadorcott. Shasta Gold may be a possibility to consider for the warmer areas due to higher acid levels late in the season, when external colour becomes more intense (T2) due to temperature drop in winter. The appearance of the Shasta Gold's fruit improved this season (older trees at Schoonbee), and there was less ribbing on the fruit and smoother rind texture. In the warmer areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit, as well as protect against the high possibility of hail damage (Marble Hall area). Gold Nugget improved considerably with smoother fruit, large fruit size and fair to good internal quality. Yosemite Gold had the largest fruit size (light crop), followed by Tahoe Gold, and then Gold Nugget. The smaller fruit size was produced on Tango, reaching up to 1XX, with a lighter crop on the young trees at Moosrivier. There were no incidences of seed in the UCR 5 fruit at the trial sites compared to Yosemite Gold with 0.4 seeds per fruit last season. African Sunset bore large fruit at Engelbrecht Trust with a fairly light crop and large fruit size (protruding navel-end).

This was the first evaluation of IRM 1&2, Leanri, Meirav 119, Mor 2, 15, 26 and UC 5; the second evaluation of Edit x Nova and Meirav 63 at Moosrivier; and the third evaluation of African Sunset, IRM 2, Nadorcott ARC & LS at the Engelbrecht Trust site, so information is limited and future evaluations will improve recommendations on these varieties. The highest seed numbers were on IRM 2 and the Mor selections this season, followed by IRM 1. All the other selections developed very low seed numbers in the fruit. Meirav 63 and 119 performed well and internally the fruit was high in juice and Brix content with deep orange colour development.

Table 4.6.6.4. Internal fruit quality data for Mandarin hybrid selections at Engelbrecht Trust (Marble Hall), Moosrivier (Marble Hall) and Schoonbee Estate (Marble Hall) during the 2017 season.

Cultivar	Rootstock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
African Sunset	CC	17/05/2017	Engelbrecht	77-85	1XX-1XXX	49.3	10.4	1.00	10.4	0.1	T4
African Sunset	CC	09/06/2017	Engelbrecht	77-84	1XX-1XXX	44.8	10.0	0.90	11.1	0.1	T3-4
African Sunset	CC	05/07/2017	Engelbrecht	80-82	1XXX	49.7	11.8	0.90	13.1	0.1	T3
African Sunset	CC	25/07/2017	Engelbrecht	80-90	1XXX	50.9	10.5	0.83	12.7	0.1	T2-3
Edit x Nova	CC	12/04/2017	Engelbrecht	59-65	2-1	59.7	11.6	0.90	12.9	0.1	T5-7
Edit x Nova	CC	26/04/2017	Engelbrecht	55-59	3-2	60.0	13.4	1.03	13.0	0.1	T4
Edit x Nova	CC	09/06/2017	Engelbrecht	63-69	2-1X	57.5	13.4	1.13	11.9	0.1	T2-3
Edit x Nova	Sunki 812	04/04/2017	Moosrivier	58-64	3-1	55.8	11.1	0.91	12.2	0.0	T5
Edit x Nova	Sunki 812	26/04/2017	Moosrivier	60-65	2-1	58.4	12.9	1.19	10.8	1.0	T2
Edit x Nova	Sunki 812	06/06/2017	Moosrivier	60-62	2	48.7	13.9	0.98	14.2	0.9	T1-2
Gold Nugget	CC	08/06/2017	Moosrivier	75-78	1XX-1XXX	42.7	9.9	1.29	7.7	0.0	T2-3

Gold Nugget	CC	04/07/2017	Moosrivier	77-82	1XX-1XXX	45.5	11.3	1.28	8.9	0.0	T2
Gold Nugget	CC	15/08/2017	Moosrivier	85-92	1XXX	46.8	12.6	0.95	13.3	0.0	T2-3
Gold Nugget	C35	18/05/2017	Moosrivier	65-70	1-1X	42.9	9.2	1.36	6.8	0.0	T4
Gold Nugget	C35	06/06/2017	Moosrivier	67-70	1-1X	46.3	12.2	1.50	8.1	0.0	T2-3
Gold Nugget	C35	04/07/2017	Moosrivier	70-75	1X-1XX	49.1	12.8	1.20	10.7	0.0	T3
Gold Nugget	C35	15/08/2017	Moosrivier	65-70	1-1X	44.7	13.3	1.11	12.0	0.0	T2
Gold Nugget	X639	18/05/2017	Moosrivier	65-69	1-1X	46.2	11.5	1.34	8.6	0.0	T4
Gold Nugget	X639	08/06/2017	Moosrivier	67-72	1-1XX	45.5	12.3	1.23	10.0	0.0	T2-3
Gold Nugget	X639	04/07/2017	Moosrivier	72-75	1XX	45.4	12.5	1.20	10.4	0.0	T3
Gold Nugget	X639	15/08/2017	Moosrivier	71-76	1X	45.7	12.9	1.02	12.7	0.0	T2
Gold Nugget	CC	17/05/2017	Schoonbee	60-62	2	48.2	11.8	1.19	9.9	0.0	T3
Gold Nugget	CC	06/06/2017	Schoonbee	63-69	2-1X	46.2	12.9	1.13	11.4	0.0	T3-4
IRM 1	Sunki 812	05/07/2017	Moosrivier	61-68	2-1X	54.1	13.4	1.13	11.9	1.8	T2
IRM 2	CC	26/04/2017	Engelbrecht	57-61	3-2	51.0	11.6	1.33	8.7	0.2	T4
IRM 2	CC	17/05/2017	Engelbrecht	60-66	2-1	49.7	12.2	1.35	9.0	0.1	T2
IRM 2	CC	09/06/2017	Engelbrecht	68-75	1X-1XX	61.8	12.0	1.15	10.4	0.1	T2-3
IRM 2	CC	25/07/2017	Engelbrecht	72-80	1XX-1XXX	55.4	13.2	1.10	12.0	0.1	T2
IRM 2	Sunki 812	26/04/2017	Moosrivier	60-62	2	42.0	11.7	1.41	8.3	2.4	T4
IRM 2	Sunki 812	08/06/2017	Moosrivier	60-65	2-1	47.5	12.7	1.14	11.1	2.3	T3
IRM 2	Sunki 812	05/07/2017	Moosrivier	60-67	2-1	50.9	13.4	1.20	11.2	1.1	T2
IRM 2	Sunki 812	25/07/2017	Moosrivier	57-65	3-1	51.6	13.2	1.17	11.3	5.2	T2
Leanri	Sunki 812	26/04/2017	Moosrivier	60-65	2-1	55.6	13.1	1.06	12.4	5.0	T2
Meirav 63	CC	12/04/2017	Engelbrecht	61-68	2-1X	61.2	12.9	1.40	9.2	0.2	T4-6
Meirav 63	CC	26/04/2017	Engelbrecht	52-61	4-2	56.9	13.4	1.33	10.1	0.1	T4
Meirav 63	CC	09/06/2017	Engelbrecht	67-74	1-1XX	52.1	13.9	1.19	11.7	0.1	T1
Meirav 63	Sunki 812	26/04/2017	Moosrivier	55-60	3-2	50.0	13.2	1.67	7.9	0.8	T5
Meirav 63	Sunki 812	05/07/2017	Moosrivier	57-60	3-2	56.0	14.0	1.53	9.2	0.0	T2
Meirav 119	Sunki 812	26/04/2017	Moosrivier	56-61	3-2	53.6	12.9	1.50	8.6	0.6	T3
Mor 2	Sunki 812	08/06/2017	Moosrivier	63-69	2-1X	42.1	12.3	1.08	11.4	0.0	T3
Mor 2	Sunki 812	04/07/2017	Moosrivier	61-67	2-1	50.7	13.0	1.08	12.0	0.0	T2-3
Mor 2	Sunki 812	25/07/2017	Moosrivier	62-67	2-1	50.0	14.0	1.33	10.5	1.3	T2
Mor 15	Sunki 812	08/06/2017	Moosrivier	70-76	1X-1XX	48.5	11.1	1.14	9.7	2.6	T2-3
Mor 26	Sunki 812	08/06/2017	Moosrivier	63-67	2-1	46.9	13.0	1.30	10.0	3.5	T3-4
Mor 26	Sunki 812	25/07/2017	Moosrivier	55-65	3-1	49.5	14.2	1.10	12.9	1.5	T2
Nadorcott ARC	CC	09/06/2017	Engelbrecht	65-73	2-1XX	55.3	12.6	1.20	10.5	0.1	T2-3
Nadorcott ARC	CC	05/07/2017	Engelbrecht	62-70	2-1X	48.4	12.7	1.00	12.7	0.1	T2
Nadorcott LS	CC	09/06/2017	Engelbrecht	63-71	2-1X	56.8	11.9	1.09	10.9	0.1	T2-3
Shani SL	CC	26/04/2017	Engelbrecht	60-63	2	51.8	12.3	1.55	7.9	0.2	T4
Shani SL	CC	09/06/2017	Engelbrecht	63-69	2-1X	58.2	13.1	1.60	8.2	0.2	T2-3
Shani SL	CC	05/07/2017	Engelbrecht	64-68	1-1X	48.0	13.5	1.20	11.3	0.1	T2
Shani SL	Sunki 812	26/04/2017	Moosrivier	55-61	3-2	57.7	12.6	1.74	7.2	0.0	T4
Shani SL	Sunki 812	18/05/2017	Moosrivier	60-62	2	53.0	12.6	1.67	7.5	0.0	T2
Shani SL	Sunki 812	08/06/2017	Moosrivier	60-65	2-1	51.0	13.6	1.70	8.0	0.0	T3
Shasta Gold	CC	25/07/2017	Moosrivier	75-80	1XX-1XXX	48.4	12.2	1.64	7.5	0.0	T2
Shasta Gold	CC	15/08/2017	Moosrivier	70-75	1X-1XX	47.0	12.4	1.55	8.0	0.0	T2
Shasta Gold	C35	25/07/2017	Moosrivier	73-77	1XX	50.2	12.6	1.80	7.0	0.0	T2
Shasta Gold	C35	15/08/2017	Moosrivier	72-80	1XX-1XXX	48.7	12.7	1.69	7.5	0.0	T2-3

Shasta Gold	X639	25/07/2017	Moosrivier	75-82	1XX-1XXX	49.0	12.6	1.77	7.1	0.0	T2-3
Shasta Gold	X639	15/08/2017	Moosrivier	79-84	1XXX	49.6	12.9	1.66	7.8	0.0	T2-3
Tahoe Gold	CC	18/05/2017	Moosrivier	65-70	1-1X	50.6	12.9	2.41	5.4	0.0	T2
Tahoe Gold	CC	08/06/2017	Moosrivier	67-70	1-1X	44.5	13.2	1.93	6.8	0.0	T3
Tahoe Gold	C35	06/06/2017	Moosrivier	69-75	1X-1XX	51.7	12.6	1.64	7.7	0.0	T3-4
Tahoe Gold	X639	18/05/2017	Moosrivier	75-78	1XX-1XXX	51.7	10.4	1.59	6.5	0.0	T3
Tahoe Gold	X639	08/06/2017	Moosrivier	74-79	1XX-1XXX	56.1	10.0	1.51	6.6	0.0	T3
Tahoe Gold	CC	08/06/2017	Schoonbee	72-81	1XX-1XXX	49.7	10.7	1.09	9.8	0.0	T2
Tango	CC	04/04/2017	Moosrivier	56-65	3-1	56.1	10.3	1.54	6.7	0.0	T5-6
Tango	CC	26/04/2017	Moosrivier	57-60	3-2	55.9	10.9	1.45	7.5	0.0	T3
Tango	CC	26/04/2017	Moosrivier	60-63	2	54.5	10.3	1.61	6.4	0.0	T3
Tango	CC	06/06/2017	Moosrivier	60-65	2-1	40.4	11.2	1.32	8.5	0.0	T2-3
Tango	C35	04/04/2017	Moosrivier	60-63	2	54.2	8.9	1.71	5.2	0.0	T5
Tango	C35	08/06/2017	Moosrivier	58-60	3-2	42.7	12.4	1.35	9.2	0.0	T3
Tango	SC	26/04/2017	Moosrivier	55-62	3-2	45.9	11.9	1.62	7.3	0.0	T4
Tango	SC	26/04/2017	Moosrivier	56-60	3-2	56.3	10.7	1.64	6.5	0.0	T3
Tango	X639	08/06/2017	Moosrivier	63-66	2-1	49.2	12.2	1.33	9.2	0.0	T2-3
Tango	CC	17/05/2017	Schoonbee	63-73	2-1XX	52.1	12.7	1.01	12.6	0.0	T3
Tango	CC	08/06/2017	Schoonbee	63-69	2-1X	43.1	11.7	0.79	14.8	0.0	T3-4
Yosemite Gold	C35	07/04/2017	Moosrivier	75-77	1XX	43.1	12.5	1.51	8.3	0.0	T2
Yosemite Gold	CC	08/06/2017	Schoonbee	70-77	1X-1XX	57.5	12.7	1.22	10.4	0.0	T2

4.6.7 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise and Weipe)
Project 899B by J. Joubert (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat 'n baie belangrike punt uitlig wanneer dit by die keuse van kultivars vir aanplantings kom, sowel as die onderstam wat gebruik word. Die resultate van die 2017 seisoen vir hierdie warm produksie areas het steeds aangedui dat Tango die vroegste ryp geword het met die kleinste 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 'n 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 Andre, Nova en Nova SL het gevolg met goeie kleur ontwikkeling en lae tot saadlose vrugte. Volgende om ryp te word sal Samba wees, gevolg deur Furr met hoë saadtellings in die vrugte. Mor 26 volg nou, met 'n ligte oes op die bome (tussenstam opsie) en goeie interne kwaliteit. Tambor 1 en Tanor Late word laaste ryp by hierdie proef perseel en eindig die mandaryn seisoen vir die warm produksie area.

Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

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 2017 season still indicated that for the warm production areas Tango matures first with the smallest fruit size and good internal quality (acid levels drop early in season). Tahoe Gold followed, with improved external colour. Tango, Tahoe Gold, Yosemite Gold, Gold Nugget and Shasta Gold were completely seedless this season. Gold Nugget and Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at these trial sites, with average to good internal quality, as well as good external colour development. Shasta Gold was the last selection to mature at the end of June to middle of July, with the highest acids for this season, ending off the Mandarin Hybrid season for this trial.

Etna was first to mature from the new additional selections, followed by Sirio with low-seeded fruit this season. Saint Andre, Nova and Nova SL follows with good external colour development and low seeded to seedless fruit. Next to mature will be Samba, followed by Furr with high seed numbers in the fruit. Mor 26 follows, cropping light yields on the trees (interstock option) and good internal quality. Tambor 1 and Tanor Late matures last at this trial site, ending of the mandarin season for the hot areas.

Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot, dry production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Alicedale (Tshipise) and NGB (Weipe) in the Limpopo region. The following varieties were evaluated: Etna, Furr, Gold Nugget, Mor 26, Nova (Control), Nova SL, Or 4, Page, Saint Andre, Samba, Shasta Gold, Sirio, Tahoe Gold, Tambor 1, Tango, Tanor Late, Tasty 1, Tast 2 and Yosemite Gold.

Table 4.6.7.1. List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2017 season.

Selection	Rootstock	Topworked
Etna	X639	2013/2014
Furr (Clemcott)	X639	2013/2014
Gold Nugget	X639	2010
Mor 26	X639	2013/2014
Nova	X639	2013/2014
Nova SL	X639	2013/2014
Page	X639	2013/2014
Saint Andre	X639	2013/2014
Samba	X639	2013/2014
Shasta Gold	X639	2010
Sirio	X639	2013/2014
Tahoe Gold	X639	2010
Tambor 1	X639	2014
Tango	X639	2010

Tanor Late	X639	2013/2014
Tasty 1	X639	2013/2014
Tasty 2	X639	2013/2014
Yosemite Gold	X639	2010

Table 4.6.7.2. List of Mandarin Hybrid selections evaluated at NGB (Weipe) during the 2017 season.

Selection	Rootstock	Topworked
Shasta Gold	X639	2011
Tahoe Gold	X639	2011
Yosemite Gold	X639	2011
Gold Nugget	X639	2011
Tango	X639	2011

Results and discussion

Limited information was available at Alicedale (new trial site) due to the first crop on the newly topworked mandarin trees. There was no fruit on the Orri and Winola trees. 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 overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. Fruit size at Alicedale and NGB remained the same this season and peaked at large (count 1-1XX) and the fruit on all the trees were completely seedless. The internal quality of the fruit was back to fair (fair/good in 2016) and developed juice (avg 47%), Brix (11.1) and acid levels below 1.0% avg and an external colour at T3. Future evaluations will determine the feasibility of Gold Nugget in the hot areas. Based on the internal quality results in Table 4.6.8.3, estimated maturity will be the middle to end of June.

Shasta Gold

Shasta Gold developed fairly round fruit (Minneola tangelo type) on the trees at the trial site. There was ribbing on most of the fruit, as well as sunburn. There was a similar crop on the Shasta Gold trees (40-50 kg/tree) this season. The tree size remained on the smaller and compact side. There were a lot of thorns on the bearing branches of the trees. Rind texture was rough (scale 4-5). The flavour was fair with high rind oil content. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless at both locations. The fruit size peaked from large to very large (count 1X-1XXX). The internal quality was good with high juice (avg 52%), Brix (10) levels and acid levels (above 1.0% final evaluation). Based on the internal quality results in Table 4.6.8.3, maturity will be middle of June to the end of July.

Tahoe Gold

Tahoe Gold produced a good crop on the trees at Alicedale and NGB. This selection developed a small tree size when compared to the other UC5 varieties (compact tree). The tree bears fruit in bundles in a similar way to grapefruit. The fruit size remained the same this season and peaked from large to very large (count 1X-1XX) 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 53%, Brix averaging 11 and acid levels were acceptable (1.0%). Based on the internal quality results in Table 4.6.8.3, estimated maturity was the middle of May to middle of June.

Tango

Tango remained completely seedless at NGB and Alicedale this season (low seed numbers last year at Alicedale). There was a good crop (263.5 kg/tree at Alicedale) on the trees. 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 54%), Brix improved for this selection (average 10 with final evaluation), acid levels (below 1.0) decreased rapidly early in the season (indicating a short shelf life) and deep orange coloured fibre. Fruit size increased and peaked at count 2 to 1X (medium to large). Based on the internal quality results in Table 4.6.8.3, estimated maturity will be middle of April.

Yosemite Gold

Yosemite Gold cropped a poor yield (off year) on X639 at Alicedale (18.5 kg/tree) 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 channeled into fruit set and crop (dwarfing rootstocks). 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 lower Brix (10.2) and acid levels (1.1%) with lower juice for the season (average 45%). External colour developed along with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 4.6.8.3, estimated maturity will be the middle to end of June.

Additional selections (second crop)

Etna bore a good crop (78.5kg/tree) with medium to very large fruit (count 2 to 1X) and average/good internal quality (low juice and Brix). External colour was delayed at peak maturity (T3 to T4) and all the fruit evaluated was seedless. Furr (Clemcott) was included as a control for the hot production areas. Fruit size was large to very large (count 1XX -1XXX), good internal quality with fairly low acids (0.88%) early in the season and very high number of seeds in the fruit (avg. 12 seeds). Mor 26 cropped very low numbers of fruit on the trees (count 1 to 1X). External colour was delayed (T3 to T4) with optimum maturity and the fruit was low seeded (0.4 seeds per fruit).

Nova was included as a control for the other early selections in the trial, the fruit peels fairly difficult and low numbers of seed developed in the fruit (1.0 seeds). External colour was late and the fruit size varied between count 1 and count 1XX (medium to large) with a 109.5 kg per tree yield produced. Nova SL (ARC) produced a very course rind texture on the fruit with medium to very large fruit size. The acid levels in the fruit remained higher compared to Nova (control) and the external colour development delayed this season at peak maturity. Fruit was seedless compared to one evaluation counting 1.0 seeds per fruit at the Nova.

Orri trees were very aggressive growing, producing no crop on the trees this season. Page had a fair yield on the trees (59.2 kg/tree) with medium to large fruit size (count 2 to 1XX). Internal quality was fair to good, acid levels dropped early in the season (0.76 with first evaluation) with delayed colour development (T2 to T3). The fruit was completely seedless compared to last season's 0.6 seeds per fruit and the rind texture was very smooth with deep orange colour development.

Saint Andre originated from a Furr orchard in the Eastern Cape. The fruit matures before Nova with good internal quality juice (51%) and Brix (above 12), but fairly low acids (average 0.7). Seed count were higher (0.8 seeds per fruit) compared to Nova and Nova SL and fruit size varied between count 1 and count 1XX. Samba produced an average second crop on the large fast growing thornless trees (84 kg/tree). Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. Seed counts were very low,

average 0.1 seeds per fruit and the fruit size peaked from count 1 to count 1XX. Sirio produced large to very large coarse fruit (count 1X to 1XXX) with average internal quality (low juice) and improved colour development on the trees.

Tambor 1 cropped fruit for the first time this season (68 kg/tree). Fruit size peaked at count 1XXX (very large) with 0.6 seeds per fruit. The internal quality was good; juice of 55%, Brix of 9.4 and high acids early in the season of 1.6 and colour development peaked at T4. Tanor Late cropped a very good yield (146.5kg/tree) on the trees compared to 2016. Young Tanor Late trees will produce large fruit size (1XXX) on the trees and will decrease when the trees mature. This selection will be late maturing (high acids and delayed colour). Tasty 1 improved this season and cropped 174.5 kg per tree compared to Tasty 2 with 77.5 kg per trees. Fruit size varied from large to very large (count 1X to 1XXX) fruit and the internal quality was fair to average (good Brix and low acids) with low seed counts.

Conclusion

The external colour delay (internal quality improved with more mature trees) in the hotter areas remained a problem; future evaluations will confirm this. Degreening may be an option for the Gold Nugget and TDEs, but ethylene reacted slowly or not at all for Tango (W. Murcott selection) and Nadorcott. Shasta Gold may be a possibility to consider for the hot areas due to higher acid levels late in the season, when external colour becomes more intense (T1-2) due to temperature drop (winter time). The appearance of Shasta Gold's fruit in the Tshipise and Weipe area (hot) may be a problem. In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack-out percentage (Shasta had severe sunburn at Alicedale and NGB). Gold Nugget improved considerably with fair/good internal quality, better production and large fruit size. Tahoe Gold had the largest fruit size, followed by Yosemite Gold, Shasta Gold and then Gold Nugget. The smallest fruit size was produced on Tango. Tango cropped the best yield (263.5 kg/tree) on the trees followed by Shasta Gold with 211.5 kg per tree.

This was the second evaluation of Etna, Furr (control), Mor 26, Nova (Control), Nova SL, Page, Saint Andre, Samba, Sirio, Tambor 1 (control), Tanor Late, Tasty 1 and Tasty 2, so information is limited and future evaluations will improve recommendations on these varieties. The promising selections at this early stage was Nova SL (cropping 129.5 kg/tree), Page, Saint Andre (cropping 95.5kg/tree) and Samba (cropping 84 kg/tree) with good internal quality fruit, fair to good colour development and crop on the trees. Seed numbers on these selections was very low to completely seedless in the combination trial block with cross pollinating cultivars included.

Table 4.6.7.3. Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) and NGB (Weipe) during the 2017 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Etna	X639	05/04/2017	Alicedale	65-70	1-1X	41.1	8.5	0.97	8.8	0.0	T3-4
Etna	X639	25/05/2017	Alicedale	63-70	2-1X	47.8	9.2	0.83	11.1	0.0	T3-4
Furr	X639	03/05/2017	Alicedale	76-80	1XX-1XXX	51.8	13.3	0.88	15.1	12.0	T2
Gold Nugget	X639	03/05/2017	Alicedale	70-76	1X-1XX	48.2	11.5	0.89	12.9	0.0	T3
Gold Nugget	X639	24/05/2017	NGB	65-69	1-1X	45.8	10.6	0.75	14.1	0.0	T3
Mor 26	X639	03/05/2017	Alicedale	65-70	1-1X	55.6	10.8	0.84	12.9	0.0	T4
Mor 26	X639	25/05/2017	Alicedale	65-70	1-1X	49.7	9.1	0.66	13.8	0.4	T3
Nova	X639	05/04/2017	Alicedale	64-73	1-1XX	46.7	12.9	0.80	16.1	0.0	T4
Nova	X639	03/05/2017	Alicedale	65-75	1-1XX	50.0	11.2	0.76	14.7	0.0	T2
Nova	X639	25/05/2017	Alicedale	73-78	1XX	40.2	9.9	0.78	12.7	1.0	T2

Nova SL	X639	05/04/2017	Alicedale	71-75	1X-1XX	43.1	12.3	0.93	13.2	0.0	T4
Nova SL	X639	03/05/2017	Alicedale	72-75	1XX	50.5	12.2	1.00	12.2	0.0	T3
Page	X639	05/04/2017	Alicedale	61-65	2-1	45.2	11.6	0.76	15.3	0.0	T3-4
Page	X639	03/05/2017	Alicedale	67-73	1-1XX	52.2	11.7	0.89	13.1	0.0	T2
Saint Andre	X639	05/04/2017	Alicedale	65-71	1-1X	41.9	12.7	0.72	17.6	0.0	T3-4
Saint Andre	X639	03/05/2017	Alicedale	69-72	1X-1XX	50.9	12.3	0.73	16.8	0.8	T2
Samba	X639	05/04/2017	Alicedale	65-70	1-1X	50.3	11.0	0.81	13.6	0.0	T3
Samba	X639	03/05/2017	Alicedale	71-74	1X-1XX	53.4	11.5	0.89	12.9	0.1	T2
Shasta Gold	X639	25/05/2017	Alicedale	80-85	1XXX	50.3	9.8	1.39	7.1	0.0	T2-3
Shasta Gold	X639	24/05/2017	NGB	72-78	1XX-1XXX	52.1	10.3	1.23	8.4	0.0	T3
Shasta Gold	X639	21/06/2017	NGB	77-81	1XX-1XXX	52.3	9.3	1.00	9.3	0.0	T2-3
Shasta Gold	X639	12/07/2017	NGB	70-81	1X-1XXX	51.7	10.5	1.08	9.8	0.0	T2
Sirio	X639	03/05/2017	Alicedale	70-80	1X-1XXX	47.4	13.5	1.07	12.6	0.0	T2
Sirio	X639	25/05/2017	Alicedale	78-82	1XXX	41.2	11.2	1.00	11.2	0.3	T2
Tahoe Gold	X639	04/03/2017	Alicedale	70-76	1X-1XX	55.0	10.6	1.26	8.4	0.0	T4
Tahoe Gold	X639	21/06/2017	NGB	62-66	1-1XX	50.4	11.4	0.73	15.6	0.0	T2-3
Tambor 1	X639	25/05/2017	Alicedale	80-85	1XXX	55.0	9.4	1.63	5.8	0.6	T4
Tango	X639	25/05/2017	Alicedale	65-70	1-1X	54.0	9.6	0.73	13.2	0.0	T4
Tango	X639	02/05/2017	NGB	65-70	1-1X	54.2	11.3	0.85	13.3	0.0	T4
Tango	X639	24/05/2017	NGB	61-63	2	54.2	10.4	0.74	14.1	0.0	T2
Tanor Late	X639	25/05/2017	Alicedale	85-91	1XXX	41.8	9.6	1.07	9.0	0.0	T3-4
Tasty 1	X639	03/05/2017	Alicedale	70-73	1X-1XX	52.4	13.3	0.75	17.7	0.0	T2
Tasty 1	X639	25/05/2017	Alicedale	70-77	1X-1XX	43.3	12.5	0.55	22.7	1.1	T2
Tasty 2	X639	03/05/2017	Alicedale	72-78	1XX-1XXX	51.6	12.8	0.94	13.6	0.0	T3
Tasty 2	X639	25/05/2017	Alicedale	79-82	1XXX	38.3	10.4	0.89	11.7	0.0	T2
Yosemite Gold	X639	25/05/2017	Alicedale	80-84	1XXX	41.5	10.4	1.37	7.6	0.0	T2
Yosemite Gold	X639	24/05/2017	NGB	70-77	1X-1XX	48.4	10.0	0.85	11.8	0.0	T3

4.6.8 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino)

Project 963C by J. Joubert (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die twee produksie areas (Nelspruit and Marble Hall), a.g.v. die klimaatsone (intermediêre areas) en boom ouderdom (2011). Die resultate vir die Nelspruit produksie area het aangedui dat Nadorcott LS eerste gereed was vir die oesproses (twee tot drie weke voor die ARC seleksie), gevolg deur Nadorcott ARC met medium tot groot vrugte vir hierdie seisoen en baie goeie kleur ontwikkeling. Edit x Nova en Meirav 63 het ook onder die vroeë seleksies ingepas. IRM 2 en Shani SL was volgende gereed vir oes gewees meer na die middel van die mandaryn seisoen; met minder ribbing op die vrugte in vergelyking met IRM 1 by die ander proef persele. Al die vrugte geëvalueer het baie

lae saadinhoud aangedui a.g.v. die kruisbestuivings impak van aangrensende varieteite met saad, behalwe vir IRM 2.

Summary

The quality of the Mandarin Hybrid fruit was similar in the two different production areas (Nelspruit and Marble Hall), due to the similar climatic region (intermediate areas) and tree age (2011). The results indicated that in the Nelspruit production area, Nadorcott LS matures first (two to three weeks before ARC selection), followed by Nadorcott ARC with medium to large fruit size for this season and excellent colour development. Edit x Nova and Meirav 63 also indicated to be fairly early maturing selections. IRM 2 and Shani SL matures next towards the middle of the mandarin season with less ribbing on the fruit compared to IRM 1 and Phoenix at the other trial sites. All the fruit evaluated this season had very low seed numbers due to cross pollination impact from the seeded varieties close by, except for IRM 2.

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. The following varieties were evaluated: Edit x Nova, Irradiated I22, IRM 2, Meirav 63, Nadorcott ARC & LS, Shani SL and Valley Gold.

Table 4.6.8.1. List of Mandarin Hybrid selections evaluated at Karino-koöp (Nelspruit) during the 2017 season.

Selection	Rootstock	Planted
Edit x Nova	CC	2011
Irradiated I22	CC	2011
IRM 2	CC	2011
Meirav 63	CC	2011
Nadorcott ARC	CC	2011
Nadorcott LS	CC	2011
Shani SL	CC	2011
Vallei Gold	CC	2011

Results and discussion

The trees at Karino-koöp were evaluated for the third time this season; this having an impact on the quality and quantity of the fruit. More information was available and future evaluations will be conducted. There was a new trial site planted on the Karino premises to expand the evaluation opportunities and the trees will bear fruit for the first time in 2017

For Mandarin Hybrids, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which the fruit is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

IRM 2

The tree shape of the IRM 2 was very upright (V-shaped) with no thorns. IRM 2 produced a alternative crop on the trees and fruit size peaked from small/medium to large (count 3 to 1). The seed numbers increased this season from 0.3 seeds to 3.1 seeds per fruit, peels easily with some ribbing on the fruit (typical Murcott characteristic). Juice levels increased compared to 2016 and averaged above 52%, Brix was very good (up to 13) and acids were above 1.3%. The external colour was deep orange and peaked at T2. Based on the internal quality results in Table 4.6.10.2, estimated maturity will be the middle to end of June

Nadorcott ARC & LS

The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections developed good internal quality with high juice levels (from 54 to 61%), Brix averaging 11 and acceptable acids (avg. 1.1%). Nadorcott LS produced a better crop on the trees compared to the ARC selection and the fruit size was smaller this season due to the crop load; varied from count 2-1. Both selections evaluated were completely seedless at the Karino trial site. Maturity seems to be two to three weeks earlier on the LS selection, but information was limited due to the third year of evaluation (end of April to the end of May), according to Table 4.6.10.2.

Additional selection

The internal quality of Edit x Nova was good with high juice levels above 58%, no granulation problems in the fruit compared to Nova. Brix (above 12) and lower acids (0.8%), 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 no crop on the tree this season and evaluations will continue next season.

Meirav 63 developed a deep orange rind colour (T1 to T2 with peak maturity). Internal quality was good with high juice content (above 58%), Brix of 11.8 and good acids (average 1.1%). The seed content decreased from 1.2 to 0.3 seeds per fruit evaluated at the Karino trial site (cross pollination).

Shani SL bore a fair to good crop on the trees with medium to large fruit size (count 2 to count 1XX) as well as deep orange rind colour (T2-T3). Internal quality was good early in the season with good juice (54%), Brix (10.4) and acids (average 1.4%) levels. The fruit was completely seedless.

Conclusion

This was the second evaluation of Edit x Nova, Irradiated I22, Meirav 63 and Shani SL; and the third evaluation of IRM 2, Nadorcott ARC and Nadorcott LS at the Karino site, so information is limited and future evaluations will improve recommendations on these varieties. All the selections developed very low seed numbers in the fruit compared to the previous season, where they were completely seedless for this trial, except for IRM2 with average 3.1 seeds per fruit. There was a good external colour development on the Nadorcott, Meirav 63 and Shani SL selections (deep orange).

Table 4.6.8.2. Internal fruit quality data for Mandarin hybrid selections at Karino- koöp (Nelspruit) during the 2017 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Edit x Nova	CC	13/04/2017	58-63	1-1X	65.0	12.0	0.71	16.9	0.0	T5
Edit x Nova	CC	24/04/2017	59-63	1-1XX	63.1	12.4	0.78	15.9	0.0	T4
Edit x Nova	CC	06/06/2017	56-59	1X	58.5	12.7	0.82	15.5	0.0	T2
IRM 2	CC	13/04/2017	60-63	2	53.9	9.6	1.62	5.9	3.3	T4
IRM 2	CC	24/04/2017	58-62	3-2	55.5	10.7	1.46	7.3	2.9	T4
IRM 2	CC	30/06/2017	60-65	2-1	49.1	13.0	1.35	9.6	3.1	T2
Meirav 63	CC	13/04/2017	55-57	3	59.8	10.8	1.42	7.6	0.0	T4
Meirav 63	CC	24/04/2017	60-64	2-1	58.4	11.1	1.06	10.5	0.8	T2-3
Meirav 63	CC	21/07/2017	60-65	2-1	62.3	13.5	0.78	17.3	0.0	T1-2

Nadorcott ARC	CC	13/04/2017	60-65	2-1	57.6	9.6	1.01	9.5	0.0	T5
Nadorcott ARC	CC	24/04/2017	60-64	2-1	56.8	9.9	1.01	9.8	0.0	T5-6
Nadorcott ARC	CC	30/06/2017	59-63	2	53.5	12.7	1.25	10.2	0.0	T2
Nadorcott ARC	CC	21/07/2017	59-63	2	61.0	11.5	1.15	10.0	0.0	T2
Nadorcott LS	CC	13/04/2017	62-65	2-1	58.6	9.6	1.05	9.1	0.0	T5
Nadorcott LS	CC	24/04/2017	60-65	2-1	60.4	9.9	1.05	9.4	0.0	T4
Nadorcott LS	CC	06/06/2017	60-64	2-1	59.0	13.3	0.95	14.0	0.0	T2
Nadorcott LS	CC	30/06/2017	59-63	2	54.5	12.2	1.04	11.7	0.0	T2
Nadorcott LS	CC	21/07/2017	55-60	3-2	60.0	13.0	1.17	11.1	0.0	T2
Shani SL	CC	24/04/2017	60-65	2-1	54.4	10.7	1.43	7.5	0.0	T5
Shani SL	CC	06/06/2017	76-80	1XX	52.6	10.1	1.23	8.2	0.0	T2-3
Shani SL	CC	30/06/2017	59-65	2-1	54.3	13.4	1.43	9.4	0.0	T2
Valley Gold (B17)	CC	13/04/2017	62-65	2-1	60.0	10.8	1.75	6.2	0.0	T5
Valley Gold (B17)	CC	24/04/2017	62-65	2-1	58.2	11.9	1.34	8.9	2.0	T5-6
Valley Gold (B17)	CC	30/06/2017	60-64	2-1	60.0	12.7	1.21	10.5	0.0	T2

4.6.9 PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Karino)

Project 963B by J. Joubert (CRI)

Opsomming

In die Nelspruit produksie area word M7 eerste ryp met die beste interne kwaliteit (laer sure) vir hierdie proef, gevolg deur Fukumoto 2 met medium tot groot vruggroote vir hierdie seisoen en aanvaarbare kleur ontwikkeling. Newhall word volgende ryp met goeie sap vlakke en vertraagde eksterne kleur op die vrugte. Clark, Fischer en Dream volg, om die middel van die nawel soetlemon reeks te vul, met medium tot groot vruggroote en hoë Brix vlakke (gemiddeld 10). Hutton se rypwordings inligting was beperk a.g.v. die evaluasie data beskikbaar en swak interne kwaliteit van die vrugte (lae sap en suur).

Summary

In the Nelspruit production area, M7 matures first with the best internal quality (lower acids) for the trial, followed by Fukumoto 2 with medium to large fruit size for this season and acceptable colour development. Newhall matures next with good juice levels and delayed external colour on the fruit. Clark, Fischer and Dream matured next, towards the middle of the navel orange range, with medium to large fruit size and high Brix levels (average 10). Due to limited evaluation data and poor internal quality (low juice and acids) this season, maturity estimates were not possible for Hutton.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (juice, Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in intermediate production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from Karino-koöp (Nelspruit) in the Mpumalanga region. The following early to mid maturing selections were evaluated: Clarke, Dream, Fischer, Fukumoto 2, Hutton, M7 and Newhall.

When the ratio between sugar and acid is 10:1, the navel fruit are considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases

to 11:1, after which the fruit is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 4.6.9.1. List of navel selections evaluated at Karino-koöp (Nelspruit) during the 2017 season.

Selection	Rootstock	Planted
Clarke	CC/SC	2011
Dream	SC	2011
Fischer	SC	2011
Fukumoto 2	SC	2011
Hutton	CC	2011
Newhall	SC	2011
M7	CC/SC	2011

Results and discussion

The trees at Karino-koöp were evaluated for the third 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 average 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 T2-3. The navel-end was fairly open and the fruit shape oblong; rind texture was fairly coarse. Clarke produced fruit with poor juice levels (up to 45%), low Brix 9.2 and acids 0.8% from the second evaluation. Based on the internal quality results in Table 4.6.9.2, estimated maturity will be the end of May to the middle of June.

Dream

The juice levels of the Dream fruit were better compared to Clarke, averaging 54%, higher Brix of up to 10 and similar acids (0.8%). Dream fruit developed a fairly smooth rind, small navel-end and fairly round fruit shape. The external colour development improved (older trees) and peaking at T2 with the last evaluation. Maturity seems to be end of May to middle June according to Table 4.6.9.2.

Fischer (control)

The acid levels on Fischer navel improved, averaging 0.8% for the season. Juice and Brix improved up to 57% and 10 respectively. Externally the fruit colour development peaked from T3 to T5. Fruit size was smaller for navel production, medium fruit size (count 88 to 72). To estimate maturity was difficult due to the low acid levels early in the season, based on the internal quality results in Table 4.6.9.2, 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 72 to count 64). Juice levels were better (50%), lower Brix of 10 and very low acids (0.5%) with a delayed external colour of T3-4. 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 settled down (irratc last season) for the season and peaked at large fruit (count 64 to count 56). Juice levels decreased compared to the 2016 season and averaged at 43%, still low 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 T1 and T2 with the final evaluation. To estimate

maturity was difficult due to the low acid levels early in the season, future evaluations will provide more information.

M7

M7 produced a good (CC) to very good (SC) yield on the trees for the season, as well as early external colour development (Carrizo T3, Swingle T3). There were chimeras and mutations on a number of the fruit in the trial block. The juice levels (up to 55%) were the best for the season compared to the other navel selections at the trial site. Fruit size on CC was bigger (count 72 to count 64) compared to SC (count 88 to count 64) due to the crop load on the trees. Brix and acid content on both rootstocks was good to very good, up to 12 and 0.8% respectively. Based on the internal quality results in Table 4.6.9.2, 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 improved acids (0.8%) taking into consideration the Swingle rootstock combination. In general, Swingle increases acid levels in the fruit, as well as delaying external colour development. Brix average decreased to 9.5 and juice levels peaked at a good 51%. The Newhall fruit remained fairly green (T2) when the final evaluation was completed. Maturity seems to be middle of April to end of April.

Conclusion

This was the third evaluation of all the navel selections at this new trial site in Karino, so information is limited and future evaluations will improve recommendations on these varieties. The juice levels on most of the combinations improved from low to average/good; above the minimum export requirement of 48% with the exception of one Clarke and Hutton evaluation. Acids remained low from the beginning of the season up to peak maturity. The external colour development on all the selections was delayed for the season, not the ideal situation with the low internal quality and more specifically the low acid levels.

Future evaluations will be crucial to determine the performance of these early to mid navel selections for the Karino area. There was severe hail damage this season in the area and poor external appearance of the fruit.

Table 4.6.9.2. Internal fruit quality data for Navel selections at Karino- koöp (Nelspruit) during the 2017 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Colour
Clarke	CC	08/05/2017	75-79	72-64	42.3	9.5	0.85	11.2	T3
Clarke	SC	06/06/2017	85-88	56-48	45.2	9.1	0.75	12.1	T2-3
Dream	SC	29/03/2017	75-80	72-64	50.4	8.9	0.75	11.9	T5-7
Dream	SC	24/04/2017	71-75	88-72	56.0	10.3	0.77	13.4	T6
Dream	SC	08/05/2017	72-80	88-64	55.9	10.1	0.87	11.6	T3
Fisher	SC	24/04/2017	69-77	88-72	56.7	10.4	0.78	13.3	T5-7
Fukumoto 2	SC	29/03/2017	72-78	88-64	48.4	8.9	0.50	17.8	T4-5
Fukumoto 2	SC	13/04/2017	75-80	72-64	50.2	10.4	0.52	20.0	T4-5
Fukumoto 2	SC	24/04/2017	74-78	72-64	50.6	10.3	0.50	20.6	T3-4
Hutton	CC	06/06/2017	78-85	64-56	46.8	8.6	0.81	10.6	T2
M7	CC	13/04/2017	77-81	72-64	50.3	12.1	0.65	18.6	T3-4
M7	CC	24/04/2017	73-80	72-64	54.9	11.1	0.73	15.2	T3
M7	SC	22/03/2017	70-77	88-72	47.9	11.8	0.85	13.9	T4-5
M7	SC	13/04/2017	71-76	88-72	49.6	12.0	0.70	17.1	T3
M7	SC	24/04/2017	71-80	88-64	53.5	12.2	0.80	15.3	T3
Newhall	SC	29/03/2017	71-80	88-64	49.7	8.4	0.72	11.7	T5-6
Newhall	SC	24/04/2017	77-82	72-56	53.7	8.0	0.68	11.8	T3-4
Newhall	SC	13/04/2017	71-77	88-72	51.1	10.3	0.69	14.9	T5
Newhall	SC	08/05/2017	70-75	88-72	51.2	11.1	1.06	10.5	T2

4.6.10 PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Marble Hall and Groblersdal)

Project 941A by J. Joubert (CRI)

Opsomming

Die Navel proef by Engelbrecht en Schoonbee was vir die derde keer hierdie seisoen geëvalueer, en Moosrivier sal eers weer in 2018 geëvalueer word (verskuif proef perseel). Hierdie produksie area ontwikkel lae suur vlakke in die navel vrugte vroeg in die seisoen en kompensasië vir laer uitvoer suur standaarde moet versoek word. M7 en Golden Buckeye word eerste ryp by die Engelbrecht en Schoonbee proef perseel met vertraagde kleur ontwikkeling (Golden Buckeye), gevolg deur Painter Early met goeie Brix vlakke. Volgende in lyn sal Clarke en Hutton wees wat die middel van die navel seisoen verteenwoordig; wat medium tot groot vrugte op Carrizo onderstam ontwikkel. Evaluasies sal voortgaan as gevolg van die beperkte inligting beskikbaar.

Summary

The navel trial at Engelbrecht and Schoonbee was evaluated for the third time this season, and the Moosrivier site will only be evaluated in 2018 for the first time (relocate to new trial site). This production region develops low acid levels on the navel fruit early in the season and compensation for lower export acid standards must be required. M7 and Golden Buckeye matures first at the Engelbrecht and Schoonbee trial site with delayed colour development (Golden Buckeye), followed by Painter Early with good Brix levels. Next in line will be Clarke and Hutton representing the middle of the navel season; developing medium to large fruit on Carrizo rootstock. Evaluations will continue due to limited information available.

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 Engelbrecht Trust (Marble Hall) and Schoonbee Estate (Groblersdal) from the Limpopo region. The following early, mid and late maturing selections were evaluated: Clarke, Golden Buckeye, Hutton, M7 and Painter Early.

For navels, the fruit is considered to be at peak maturity when the ratio between sugar and acid is 10:1. 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 overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instance of quality and rind issues.

Table 4.6.10.1. List of navel selections evaluated at Engelbrecht Trust (Marble Hall) during the 2017 season.

Selection	Rootstock	Planted
Clarke	CC	2011
Hutton	CC	2011
M7	CC	2011

Table 4.6.10.2. List of navel selections evaluated at Schoonbee Estate (Groblersdal) during the 2017 season.

Selection	Rootstock	Topwork
Golden Buckeye	CC	2012
Painter Early	CC	2012

Results and discussion

The new trial site at Moosrivier will come into production next year to improve the navel range and evaluations for this intermediate production region.

Painter Early developed the lowest juice (42%) and M7 (early selection) the lowest acid (0.6%) levels in the fruit with optimum external colour (T2) whilst Clarke, Hutton and M7 developed the best juice of average 48%. M7 followed by Clarke produced the best Brix above 12 and Clarke, as well as Hutton and Painter Early acids above 0.8% (Table 4.6.10.3). M7 developed low acids in the fruit (0.6%), the lowest for the trial site in combination with Golden Buckeye (indication of early selections). The lowest Brix was on Golden Buckeye in combination with Carrizo rootstock (high IQ and acid inducing rootstock). Brix:acid ratios peaked from 9.3 to 19.8 (well overmatured) due to the fairly low acids. External colour improved on 5 of the 6 selections, except for Golden Buckeye. Evaluations will continue before any recommendations are made.

Conclusion

The internal quality of the fruit was poor to fair and the juice levels remained low compared to 2016; average below the minimum standards (48.5%), except for Hutton. All the Brix levels for this trial improved (average 11.0), except for Golden Buckeye with 8.3. The acid levels improved (average 0.8%) from the second evaluation and the shelf life of the fruit will be better (shelf life remained short). Colour development was better on the overmatured fruit early in the season (peaked between T2 and T3), except for Golden Buckeye st T3-4.

Table 4.6 .10.3. Internal fruit quality data for Navel selections at Engelbrecht Trust (Marble Hall) and Moosrivier Estate (Groblersdal) during the 2017 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Colour
Clarke	CC	09/06/2017	Engelbrecht	76-82	72-56	49.0	11.5	0.85	13.5	T2-3
Clarke	CC	25/07/2017	Engelbrecht	70-85	88-56	46.5	12.5	0.73	17.1	T2
Golden Buckeye	CC	17/05/2017	Schoonbee	73-80	72-64	48.7	8.0	0.66	12.1	T3
Golden Buckeye	CC	08/06/2017	Schoonbee	78-84	64-56	44.3	8.4	0.80	10.5	T3
Golden Buckeye	CC	04/07/2017	Schoonbee	80-85	64-56	43.1	9.4	0.60	15.7	T3-4
Hutton	CC	09/06/2017	Engelbrecht	65-70	105-88	51.1	9.5	0.70	13.6	T3-4
Hutton	CC	25/07/2017	Engelbrecht	76-80	72-64	45.0	12.3	0.96	12.8	T2
M7	CC	12/04/2017	Engelbrecht	75-85	72-56	46.9	11.9	0.69	17.2	T2-5
M7	CC	26/04/2017	Engelbrecht	75-83	72-56	48.3	12.2	0.60	20.3	T2
Painter Early	CC	17/05/2017	Schoonbee	75-81	72-64	43.0	9.2	0.83	11.1	T2
Painter Early	CC	08/06/2017	Schoonbee	73-81	72-64	48.0	12.3	0.72	17.1	T2

4.6.11 PROGRESS REPORT: Evaluation of Lemon selections in the intermediate production areas (Marble Hall)

Project 941B by J. Joubert (CRI)

Opsomming

Hierdie was die tweede jaar wat die suurlemoen kultivars by Moorsivier in Marble Hall geëvalueer word. Dit was Willowtree Long se tweede oes by hierdie proef blok vir die noordelike areas as gevolg van die hoë temperature en die tipiese suurlemoen groeitempo (groeikragtig). Eureka, Lisbon en Genoa het 'n beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseel; Genoa set slegs een hoofset per seisoen. Lisbon Yen Ben ontwikkel die kleinste vruggrootte op die bome (telling 6 tot 5) in vergelyking met die ander seleksies en was meer bestand teen koue toestande. Al die seleksies was aangeplant aan die einde van die 2013 seisoen op X639, vir die aanpasbaarheid by die hoër pH gronde en verenigbaarheid met Eureka-tipe suurlemoene.

Summary

This was the second year to evaluate the lemon cultivars at the Moorsivier trial site in Marble Hall. Willow Tree Long bore their second fruit at this trial site for the northern areas due to high temperatures and the typical lemon growth rate (aggressive). Eureka, Lisbon en Genoa had a better fruit set compared to the rest of the lemon selections at the trial site; Genoa setting one main crop on the trees per season. Lisbon Yen Ben developed the smallest fruit on the trees (count 6 to 5) compared to the other selections and was more tolerant to cold conditions. All the selections were planted at the end of the 2013 season on X639 rootstock, due to the higher pH soils in the production area as well as compatibility with the Eureka-type lemons.

Objectives

- To find Lemon selections suitable for the intermediate production area.
- To produce lemon selections with Eureka like fruit shape (elongated), high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted at Moorsivier (Marble Hall) on Eureka, Feminello, Genoa, Limoneira, Lisbon, Lisbon Yen Ben and Willowtree Long.

Table 4.6.11.1. List of lemon selections evaluated at Moorsivier (Marble Hall) during the 2017 season.

Selection	Rootstock	Topworked
Eureka	X639	2013
Feminello	X639	2013
Genoa	X639	2013
Limoneira	X639	2013
Lisbon	X639	2013
Lisbon Yen Ben	X639	2013
Willowtree Long	X639	2013

Results and discussion

Lisbon Yen Ben (33.1%) and Lisbon (34.6%) developed the lowest juice percentages for the season, but Femminello had the highest juice percentage of 50%. Genoa produced the biggest fruit size and still peaked from count 4 to count 1 through the season. The highest seed content per fruit was on Lisbon and Genoa (11.0 seeds per fruit), followed by Eureka (9.0 seeds per fruit). The external colour ranged from T2 to T3 at the second evaluation at the trial site for all the selections.

Conclusion

Eureka produced elongated fruit; Willow Tree Long was the only other selection with a more elongated type fruit on the trees, the rest were fairly round.

For the second season an average to good crop was produced on the trees. The lemon selections were not that vigorous and tree canopy was less dense on the X639 rootstocks. High temperatures can affect the fruit set and as well as the juice percentages. The four commercial Lemon selections; Eureka, Lisbon, Limoneira and Genoa performed well and were more suitable for the intermediate production areas compared to the experimental selections, but Femminello developed the highest juice of 50%. Lisbon Yen Ben keeps on producing small fruit size (count 4-3), although the cultivar remains more tolerant to low temperatures.

Table 4.6.11.2. Internal fruit quality data for Lemon selections at Moosrivier (Marble Hall) during the 2017 season.

Cultivar	Root-stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Avg. seed	Colour
Eureka	X639	26/04/2017	56-61	5-4	39.3	9.0	T2
Eureka	X639	04/07/2017	62-65	4-3	37.4	5.9	T2
Eureka	X639	15/08/2017	70-75	2-1	43.1	5.9	T2
Femminello	X639	26/04/2017	53-56	6-5	40.8	2.5	T2
Femminello	X639	04/07/2017	60-63	4-3	50.0	5.3	T2
Femminello	X639	15/08/2017	73-79	1	46.5	3.5	T2
Genoa	X639	04/07/2017	69-73	2-1	36.0	10.0	T2
Genoa	X639	26/04/2017	60-61	4	42.1	7.5	T2
Genoa	X639	15/08/2017	75-80	1	45.7	7.1	T2
Limoneira	X639	26/04/2017	60-64	4-3	40.3	7.2	T3
Limoneira	X639	04/07/2017	63-66	3	44.4	9.2	T2
Limoneira	X639	15/08/2017	70-75	2-1	45.2	7.6	T2
Lisbon	X639	26/04/2017	62-66	4-3	41.9	10.0	T3
Lisbon	X639	04/07/2017	64-69	3-2	34.6	6.6	T2
Lisbon	X639	15/08/2017	70-75	2-1	37.9	4.2	T2
Lisbon	X639	26/09/2017	49-53	6	38.4	5.8	T3
Lisbon Yen Ben	X639	04/07/2017	60-65	4-3	33.1	2.2	T2
Willowtree Long	X639	26/04/2017	58-60	5-4	42.9	8.8	T3
Willowtree Long	X639	15/08/2017	65-70	3-1	45.7	7.0	T2

4.6.12 PROGRESS REPORT: Evaluation of Lemon selections in the intermediate production areas (Letsitele)

Project 75D by J. Joubert (CRI)

Opsomming

Die 2017 seisoen was die eerste drag op die bome gewees vir die suurlemoen proef by Letsitele. Dit was Willowtree Long se eerste vrugset by hierdie proef blok vir die noordelike areas as gevolg van die hoë temperature en die tipiese suurlemoen groeitempo (groeikragtig). Lisbon en Eureka het 'n beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseel. Femminello ontwikkel die kleinste vruggrootte op die bome (telling 5 tot 4) in vergelyking met die ander seleksies. Hoë temperature gedurende blom periodes het swak vrugset tot gevolg gehad vir sekere van die seleksies. Al die seleksies was geplant aan die einde van November 2012 op X639, vir die verenigbaarheid met Eureka-tipe suurlemoene.

Summary

The 2017 season produced the first crop on the trees for the Lemon trial at Letsitele. Willowtree Long bore their first fruit at this trial site for the northern areas due to high temperatures and the typical lemon growth rate (aggressive). Lisbon and Eureka had a better fruit set compared to the rest of the lemon selections at the trial

site. Femminello developed the smallest fruit on the trees (count 5 to 4) compared to the other selections. High temperatures during the flowering periods induced poor fruit set on some of the selections. All the selections were planted in November 2013 on X639 rootstock, due to the compatibility with the Eureka-type lemons.

Objectives

- To find Lemon selections suitable for the intermediate production area.
- To produce lemon selections with Eureka like fruit shape (elongated), high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted at Bosveld Citrus (Letsitele) on Eureka, Femminello, Genoa, Limoneira, Lisbon and Willowtree Long.

Table 4.6.12.1. List of lemon selections evaluated at Bosveld Citrus (Letsitele) during the 2017 season.

Selection	Rootstock	Planted
Eureka	X639	2013
Femminello	X639	2013
Genoa	X639	2013
Lisbon	X639	2013
Limoneira	X639	2013
Willow Tree Long	X639	2013

Results and discussion

Limoneira (42.5%) and Lisbon (40.2%) developed the lowest juice percentages for the season, but Eureka had the highest juice percentage of 52.4%. Genoa produced the biggest fruit size and peaked from count 4 to count 2 through the season. The highest seed content per fruit was on Genoa (22.4 seeds per fruit), followed by Eureka (9.9 seeds per fruit). The external colour ranged from T2 to T3 at the first evaluation at the trial site.

Conclusion

Eureka produced elongated fruit; Willow Tree Long was the only other selection with a more elongated type fruit on the trees, the rest were fairly round.

For the first season a reasonable crop was produced on the trees. The lemon selections were not that vigorous and tree canopy was less dense. High temperatures can affect the fruit set and as well as the juice percentages. The four commercial Lemon selections; Eureka, Genoa, Femminello and Willowtree Long performed well and seems to be more suitable for the hot production areas compared to the other commercial selections.

Table 4.6.12.2. Internal fruit quality data for Lemon selections at Bosveld Citrus (Letsitele) during the 2017 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Avg. seed	Colour
Eureka	X639	04/05/2017	58-65	5 - 3	52.4	9.9	T2
Femminello	X639	04/05/2017	55-60	5 - 4	51.7	3.8	T3
Genoa	X639	04/05/2017	61-70	4 - 2	48.4	22.4	T2
Limoneira	X639	04/05/2017	61-67	4 - 3	42.5	0.0	T2
Lisbon	X639	04/05/2017	61-63	4 - 3	40.2	0.0	T2

Willowtree Long	X639	04/05/2017	61-67	4 - 3	50.5	9.3	T2
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4.6.13 **PROGRESS REPORT: Evaluation of Grapefruit on different rootstocks in a semi-desert production area (Kakamas)**

Project 922 by J. Joubert and W. Swiegers (CRI)

Opsomming

Visuele evaluasies van die Star Ruby en Nelruby entlas, met 'n gesonde entlas verbinding, het bewys dit is verenigbaar met die kombinasies (moet bevestig met opvolg inspeksies). Sunki 812 is 'n hibried onderstam kruising tussen Sunki mandaryn en Beneke trifoliaat (Sunki 812). Die boomgrootte van hierdie kombinasie word as medium beskou (vergelyk met Carrizo boomgrootte en groeikragtigheid), alhoewel Sunki 812 onderstam as boom op sy eie baie groeikragtig is en 'n groot boom oplewer. In kombinasie met Star Ruby was die boomgrootte kleiner as Citrange 35 en Benton citrange (verdwergde onderstamme). Die oes produksie het verbeter hierdie seisoen met die tweede oes op die bome, (Nelruby het steeds beter presteer as Star Ruby in hierdie proef) met 'n ooreenstemmende afname in vruggrootte vir beide seleksies, met pieke by telling 48 (klein vrugte).

Saad tellings op die Nelruby vrugte was hoër in vergelyking met Star Ruby wat feitlik saadloos toets (gemiddeld 0.1 sade per vrug). Kleur ontwikkeling op albei seleksies en al die onderstam kombinasies was vertraag met die beste vir Star Ruby op Terrabella. Beide die verdwergde onderstamme, Citrange 35 en Benton citrange het baie goed presteer wanneer die impak van die hoë pH van die grond in ag geneem word. Verdere evaluasies sal die aanpasbaarheid van hierdie onderstamme bevestig.

Evaluasies tot op datum toon aan dat hierdie onderstamme waardevol kan wees vir die sitrus produsente; meer spesifiek Sunki 812, waar hoë pH vlakke en kalkagtige gronde voorkom. Sunki 812 was vir sy hoë verdraagsaamheid teen Phytophthora, sitrus aalwurms en tristeza, asook beter weerstand vir hoër pH en kalkagtige gronde, geselekteer.

Summary

Visual evaluations of Star Ruby and Nelruby bud-unions, indicated that the unions were in good condition and the combinations compatible (need to confirm with follow-up inspections). Sunki 812 is a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliolate (Sunki 812). The tree size of this combination is described as medium (similar to Carrizo tree size and growth rate), although Sunki 812 rootstock as a tree on its own is aggressive and develops into a fairly large tree. In combination with Star Ruby, the tree was smaller compared to Citrange 35 and Benton citrange (dwarfing rootstocks). Yield production was better this season due to the second crop on the trees. Nelruby outperformed Star Ruby at this early stage of the trial, with a corresponding decrease in fruit size for both selections, peaking at count 48 (small fruit).

Seed counts on the Nelruby fruit were higher compared to Star Ruby being virtually seedless (average 0.1 seeds per fruit). Colour development on both selections and all the rootstock combinations was good with the best being Star Ruby on Terrabella. The dwarfing rootstocks, Citrange 35 and Benton citrange performed very well, bearing in mind the impact of the high pH of the soil. Future evaluations will determine the adaptability of these rootstocks.

Evaluations to date show that these rootstocks could be of value to citrus producers, particularly Sunki 812, should high pH levels and calcareous soils be a problem. Sunki 812 was selected for its high tolerance to Phytophthora, citrus nematodes and tristeza, as well as better tolerance of high pH and calcareous soils.

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 Citumelo, Terrabella and X639 were propagated by Cedarberg Nursery, a CIS accredited nursery in the Citrusdal region of the Western Cape.

Star Ruby and Nelruby grapefruit was budded onto the following rootstocks at Cedarberg nursery in 2010: Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, Sunki 812, Swingle Citumelo, Terra Bella and X639. The trees were planted at Karsten in March 2012.

Table 4.6.13.1. Number of trees per rootstock in the Star Ruby and Nelruby Grapefruit trial at Kakamas.

Selection	Rootstock	No. of trees
Star Ruby	BC	6
Star Ruby	C35	6
Star Ruby	CC	6
Star Ruby	RL	3
Star Ruby	Sunki 812	4
Star Ruby	SC	6
Star Ruby	TB	6
Star Ruby	X639	5
Nelruby	BC	6
Nelruby	C35	6
Nelruby	Sunki 812	5
Nelruby	SC	6
Nelruby	TB	6
Nelruby	X639	6

Results and discussion

The Grapefruit trial was harvested for the second time this season with a fair to good crop on the trees. Limited information was available at Karsten due to the second crop on the young grapefruit trees.

Star Ruby

The lowest crop production for the 2017 season was in combination with BC yielding 49.9kg/tree (2016; C35 - 15.5 kg/tree) and the best on SC yielding 67.8 kg/tree (2016; X639 - 32.1 kg/tree), selected for intermediate pH soil conditions (Table 4.6.13.4). The second highest crop was produced on RL with 62 kg/tree, and the average yield for the Star Ruby trial was 59.4KG (2016 - 25 kg). Internally fruit quality was good with Brix ranging from 8.8 up to 10.6 (average 9.8) and juice levels above 52% (Table 4.6.13.2).

The acid content remained fairly high this season from 1.1 to 1.3% (similar to 2016), decreasing the Brix:acid ratio to 8.0. Fruit size was smaller compared to 2016 and peaked at count 48 (75%), followed by count 40 (63%) and count 64 (50%), producing a smaller fruit size on the trees for this season, due to the better crop.

Nelruby

Nelruby on SC produced the best juice content (58.1%), as well as the third highest Brix:acid ratio of 8.7, followed by BC with the highest Brix level (10.4) and acid of 1.1% (Table 4.6.13.2). The external colour development on all 8 rootstock combinations peaking at T1 to T4/5, except for Terrabella between T1 and T2. All the combinations peaked at count 48 (100%) (2016 peaked at count 27), followed by count 64 (67%) and count 40 (100%). The best crop on the Nelruby trees was in combination with SC (83.9 kg/tree), followed by C35 (80.2 kg/tree) and BC (75.3 kg/tree). Both C35 and BC develop smaller trees size due to their dwarfing characteristics.

Conclusions

Star Ruby in combination with Sunki 812 developed very small tree size compared to the other rootstocks (smaller than C35 and BC). The seed content in the Star Ruby fruit remained significantly lower in comparison with the Nelruby fruit. Fruit size distribution from Nelruby was more even compared to the fruit sizes on Star Ruby (count 64, 48 and 40). The smaller fruit size (both selections peaked at count 48) was due to the better crop on the trees. Nelruby cropped a better yield on the trees (average 69.3 kg/tree versus 59.4 kg) compared to Star Ruby this season. Star Ruby had improved colour development (deeper red blush on rind) where Nelruby was more yellowish.

Star Ruby and Nelruby was evaluated on eight and six rootstocks respectively, the most important combination of the above mentioned was Sunki 812 (Sunki mandarin x Beneke trifoliata). Sunki 812 was selected for replant conditions, very specific high pH and calcareous soils. The first and second evaluation and harvest indicated that the other rootstocks outperformed Sunki 812 and future evaluations will be crucial to determine the best combination in these semi-desert conditions.

Table 4.6.13.2. Internal fruit quality of Star Ruby and Nelruby Grapefruit on different rootstocks at Karsten Boerdery (Kakamas) on 24th May 2017.

Cultivar	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Nelruby	BC	57.5	10.4	1.13	9.20	1.3	T1-5
Nelruby	C35	56.3	9.7	1.13	8.58	1.1	T1-4
Nelruby	Sunki 812	54.7	9.8	1.09	9.03	1.8	T1-6
Nelruby	SC	58.1	9.6	1.11	8.65	2.9	T3-5
Nelruby	TB	55.7	9.9	1.15	8.65	0.0	T1-2
Nelruby	X639	57.3	9.3	1.15	8.09	1.8	T1-5
Star Ruby	BC	59.3	10.1	1.22	8.28	0.0	T1-4
Star Ruby	C35	56.0	10.0	1.31	7.66	0.0	T1-5
Star Ruby	CC	54.4	9.3	1.22	7.62	0.2	T1-4
Star Ruby	RL	52.2	8.8	1.12	7.86	0.1	T2-4
Star Ruby	Sunki 812	59.5	10.6	1.26	8.41	0.0	T1-4
Star Ruby	SC	58.1	10.0	1.19	8.44	0.2	T1-4
Star Ruby	TB	56.7	10.0	1.21	8.26	0.1	T1-4
Star Ruby	X639	57.6	9.5	1.23	7.72	0.2	T1-5

Table 4.6.13.3. Fruit size distribution at Karsten Boerdery during the 2017 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	BC	27	4.35	Nelruby	BC	27	8.41
Star Ruby	BC	32	1.92	Nelruby	BC	32	3.91
Star Ruby	BC	36	8.49	Nelruby	BC	36	10.80
Star Ruby	BC	40	11.02	Nelruby	BC	40	14.86
Star Ruby	BC	48	36.70	Nelruby	BC	48	37.03
Star Ruby	BC	64	37.51	Nelruby	BC	64	25.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C35	27	5.45	Nelruby	C35	27	7.75
Star Ruby	C35	32	3.66	Nelruby	C35	32	6.34
Star Ruby	C35	36	9.65	Nelruby	C35	36	16.21
Star Ruby	C35	40	16.35	Nelruby	C35	40	17.55
Star Ruby	C35	48	31.01	Nelruby	C35	48	32.77
Star Ruby	C35	64	33.87	Nelruby	C35	64	19.38
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit

Star Ruby	CC	27	17.10	Nelruby	Sunki 812	27	3.26
Star Ruby	CC	32	6.67	Nelruby	Sunki 812	32	4.02
Star Ruby	CC	36	13.14	Nelruby	Sunki 812	36	12.55
Star Ruby	CC	40	16.91	Nelruby	Sunki 812	40	19.70
Star Ruby	CC	48	28.31	Nelruby	Sunki 812	48	41.53
Star Ruby	CC	64	17.87	Nelruby	Sunki 812	64	18.95
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	RL	27	11.78	Nelruby	SC	27	3.90
Star Ruby	RL	32	7.34	Nelruby	SC	32	4.17
Star Ruby	RL	36	17.76	Nelruby	SC	36	13.62
Star Ruby	RL	40	20.46	Nelruby	SC	40	22.55
Star Ruby	RL	48	33.20	Nelruby	SC	48	42.59
Star Ruby	RL	64	9.46	Nelruby	SC	64	13.16
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	2.50	Nelruby	TB	27	1.24
Star Ruby	Sunki 812	32	3.16	Nelruby	TB	32	2.48
Star Ruby	Sunki 812	36	8.95	Nelruby	TB	36	8.00
Star Ruby	Sunki 812	40	16.45	Nelruby	TB	40	17.41
Star Ruby	Sunki 812	48	37.76	Nelruby	TB	48	44.88
Star Ruby	Sunki 812	64	31.18	Nelruby	TB	64	25.99
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	SC	27	9.62	Nelruby	X639	27	2.61
Star Ruby	SC	32	7.71	Nelruby	X639	32	2.44
Star Ruby	SC	36	17.68	Nelruby	X639	36	8.33
Star Ruby	SC	40	21.14	Nelruby	X639	40	19.43
Star Ruby	SC	48	30.68	Nelruby	X639	48	42.56
Star Ruby	SC	64	13.17	Nelruby	X639	64	24.64
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	TB	27	3.01				
Star Ruby	TB	32	2.28				
Star Ruby	TB	36	11.75				
Star Ruby	TB	40	18.31				
Star Ruby	TB	48	42.53				
Star Ruby	TB	64	22.13				
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	X639	27	2.96				
Star Ruby	X639	32	4.71				
Star Ruby	X639	36	12.83				
Star Ruby	X639	40	17.76				
Star Ruby	X639	48	38.93				
Star Ruby	X639	64	22.81				

Table 4.6.13.4. Production per tree of Star Ruby and Nelruby Grapefruit trees on different rootstocks at Karsten Boerdery (Kakamas) during the 2017 season.

Cultivar	Rootstock	2016 Kg/tree	2017 Kg/tree
Nelruby	BC	39.1	75.3
Nelruby	C35	40.3	80.2
Nelruby	Sunki 812	34.0	51.8
Nelruby	SC	43.1	83.9
Nelruby	TB	31.3	62.3

Nelruby	X639	33.8	62.1
Star Ruby	BC	20.3	49.9
Star Ruby	C35	15.3	58.4
Star Ruby	CC	25.5	61.7
Star Ruby	RL	23.8	62.0
Star Ruby	Sunki 812	20.9	58.5
Star Ruby	SC	31.0	67.8
Star Ruby	TB	27.3	58.2
Star Ruby	X639	32.1	58.6

4.6.14 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (East Cape Midlands)

Project 57A by W. Swiegers and Z. Zondi (CRI)

Opsomming

Hierdie spesifieke Satsuma proef is deel van n kommersiële aanplanting. Die proef se ligging is goed geskik vir Satsuma produksie a.g.v. die hoë koue eenhede wat nodig is vir kleurontwikkeling. Die boorde is op verskillende tye aangeplant vanaf 1991 tot 2000 en die bome is volwasse met genoegsame boom volume. Van die seleksies is ook op verskillende onderstamme geplant. Die volgorde van rypwording is:: Miho Wase, Okitsu, Miyagawa Wase, Ueno en Dobasi Beni. Die oesvenster vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

Summary

This specific Satsuma trial is a commercial planting. The trial location is in an area well suited for Satsuma production due to enough cold units for colour break. The orchards were planted between 1991 and 2000. The trees are mature with large tree canopies. Some of the selections were planted on different rootstocks. The order of ripening was as follows; Miho Wase, Okitsu, Miyagawa Wase, Ueno and Dobasi Beni. Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on early to late Satsuma selections from the East Cape Midlands part of the Eastern Cape. The following selections were evaluated: Miho Wase, Okitsu, Miyagawa Wase, Dobashi Beni and Ueno.

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 4.6.14.1 List of Satsuma selections evaluated at Saxfold Park (Adelaide) during 2017.

Selection	Rootstock	Planted
Ueno	Carrizo	1997
Dobashi Beni	Unknown	Unknown
Miyagawa Wase	Swingle	1997
Miho Wase	Unknown	Unknown
Okitsu	Carrizo	1997

Results and discussion

Miho Wase

Miho Wase was the first selection to mature this season. The rind was smooth, and the fruit peeled easily. It developed a very good fruit size (count 1). Miho Wase is known to have fruit size counts of 2 - 3. The fruit was seedless. The fruit colour on the colour plate was T6. The ratio was already at 10.8 close to over maturity. Fruit matured internally prior to good colour development. 54.4% was a good juice percentage for the Miho Wase fruit. Miho Wase had the lowest Brix° with maturity at 9.4° as well as the lowest Acid %, 0.87%.

Dobashi Beni

Dobashi Beni was the late selection Satsuma for this trial site, with peak maturity being late April beginning of May. The fruit size was smaller than last year with a 2 count, although it is still on its way toward peak maturity. The juice percentage of the Dobashi Beni were the third highest of all the selections with 56.2%. Dobashi Beni had the highest Brix° and Acid % at 11.1° and 1.33% and it is still building towards peak maturity. Dobashi Beni had a seed count of 0.5 - 0.1 and the fruit colour on the colour plate was T6 - T7. The colour development was better than that of Miho Wase. The fruit of the Dobashi Beni was flat with smooth rind that peeled easily.

Miyagawa Wase

The fruit size of Miyagawa Wase at peak maturity was count 2, smaller than last year count of 1 – 1x. The juice % at peak maturity was the highest and also higher than last year with a good juice percentage of 59.4%. The Brix° and acid percentage were very good at peak maturity, being 9.4° and 0.96% respectively. Colour development was delayed compared to the internal maturity. There were no seeds in Miyagawa Wase. The colour on the colour plate at peak maturity was T6 – T7. The delayed rind colour could be due to the Swingle citrumelo rootstock. Fruit had better taste than Miho Wase, and the fruit was smooth and flat. Internal colour was a deep orange.

Ueno

This selection is a mid to late maturing selection for the Satsumas trial site. Ueno had an above average fruit size count with a 1x count. Ueno juice % increased with maturity and it also had the highest juice % of the 4 selections with 57.5% at peak maturity. The Brix° of Ueno was the second highest at build up towards peak maturity being 10.3° with a very good acid percentage of 1.23%. The acid percentage of Ueno was also the second highest. The selection had seeds 0.1 and 1, and Ueno colour on the colour plate towards build up to peak maturity was T6. Peelability was found to be easy.

Okitsu

Okitsu fruit size count was a count 1 at peak maturity. One of only 2 selections to get a fruit size count of 1. Okitsu juice percentage was the second highest percentage of the the selections with 57.6% at peak maturity. The Brix° of Okitsu at peak maturity was 9.8° with a very good acid percentage of 0.95%. There were no seeds and Okitsu colour on the colour plate at peak maturity was T6. Okitsu also had a delayed colour development compared to internal maturity. This is also an early maturing selection for this trial site. The fruit peelability was easy.

Conclusion

Both early maturing selections Miho Wase and Okitsu had the best fruit size count with a count 1, while Dobashi Beni, Ueno and Miyagawa Wase peaked at a size count of 2. Miyagawa Wase and Okitsu had the best juice

percentages: 59.4% and 57.6% respectively. Dobashi Beni had the highest Brix° of all the Satsuma selections (11.1°). Ueno also had the highest acid percentage of all the Satsuma selections (1.33%). Ueno had the highest seed count with 1 seed per fruit. All the selections rind colour development was delayed compared to their internal maturity development.

Table 4.6.14.2. Internal fruit quality data for Satsuma selections in the Adelaide region (Saxfold) of the East Cape Midlands during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-03-23	Dobashi Beni	Unknown	3	50,0	10,4	1,42	7,3	0,5	T8
2017-04-10	Dobashi Beni	Unknown	2	56,2	11,1	1,33	8,3	0,1	T6-7
2017-03-23	Miho Wase	SC	1	54,4	9,4	0,87	10,8	0,0	T6
2017-03-23	Miyagawa Wase	SC	2	59,4	9,4	0,96	9,8	0,0	T6-7
2017-03-23	Okitsu	CC	1	57,6	9,8	0,95	10,3	0,0	T6
2017-03-23	Ueno	CC	2	57,8	9,4	1,58	5,9	0,1	T8
2017-04-10	Ueno	CC	2	53,9	10,3	1,23	8,4	1,0	T6

4.6.15 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)

Opsomming

Hierdie spesifieke Satsuma proef is 'n relatiewe nuwe proef en 2017 was die bome se tweede drag. Die bome was in 2012 getopwerk na die volgende seleksies toe, wat ook dien as die volgorde van rypwording; Miho Wase, Miyagawa Wase, Sonet 2, Ueno, Imamura, Sugiyama, Aoshima en die seisoen was afgesluit met Dobashi Beni. Die rypwordings volgorde gaan bietjie verskil a.g.v. jong bome. Pluk periodes vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powwerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

Summary

This specific Satsuma trial is a relatively new trial and 2017 was the second 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, Miyagawa Wase, Sonet 2, Ueno, Imamura, Sugiyama, Aoshima and the season was finished off with Dobashi Beni. The order of ripening might change a bit due to the young trees. Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on early to late Satsuma selections from the Sundays River Valley part of the Eastern Cape. The following selections were evaluated: Miho Wase,

Miyagawa Wase, Ueno, Aoshima, Sugiyama, Imamura, Dobashi Beni and Sonet 2.

For Satsuma mandarins, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which the fruit is considered over mature. This process from is approximately three weeks long. Fruit harvested before and after this period would result in higher instances of quality and rind issues.

Table 4.6.15.1. List of Satsuma selections evaluated at Invercloy (Kirkwood) during 2017.

Selection	Rootstock	Topworked
Aoshima	Carrizo	2012
Miho Wase	Carrizo	2012
Miyagawa Wase	Carrizo	2012
Sugiyama	Carrizo	2012
Ueno	Carrizo	2012
Dobashi Beni	Carrizo	2012
Imamura	Carrizo	2012

Table 4.6.15.2. List of Satsuma selections evaluated at Penhill (Addo) during 2017.

Selection	Rootstock	Planted
Sonet 2	Carrizo	2011

Results and discussion

Aoshima

Aoshima were the second last selection to reach peak maturity. The fruit size count for Aoshima was very good with the count being 1xxx. This was also one of the selections at the trial site with the biggest fruit size count. The juice percentage did increase from last year from 42.4% to 47.1%, but this juice percentage of Aoshima was one of the lowest this season. Brix also increased from the previous season, the Brix was 8.2°, one of the higher Brix for the season compare to some of the other selections. There were some seeds in the fruit; 0.6 seeds per fruit. The external colour development of the Aoshima was not very good, with a T7 on the colour plate. T 7 on the colour plate was while the fruit were over mature.

Miho Wase

Miho Wase was again the first selection to mature to peak maturity this year. Miho Wase are also used as the control for the early maturing selections for this site. The selection had a slightly smaller fruit size count of 1x this season compared to the previous season's 1xx. Juice percentage for Miho Wase was the 3rd highest this season with 55.4% juice. Miho Wase had a Brix° of 8.5° and acid percentage of 0.66% with a 12.9 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.

Miyagawa Wase

Miyagawa Wase followed Miho Wase to mature to peak maturity. Fruit size count was very good with a count of 1xx; the second highest count for this trial site. Juice percentage for Miyagawa Wase was the 2nd highest being 56.5%. Having a ratio of 10.8 close to over maturing, the Brix° was 7.9° and the acid percentage was 0.73%. Miyagawa Wase colour development was once again delayed this season being a T6 on the colour plate, slightly better than last season's T7. There were no seeds in the Miyagawa Wase and it had a smooth rind and peeled easy.

Sugiyama

Sugiyama are one of the late maturing selections for this Satsuma trial site. It was over mature in the second week of May. It reached peak maturity probably about end of April. Sugiyama had a very good fruit size count at 1xxx, one of the biggest count compared to some of the other selections. The juice percentage for Sugiyama

was the lowest from all the selections having a juice percentage of 43.6. It is a big decrease from last year's 58.2%. The Brix° and acid percentage of Sugiyama were the lowest at 7.3° and 0.59 % respectively. Seed count for the selection was a very low count at 0.1 seeds per fruit. There was also a delay in colour development with a T6 on the colour plate when the fruit was over mature.

Ueno

Ueno is a mid to late maturing selection. At this Satsuma trial site, it reached peak maturity more towards the mid maturing selection range. Ueno's fruit size count was very good ranging from 1 to 1xxx. Count 1 was towards build up to peak maturity and count 1xxx was when the fruit was over mature. The juice percentage was higher this season at 54.5%, compared to last season's 48.2%. Towards peak maturity the Brix° was 7.8° and the acid percentage was 0.84%. Although the Brix° is a bit low the acid percentage is still good and will help Ueno with its flavour development. Ueno had no seeds and the colour of Ueno on the colour plate was a T7 towards peak maturity and a T5 when it was over mature. The fruit was flat and peelability was easy.

Sonet 2

Sonet 2 is one of the early maturing Satsuma selections. Fruit size count for Sonet 2 was count 2 – 3, the smallest size count from all the Satsuma selections that was evaluated. Juice percentage for this selection was the highest from all the selections, with a very good juice percentage ranging between 63 – 64.7 % at peak maturity. Brix° at peak maturity for Sonet 2 was between 9.9 – 10.7°. The acid percentage at peak maturity for this selection ranged between 0.95 – 1.05 %. Sonet 2 had the highest Brix° and acid % of all the Satsuma selections that was evaluated in this region. The high Brix and relatively high acid helped the fruit to hang a bit longer and it also helped with the flavour of the fruit. Seed count for Sonet 2 was 2.8 – 3.8 seeds per fruit, the highest of all the selections. Colour on the colour plate was at T6, also delayed external colour development compared to the internal maturing development. The advantage is the slightly higher acid which helped to hang the fruit a little longer to get better colour. Just be careful for creasing.

Imamura

Imamura is one of the late maturing selections for this Satsuma trial site. Due to the age of the trees the selection matured a bit earlier than if it was an older tree. Imamura normally reach peak maturity mid to end of May in cool production regions. The juice percentage for Imamura was on the lower side with a juice percentage of 49.6%. Although Imamura was overmature the Brix° was also low as well as the acid percentage 7.7° and 0.60% respectively. Seed count was 0.2 seeds per fruit. The colour development was T6 on the colour plate, meaning that the external colour development was also delayed. It is important to know that the trees are still young. Internal colour was deep orange and the fruit rind varied from smooth to coarse.

Dobashi Beni

Dobashi Beni are the control selection for the mid to late maturing Satsuma selections. Dobashi Beni was the last selection to reach peak maturity at the end of April beginning of May. Fruit size count was very good ranging from 1 to 1xx. The juice percentage decreased towards peak maturity, being 50.2% during the build up towards peak maturity and 46.4% when it was over mature. Brix° stayed the same more or less from build up to being over mature and the Brix° was 7.5° not very high. The acid percentage did drop a bit as expected from 0.98% to 0.68%. Acid of 0.68% was when the selection was over mature, meaning Dobashi Beni had a good acid % during peak maturity for a good eating fruit. Internal colour is deep orange, rind is smooth and peelability is easy. Dobashi Beni did have some seeds with a seed count of 0.8 – 0.9 seeds per fruit. This selection showed the best external colour development going from T7 on the colour plate during build up towards peak maturity to T4 when it was over mature.

Conclusion

Aoshima, Sugiyama and Ueno had the best fruit size i.e. count 1xxx. Dobashi Beni, Imamura, Miho Wase and Miyagawa Wase also had a good fruit size count ranging from 1 to 1xx. Sonet 2 had the smallest fruit size count 2 – 3. Sonet 2, Miyagawa Wase and Miho Wase had the best juice percentage being 64.7%; 56.5% and 55.4%, respectively. All the other selections had a juice percentage below 55%. Sonet 2 had the highest Brix° at peak maturity being 10.7° and Sugiyama had the lowest Brix° being 7.3° of all the Satsuma selections.

Sonet 2 had the highest seed count at 3.8 seeds per fruit. Miho Wase, Miyagawa Wase and Ueno were the only selections to be seedless.

Table 4.6.15.3. Internal fruit quality data for Satsuma selections in the Addo and Kirkwood region of the Eastern Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-05-10	Aoshima	CC	1xxx	47,1	8,2	0,68	12,1	0,6	T7
2017-04-04	Dobashi	CC	1	50,2	7,5	0,98	7,7	0,8	T7
2017-05-10	Dobashi	CC	1xx	46,4	7,6	0,68	11,2	0,9	T4
2017-05-10	Imamura	CC	1xx	49,6	7,7	0,60	12,8	0,2	T6
2017-03-22	Mihowase	CC	1x	55,4	8,5	0,66	12,9	0,0	T6
2017-03-22	Miyagawa	CC	1xx	56,5	7,9	0,73	10,8	0,0	T6
2017-03-28	Miyagawa	CC	1xx	54,7	8,0	0,65	12,3	0,0	T6
2017-05-10	Sugiyama	CC	1xxx	43,6	7,3	0,59	12,4	0,1	T6
2017-03-22	Ueno	CC	1	54,5	7,8	0,84	9,3	0,0	T7
2017-05-10	Ueno	CC	1xxx	49,0	8,9	0,69	13,8	0,0	T5
2017-03-28	Sonet 2	CC	3	63,0	9,9	0,95	10,4	2,8	T 6
2017-04-04	Sonet 2	CC	2	64,7	10,7	1,05	10,2	3,8	T 6

4.6.16 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)
Project 997A by W Swiegers and Z. Zondi (CRI)

Opsomming

Die 2017 seisoen was die laaste oes vir hierdie mandaryn proef in die Oos-Kaap Middelande. Daar is opwinde nuwe seleksies getopwerk in die perseel. Nadorcott het as kontrole gedien vir hierdie proef perseel. Die volgorde van rypwording is: Tahoe Gold, Nadorcott, Gold Nugget, Tango, Yosemite Gold en Shasta Gold.

Summary

The 2017 season was the last harvest for this mandarin trial in the East Cape Midlands area. A range of new mandarin hybrids have been topworked in this site. Nadorcott was the control selection for this trial site. The order of ripening was as follows: Tahoe Gold, Nadorcott, Gold Nugget, Tango, Yosemite Gold and Shasta Gold.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Cookhouse region of the East Cape Midlands. The following varieties were evaluated: Gold Nugget, Nadorcott, Shasta Gold, Tahoe Gold, Tango and Yosemite Gold.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.16.1. List of mandarin hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2016 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Yosemite Gold	CC	2010

Results and discussion

Gold Nugget

Gold Nugget reached peak maturity at 08-08-2017 with a Brix° of 13.1° and the Acid still being at 1.07%. This indicates that the fruit will likely have an extended shelf life. It also showed that the fruit can hang well, because at 22-08- 2017 the fruit were not over mature yet and still had an acid percentage of 1%. The high Brix° and relatively high acid percentage also contribute to the good flavour of the selection. There were no seeds in the fruit as Gold Nugget is a seedless variety. Gold Nugget has a very upright growth habit and there are thorns on the branches. Fruit rind is pebbly and the selection had a good fruit size with counts from 1-1xx. The juice % of the Gold Nugget was the second lowest with a juice percentage of 49.5% at peak maturity, and the juice % decrease like last season towards peak maturity. Gold Nugget had a good colour development at the trial site and peaked at colour plate T1 before peak maturity was reached. Peak maturity was also 2 – 3 weeks earlier compared to the previous season.

Nadorcott

Nadorcott was used as a control for this mandarin varieties trial site, and more specifically for Tango. Nadorcott was the second selection to reach peak maturity. Nadorcott developed the second smallest fruit size (count 1-1x) for this trial site for a third year in a row. Fruit size count of 1-1x are still very good. Nadorcott fruit size was slightly bigger than that of Tango. This Nadorcott selection had a seed count of 0.3 – 0.9 seeds per fruit. Colour development for this variety was good again, with a (T1 colour) on the colour plate at peak maturity. Nadorcott had high Brix° around 13° at peak maturity with acid percentage still above 1.00%. The fruit have a shiny, waxy look and is flat with a smooth rind. Internal colour is also good with a deep orange colour. Trees also had good yields. The juice percentage for Nadorcott at peak maturity was around 60% one of the highest percentages.

Shasta Gold

Shasta Gold was the last selection to reach peak maturity between the 6 selections that was evaluated at this trial site. Towards the build up to peak maturity the selection had a high Brix° of 12.4° with the acid percentage still at 1.28% in the beginning of August. This selection ripening was also earlier compared to the previous season. Shasta Gold again had a very good colour development and peaked at colour plate T1 well before peak maturity. Fruit size count for Shasta Gold peaked at 1xxx and together with Yosemite Gold had the biggest fruit size count. The internal juice content was the same compared to the previous season around 59%. Shasta Gold was seedless.

Tahoe Gold

Tahoe Gold was the first selection to mature in this trial site for 2017. There were no seeds. Tahoe Gold had the highest internal juice content of all the selections for this trial site with a juice percentage of 63%. Tahoe Gold had a slightly bigger fruit size in 2017 with the fruit size count 1xx – 1xxx. Colour development was delayed compared to the other selections with a T4 on the colour plate close to peak maturity. This selection

also had the lowest Brix° and acid % of all the other selections with Brix° of 10.3° and acid % of 0.90%.

Tango

Tango reached peak maturity after the Nadorcott selection. The selections had the smallest fruit size compared with all the other selections, but it still had a good size count at count 1. Size is slightly bigger compared to last season. External colour development was very good with a T1 on the colour plate well before peak maturity was reached and the internal colour development was also very good with a deep orange colour. Tango had the lowest juice levels with an internal juice percentage of 43%. Brix° and acid percentage for Tango were the highest at this trial site with a 16.1° Brix and acid percentage of 1.35%. This high Brix° and acid percentage for this selection help the fruit to hang a bit longer on the tree, give the fruit a better shelf life and it will make a good eating fruit. Tango is also a seedless variety. The fruit also have a natural shine on the tree and a smooth rind. The production on the trees was also good.

Yosemite Gold

Yosemite Gold matured earlier this season, but the colour development was delayed compared to last season by reaching T3 on the colour plate, before peak maturity. Last season it was T1 before peak maturity. Fruit size count this season was bigger with 1xxx size towards peak maturity. Seed count was 0 seeds per fruit. Juice percentage and sugars increased towards peak maturity.

Conclusion

Yosemite- and Shasta Gold is the two selections with the largest fruit size 1xxx followed by Tahoe Gold with 1xx – 1xxx size count. Most of the selections reach T1 on the colour plate before peak maturity except Tahoe and Yosemite Gold. All six selections are likely to have a good shelf life because of the selections high Brix: Acid ratio. Nadorcott, Shasta Gold and Tahoe Gold had the best juice percentages around 60%. Nadorcott had the highest number of seeds per fruit, but it was very a very low count 0.9 seeds per fruit.

Table 4.6.16.2. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-07-06	Nadorcott	CC	1x	61,6	12,7	1,27	10,0	0,3	T 3
2017-07-20	Nadorcott	CC	1	59,9	12,6	1,15	11,0	0,9	T 1
2017-08-08	Nadorcott	CC	1	60,5	13,8	1,06	13,0	0,4	T 1
2017-07-20	Gold Nugget	CC	1	56,4	12,0	1,30	9,2	0,0	T1
2017-08-08	Gold Nugget	CC	1xx	49,5	13,1	1,07	12,2	0,0	T1
2017-08-22	Gold Nugget	CC	1	48,0	12,7	1,00	12,7	0,0	T1
2017-07-06	Shasta Gold	CC	1xxx	58,5	11,6	1,61	7,2	0,0	T3
2017-08-08	Shasta Gold	CC	1xxx	59,2	12,4	1,28	9,7	0,0	T1
2017-06-21	Tahoe Gold	CC	1xxx	63,0	10,2	1,04	9,8	0,0	T5
2017-07-06	Tahoe Gold	CC	1xx	63,9	10,3	0,90	11,4	0,0	T4
2017-07-20	Tango	CC	1	58,8	16,6	1,81	9,2	0,0	T1
2017-08-08	Tango	CC	1	56,5	17,1	1,57	10,0	0,0	T1
2017-08-22	Tango	CC	1	43,0	16,1	1,35	11,9	0,0	T1
2017-06-21	Yosemite Gold	CC	1xxx	52,2	10,7	1,23	8,7	0,0	T5
2017-07-06	Yosemite Gold	CC	1xxx	54,7	11,3	1,32	8,6	0,0	T3

4.6.17 **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)

Opsomming

Die mandaryn proef is opgedeel in 3 verskillende proef persele. Daar is twee persele in Sondagsrivier Vallel nl. Kirkwood en die 3^{de} perseel is in Addo . Die proef perseel in die Kirkwood area is 'n semi kommersiële aanplanting en bestaan uit die volgende seleksies, wat ook dien as die volgorde van rypwording: Saint Andre, Tango, Tahoe Gold, 2PH Phoenix, Gold Nugget, Mor 26, Shani SL, IRM 2, Yosemite Gold, IRM 1 en Shasta Gold. By die ander perseel in Kirkwood was die volgende seleksies geëvalueer in hulle orde van rypwording: Etna, Sirio, Bakgat en Tanor Late. Die perseel in Addo bestaan uit die volgende seleksies wat ook die volgorde van rypwording was: Edit x Nova, Meirav 119, Michal 6/47, Michal 89/64, Meirav 63, Valley Gold B24, Nova (kontrole), Nadorcott SL, Nadorcott ARC, IRM 2, IRM 1 en Shani SL

Summary

The mandarin trial is divided into 3 different trial sites. Two of the sites are in the Kirkwood region of the Sundays River Valley and the third site is in the Addo part of the Sundays River. One site in the Kirkwood region is a semi-commercial planting site, and it includes the following selections in their order of reaching peak maturity: Saint Andre, Tango, Tahoe Gold, 2PH Phoenix, Gold Nugget, Mor 26, Shani SL, IRM 2, Yosemite Gold, IRM 1 and Shasta Gold. At the other site in the Kirkwood region, we evaluated the following selections in their order of ripening: Etna, Sirio, Bakgat and Tanor Late. The trial site in Addo has the following selections also in their order of reaching peak maturity: Edit x Nova, Meirav 119, Michal 6/47, Michal 89/64, Meirav 63, Valley Gold B24, Nova (kontrole), Nadorcott SL, Nadorcott ARC, IRM 2, IRM 1 and Shani SL

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Sundays River Valley. A range of new mandarin hybrids have been added to this area. The following varieties were evaluated: Edit x Nova, Meirav 119, Michal 6/47, Michal 89/64, Meirav 63, Valley Gold B24, Nova (kontrole), 2PH Phoenix, Nadorcott SL, Nadorcott ARC, IRM 2, IRM 1, Shani SL. Etna, Sirio, Bakgat, Tanor Late, Saint Andre, Tango, Tahoe Gold, Gold Nugget, Mor 26, Yosemite Gold, Shasta Gold.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.17.1. List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2017 season.

Selection	Rootstock	Topwork
Saint Andre	Carrizo	2013
2 PH Phoenix	Carrizo	Unknown
Mor 26	Carrizo	Unknown
Gold Nugget	Carrizo	2013
Shasta Gold	Carrizo	2013
Tahoe Gold	Carrizo	2013
Yosemite Gold	Carrizo	2013

Tango	Carrizo	2013
Shani SL	Carrizo	Unknown
IRM 1	Carrizo	Unknown
IRM 2	Carrizo	Unknown

4.6.17.2. List of Mandarin hybrid selections evaluated in the Sundays River Valley (Penhill) region during the 2017 season.

Selection	Rootstock	Topwork
African Sunset (B24)	Carrizo	2011
Nova	Carrizo	2011
Michal 6/47	Carrizo	2011
Michal 89/64	Carrizo	2011
Edit x Nova	Carrizo	2011
Merav 119	Carrizo	2011
Merav 63	Carrizo	2011
Shani SL	Carrizo	2011
Nadorcott ARC	Carrizo	2011
Nadorcott SL	Carrizo	2011
IRM 1	Carrizo	2011
IRM 2	Carrizo	2011

Table 4.6.17.3 List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2017 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Bakgat	Carrizo	2012
Tanor Late	Carrizo	2012

Results and discussion

Edit x Nova

Edit x Nova kicked the season off for this Addo trial site. It had a good fruit size count ranging from 1 - 1x. Edit x Nova hang very well on the tree; it was observed in previous seasons as well. The sugars and acid percentage don't change much close to and at peak maturity, while the external colour development improves. This could be due to the good internal quality. The selection has good Brix° close to 12° and acid % just below 1.00%. This internal quality contributes to the good fruit flavour. Juice percentage for Edit x Nova was very good at 63%. There were seed for this selection during evaluations with a count of 1.6 seeds per fruit. During the first evaluation at peak maturity the colour was T5 on the colour plate and with the next evaluation still at peak maturity the colour was a T1 on the colour plate. The rind colour was deep orange as well as the internal colour.

African Sunset (B24)

Fruit size for B24 this season was very large again with a 1xxx count. B24 are a seedless variety and no seeds were detected during evaluations. Last season the colour development was good for B24, this season however it was delayed, compared to the internal maturity with a T 5 on the colour plate at peak maturity. African Sunset had a very good juice percentage of 56.9% at peak maturity. A Brix° of 11.3° at peak maturity were in line with most of the other selections at this trial site and the acid percentage were 0.95% that is very good. The fruit of B24 is flat to oblate, rind can be smooth to pebbly and sometimes there are a closed protruding navel present.

Nova

Nova are used as a control in this mandarin trial site. Fruit size count for Nova were count 1 close to peak maturity. Nova's juice percentage was 58.2% close towards peak maturity. This selection had one of the highest sugar compared to the other selections at this trial site. The high sugar was also supported with good acids. The Brix° was 14.8° and acid percentage was 1.31%. This will contribute to good shelf life for the fruit. Nova's fruit had the highest seed count 5.2 seeds per fruit. The external colour development for Nova fruit was good and in line with internal development of the fruit reaching T1 on the colour plate close to peak maturity. Peelability is not easy and rind oil can bother. Internal colour is an excellent deep orange.

Saint Andre

At peak maturity the Saint Andre has a very good juice percentage peaking at 58%. Fruit size of the Saint Andre was good having ranged from 1 to 1xx fruit size count. The sugar had a slight increase towards peak maturity as expected with the acid % stabilizing around 0.80% - 0.90%. Brix° was 9.9° towards build up to peak maturity and 11° when it was over mature. During the evaluations there were no seeds. The Saint Andre had an external colour of T7 on the colour plate towards peak maturity and above peak maturity it was T4 on the colour plate. External colour development was delayed. Rind is slightly pebbly and flesh is deep orange. Fruit is flat to round and peelability is easy.

Gold Nugget

Gold Nugget fruit size count was good with 1xx count. The juice percentage of Gold Nugget was also good ranging above 56%. At peak maturity the Gold Nugget had a T5 colour on the colour plate not as good as last season T1. Brix° stayed constant just below 11° during the ripening period. Acid percentage did decrease a bit, but the acid percentage was still good at 0.87% 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.

Shasta Gold

Shasta Gold is a late maturing mandarin hybrid. Mid-August in cold production regions. It is prone to alternate bearing. Fruit size is very big and it peaked at count 1xxx. The external colour development of the fruit was good with a T1 colour on the colour plate long before peak maturity. The selection juice percentages decrease towards peak maturity. Shasta Gold was also completely seedless. Brix° will still increase towards peak maturity and acids percentage normally stabilize around 1.00%.

Tahoe Gold

Fruit size count for Tahoe Gold was bigger this season with 1xxx count. The external colour development for this selection was not good with a T6 colour on the colour plate at peak maturity. It only reached a T1 on the colour plate when it was over mature. Brix° and acid % for Tahoe Gold this season was not as high as it would normally be for this selection. Tahoe Gold juice % was very good with a juice percentage of 64%. Tahoe Gold is a seedless variety. You have to manage this selection's alternate bearing.

Tango

Tango is mid to late maturing, seedless selection. Peak maturity for Tango was about 2 – 3 weeks earlier compared to the previous season's data. Rind colour development this season was not as good as the previous season. The previous season the selection was a T1 on the colour plate before peak maturity, this season it was T6 on the colour plate just before peak maturity. Juice percentages (around 58%) towards peak maturity is very good and better than last year's 50%. The fruit size for Tango were count 1. Tango had slightly lower Brix° and acid percentage this season. Brix° was 9.2° and acid percentage was 0.78% just before peak maturity. The rind is shiny and smooth and the flesh have a deep orange colour.

Yosemite Gold

Yosemite Gold had good external colour development and fruit was fully coloured before peak maturity was reached. The colour was T1 on the colour plate and the ratio was 10.7. The selection had a good juice percentage around 60% towards to peak maturity. Yosemite Gold had big fruit towards peak maturity with a fruit size count of 1xxx. Yosemite Gold had no seeds. Sugars are increasing towards peak maturity and acids

% is still good, not dropping too much. This will make a fruit with good internal quality and flavour.

Michal 6/47

Michal 6/47 were 2 count smaller this season with a count 3 at peak maturity. The selection had a very good juice % at peak maturity 63.5%. The colour was better this season with a T2 on the colour plate compared to the T5 - T6 from the previous season. The selection did have seeds with a maximum of 0.9 seeds per fruit. Just before peak maturity Michal 6/47 Brix° was 11.7° with an acid % of 1.00%. This good internal quality will contribute to the fruits shelf life. Michal 6/47 is an early – mid maturing experimental mandarin hybrid.

Michal 89/64

Fruit size for Michal 89/64 was also smaller this season with fruit size count 3. The juice percentage for this selection was good with a 61.4%. Michal 89/64 had a seed count of 1.1 seeds per fruit. Brix° for Michal 89/64 was good and it was just below 12° at peak maturity. Acid % was also good to support the high sugars with an acid % of 0.91%. The rind colour development was also better this season with a T3 on the colour plate. Michal 89/64 is also an early – mid maturing experimental selection, but this selection reaches peak maturity 2 weeks later than Michal 6/47.

Meirav 119

Meirav 119 is a mid-maturing experimental mandarin hybrid. It is a low seeded selection, seed count for this season ranged between 0.3 – 1.5 seeds per fruit. Fruit size count was between counts 2 - 1. The juice percentage was very good 62.6% at peak maturity but decreased as the selection reached over maturity. Meirav 119 was a T4 on the colour plate at peak maturity, and had a T1 on the colour plate at over maturity. This selection maturity was again before that of Meirav 63. Sugar and acid percentage at peak maturity was 11.4° and 0.93% respectively. The internal quality will contribute to the flavour and the flesh had a beautiful orange colour.

Meirav 63

Meirav 63 is also a mid-maturing experimental selection. Maturity is a couple of weeks behind Meirav 119. The fruit size count for Meirav 63 was count 2 – 3, it was a little bit smaller than that of Meirav 119. Meirav 63 had a slightly higher juice percentage at peak maturity 63.4%, but the juice percentage also decrease as the selection became more mature. Meirav 63 is also a low seed selection and the seed count was 0.9 – 1.2 seeds per fruit. Towards peak maturity the selection colour on the colour plate was T4 – T5 and the fruit was fully coloured with a T1 on the colour plate at peak maturity. The internal colour was a good orange colour. Brix° was also good at peak maturity 11.3° and the sugar had a good acid to support it with 0.95% acid at peak maturity. The good acid % will give the product a better shelf life.

Shani SL

The selection is a seedless selection, and no seeds were found during evaluations. Shani SL is a late maturing mandarin hybrid. The selection from the Kirkwood trial site was 1st to reach peak maturity, Shani SL from the Addo trial site matured later. The colour development of both sites was very good, T1 on the colour plate at peak maturity at the Kirkwood trial and T2 on the colour plate towards peak maturity at the Addo trial site. The selection was on a T1 on the colour plate long before peak maturity. The fruit size count was smaller this season and a big difference in size between the 2 sites. The trial site in Kirkwood had fruit size count of 1 – 1x and at the Addo site the count was 3 – 4 but it was before peak maturity. The size count might get a little bit bigger for the Addo site. Juice percentage decrease towards peak maturity, but the juice percentage was still good, 60% at peak maturity. Shani SL had some of the highest Brix° of all the selections with 14° Brix. The selection also had good acids to support the high Brix and contribute to the rich fruit flavour, the acid % at peak maturity was 1.15%.

Nadorcott ARC

The fruit size count of the Nadorcott ARC fruit ranged from count 1 to 1xx, slightly bigger count. Nadorcott SL had was one count smaller. The juice percentage of the Nadorcott ARC selection was a little bit lower, but not worth mentioning (60.7%). Juice percentage was higher this season. This selection had a very good colour development, T1 on the colour plate at peak maturity. Nadorcott ARC was seedless during evaluations.

Nadorcott LS was also completely seedless. The Nadorcott ARC had good Brix (11.6°) and acids of 0.94% at peak maturity. This good internal quality gives the fruit its good flavour. Internal colour is also an excellent orange. External colour is a deep orange with a waxy look. Yields on the tree were very good.

Nadorcott LS

Nadorcott LS reached peak maturity at the same time as ARC Nadorcott. Fruit size count for the Nadorcott LS range peaked from count 1 to 1x, the same as the previous season. This selection was completely seedless with all the evaluations completed. Nadorcott LS had a slightly higher juice percentage compared to Nadorcott ARC with a juice content of 63.3% at peak maturity. But the difference was really small. On the colour plate Nadorcott LS was a T1 at peak maturity. This selection had good Brix and acid levels at peak maturity which indicate the fruit will have a good shelf life. The rind colour is very good, dark orange. The fruit have a natural shine. Internally the fruit have a deep orange colour with an open core. Production on the trees was also good.

Phoenix

The fruit size of this selection varied between count 1xx and 1xxx, one count larger this season. At peak maturity the selection had a T5 – T6 colour on the colour plate. External colour development was delayed this season compared to last season's T1 on the colour plate. This selection has a very good juice percentage at peak maturity ranging between 54.5% - 58%. The seed count on two evaluations was seedless and one evaluation had 0.6 seed per fruit. At peak maturity the sugar was 10.5° with acid % of 0.86%.

IRM 1

The IRM 1 selection reached peak maturity after the IRM 2 at both sites. The IRM 1 fruit size count was 1x – 1xxx, the difference between the two sites and IRM 1 and IRM 2 in size are very small. IRM 1 had a very good juice percentage which increased with maturation. The selection had a very good juice content over 60% at peak maturity at both sites. Brix: acid ratio around 12 which is peak maturity Brix was above 12.5° and acids above 1%. The selection was not seedless with seed counts between 1.8 and 3.4 seeds per fruit. IRM 1 also had no problem with its external colour development reaching T3 colour on the colour plate before peak maturity and T1 at peak maturity. Rind is thin and peelability easy.

IRM 2

IRM 2 selection have coloured up completely before peak maturity (T1 on the colour plate). IRM 2 had a good fruit size development and peaked at count 1xxx. The juice levels of the IRM 2 increased with maturity up to 63.2% (peak maturity), slightly lower than IRM 1. Juice percentage was slightly higher at the Kirkwood site. IRM 2 had seeds with the evaluation ranging from 0.0 to 2.4 seeds per fruit. The Brix and acid levels were slightly lower again compared to the IRM 1 selection. IRM 2 have a very good flavour. The selection peelability is easy. IRM 1 and IRM 2 are firm fruit and prone to ribbing.

Mor 26

Size count for the selection was good with count 1xx. The juice percentage for Mor 26 was very good around 62% juice percentage. Rind colour development was slightly delayed compared to the internal maturity, with a T3 on the colour plate (peak maturity). The seed count was 1 – 2 seeds per fruit. Internal quality was good at peak maturity Brix° 11.6° and acid % was 0.93%.

Etna

Etna are an early maturing mandarin hybrid. Etna reached peak maturity early April. External colour development was not good with a T6 – T7 on the colour plate at peak maturity. Etna will be able to degreen. Fruit size count are 1x – 1xx. Juice percentage for Etna was very good, and it increased towards peak maturity to 64.1% at peak maturity. Etna had a low seed count ranging between 0.2 – 0.8 seeds per fruit. Etna was the selection with the lowest sugar and acid percentage at peak maturity. Brix° 8.9° and acid percentage was 0.75%. Etna have a deep orange internal colour.

Sirio

Sirio is also early maturing mandarin hybrid. Fruit size was big for Sirio with with a count 1xx – 1xxx. Juice percentage was above 50% for the selection at peak maturity. Seed count was low 0.0 – 1.3 seeds per fruit. Rind colour development was good with a T1 at the colour plate (peak maturity). Sirio's rind colour is a deep

orange as well as the flesh. Sirio have a good internal quality at peak maturity and it give the fruit a good flavour.

Bakgat

Bakgat rind colour development was good with a T2 on the colour plate at peak maturity. The fruit have a good acid, 0.93 % that stay stable during ripening. With the good acid the fruit will be able to hang a bit to get a T1 on the colour plate before the selection is over mature. The Brix° was also good along with the acid, being 11.4°. Bakgat fruit size count was 1 – 1x. At peak maturity Bakgat's juice % was good being 57.9%.

Tanor Late

Tanor Late is a late maturing mandarin hybrid. Fruit size count for the selection was big with a 1xxx count. Tanor Late had a good juice percentage, 56.5 % (peak maturity). Tanor Late seed count was low 0.0 – 1.5 seeds per fruit. Brix: Acid ratio at peak maturity was very good 11.7° and 0.98% acid. The good internal quality contributes to better shelf life for the fruit as well as the good flavour of the fruit. External colour development was very good being T1 on the colour plate at peak maturity. Tanor Late rind colour is a beautiful dark orange colour and internally the colour is an excellent deep orange colour. Tanor Late had a very good crop on the trees. Peelability for Tanor Late is easy. There are small thorns on the bearing branches.

Conclusion

The following selections had the largest fruit size count with a 1xxx count: IRM 1, Valley Gold B24, Phoenix, Shasta-, Tahoe-, Yosemite Gold, Sirio and Tanor Late. The selections with the highest juice percentage above 60% was: Edit x Nova, IRM 1, IRM 2, Meirav 119, Meirav 63, Michal 89/64, ARC Nadorcott, Nadorcott LS, Mor 26, Shani SL, Tahoe Gold and Etna. The selections with the highest °Brix was IRM 1, Shani SL and Nova. Bakgat and Nova was the selections that had the most seeds per fruit. Most of the selections had a colour T1 on the colour plate, and those that did have enough colour brake to degreen.

Table 4.6.17.4. Internal fruit quality data for Mandarin hybrid selections from Addo region of the Sundays River Valley (Penhill) during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-05-31	Phoenix	CC	1xx	56,7	10,7	0,97	11,0	0,8	T 6
2017-06-08	Phoenix	CC	1xx	55,0	11,4	0,76	15,0	1,0	T 5
2017-05-10	Edit x Nova	CC	1x	63,4	11,4	0,91	12,5	1,6	T 5
2017-05-24	Edit x Nova	CC	1	62,1	11,7	0,96	11,7	0,0	T 1
2017-07-05	IRM 1	CC	1xxx	61,7	12,3	1,15	10,7	3,4	T 3
2017-07-19	IRM 1	CC	1xxx	64,8	12,5	1,01	12,4	2,7	T 1
2017-08-02	IRM 1	CC	1xx	68,0	14,0	1,16	12,1	2,6	T 1
2017-06-19	IRM 2	CC	1xx	60,9	11,1	1,02	10,9	0,0	T 3
2017-07-05	IRM 2	CC	1xx	61,4	11,6	1,10	10,5	2,3	T 2
2017-07-19	IRM 2	CC	1xxx	59,5	12,3	0,88	14,0	2,3	T 1
2017-05-10	Meirav 119	CC	2	62,6	11,4	0,93	12,3	0,3	T 4
2017-05-24	Meirav 119	CC	1	58,9	11,4	0,88	13,0	1,5	T 1
2017-05-10	Meirav 63	CC	3	64,3	11,8	1,12	10,5	1,2	T 4-5
2017-05-24	Meirav 63	CC	2	63,4	11,3	0,95	11,9	0,9	T 1
2017-05-10	Michal 6/47	CC	3	63,5	11,7	1	11,7	0,9	T 2
2017-05-24	Michal 6/47	CC	3	64,3	11,8	0,71	16,6	0,0	T 1
2017-05-17	Michal 89/64	CC	3	61,4	11,8	0,91	13,0	1,1	T 3
2017-05-24	Michal 89/64	CC	3	61,4	11,9	0,74	16,9	1,1	T 1
2017-06-19	ARC Nadorcott	CC	1xx	62,0	11,8	1,16	10,2	0,0	T 3-4

2017-07-05	ARC Nadorcott	CC	1xx	60,7	11,6	0,94	12,3	0,0	T 1
2017-08-02	ARC Nadorcott	CC	1	64,0	11,5	0,84	13,7	0,0	T 1
2017-06-19	Nadorcott LS	CC	1x	63,5	10,1	0,91	11,1	0,0	T 3-4
2017-07-05	Nadorcott LS	CC	1	63,3	11,6	0,94	12,3	0,0	T 1
2017-07-19	Nadorcott LS	CC	1	63,5	11,4	0,77	14,8	0,0	T 1
2017-06-08	Shani SL	CC	4	61,3	14,4	2,16	6,7	1,0	T 4
2017-06-19	Shani SL	CC	4	59,2	15,3	2,21	6,9	0,0	T 2
2017-07-05	Shani SL	CC	3	58,8	18,7	2,56	7,3	0,0	T 2
2017-05-17	African Sunset (B 24)	CC	1xxx	59,5	10,6	0,91	11,6	0,0	T 5
2017-05-24	African Sunset (B 24)	CC	1xxx	56,9	11,3	0,95	11,9	0,0	T 5
2017-06-08	African Sunset (B 24)	CC	1xxx	56,7	10,6	0,78	13,6	0,0	T 3
2017-05-10	Nova	CC	3	56,8	14,1	1,48	9,5	2,5	T5
2017-05-17	Nova	CC	2	54,6	14,6	1,45	10,1	4,6	T5
2017-05-24	Nova	CC	1	58,2	14,8	1,31	11,3	5,2	T1

Table 4.6.17.5 Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-06-08	Phoenix	CC	1xx	54,5	10,5	0,86	12,2	0,0	T 6
2017-06-19	Phoenix	CC	1xxx	58,0	10,4	0,84	12,4	0,6	T 5
2017-07-05	Phoenix	CC	1xx	55,0	11,7	0,79	14,8	0,0	T 3
2017-07-05	IRM 1	CC	1xx	63,9	11,4	1,23	9,3	1,8	T 4
2017-07-19	IRM 1	CC	1xxx	64,3	12,7	1,15	11,0	2,1	T 1
2017-08-02	IRM 1	CC	1x	62,6	12,6	1,09	11,6	1,8	T 3
2017-06-19	IRM 2	CC	1xx	62,5	10,4	1,09	9,5	2,4	T 5
2017-07-05	IRM 2	CC	1xx	62,0	11,3	1,04	10,9	1,3	T 3
2017-07-19	IRM 2	CC	1xx	63,2	11,7	0,93	12,6	2,4	T 1
2017-06-19	Mor 26	CC	1xx	63,6	11,5	1,15	10,0	1,3	T 4-5
2017-07-05	Mor 26	CC	1xx	62,6	11,6	0,93	12,5	2,0	T 3
2017-07-19	Mor 26	CC	1xx	62,8	13,0	1,00	13,0	1,0	T 1
2017-06-08	Gold Nugget	CC	1xx	56,3	10,7	0,96	11,1	0,0	T6
2017-06-19	Gold Nugget	CC	1xx	56,5	10,9	0,87	12,5	0,0	T5
2017-07-05	Gold Nugget	CC	1xx	57,0	10,8	0,83	13,0	0,0	T3
2017-04-04	Saint Andre	CC	1	58,1	9,9	0,91	10,9	0,0	T7
2017-05-10	Saint Andre	CC	1xx	58,5	11	0,8	13,8	0,0	T4
2017-06-19	Shani SL	CC	1x	62,9	12,8	1,28	10,0	0,0	T 1-2
2017-07-05	Shani SL	CC	1x	63,0	13,3	1,19	11,2	0,0	T 1
2017-07-19	Shani SL	CC	1	60,0	14,1	1,15	12,3	0,0	T 1
2017-06-19	Shasta Gold	CC	1xxx	60,1	10,1	1,23	8,2	0,0	T2-3
2017-07-05	Shasta Gold	CC	1xxx	59,3	10,0	1,10	9,1	0,0	T1
2017-07-19	Shasta Gold	CC	1xxx	56,4	10,2	1,08	9,4	0,0	T1
2017-05-24	Tahoe Gold	CC	1xxx	62,9	9,1	1,29	7,1	0,0	T6
2017-06-08	Tahoe Gold	CC	1xxx	64,1	9,6	0,78	12,3	0,0	T6
2017-07-05	Tahoe Gold	CC	1xxx	62,9	9,9	0,74	13,4	0,0	T1
2017-05-24	Tango	CC	1	58,2	9,2	0,78	11,8	0,0	T6

2017-06-19	Tango	CC	1	58,6	11,2	0,83	13,5	0,0	T3
2017-06-08	Yosemite Gold	CC	1xxx	58,9	9,8	1,18	8,3	0,0	T5
2017-06-19	Yosemite Gold	CC	1xxx	60,5	10,6	1,00	10,6	0,0	T2-3
2017-07-05	Yosemite Gold	CC	1xxx	59,0	10,4	0,97	10,7	0,0	T1

Table 4.6.17.6 Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-05-24	Bakgat	CC	1	56,7	10,8	1,05	10,3	5,8	T6
2017-06-19	Bakgat	CC	1x	57,9	11,4	0,93	12,3	6,4	T2
2017-07-05	Bakgat	CC	1x	57,7	12,1	0,92	13,2	5,9	T1
2017-03-28	Etna	CC	1x	58,1	8,1	0,83	9,8	0,4	T7
2017-04-04	Etna	CC	1xx	64,1	8,9	0,75	11,9	0,2	T6-7
2017-05-10	Etna	CC	1xx	63,8	9,3	0,69	13,5	0,8	T3
2017-04-04	Sirio	CC	1xx	50,3	9,1	1,09	8,3	0,3	T7
2017-05-10	Sirio	CC	1xxx	53,9	10,4	0,81	12,8	1,1	T1
2017-05-24	Sirio	CC	1xxx	51,5	10,6	0,80	13,3	0,0	T1
2017-07-05	Tanor Late	CC	1xxx	58	10,7	1,07	10,6	1,5	T5
2017-07-19	Tanor Late	CC	1xxx	56,5	11,7	0,98	11,9	0	T1
2017-09-12	Tanor Late	CC	1xxx	46,1	13	0,78	16,7	0,9	T1

4.6.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)
Project 997C by W. Swiegers and Z. Zondi (CRI)

Summary

Loerie is the main trial site in the Gamtoos River Valley area, but there is a new site on the way with all of the latest selections in Patensie. Trees have been planted in 2017. Both trial sites will form part of the Gamtoos River Valley. At Loerie the season started off with Saint Andre and was followed up by Etna, Sirio, Nova ARC, Tasty 1, Tango, Tahoe Gold, 2PH Phoenix, Yosemite Gold, Mor 26, IRM 2, IRM 1, Gold Nugget, Shasta Gold and the season ended with Tanor Late.

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 Saint Andre gevolg deur Etna, Sirio, Nova ARC, Tasty 1, Tango, Tahoe Gold, 2PH Phoenix, Yosemite Gold, Mor 26, IRM 2, IRM 1, Gold Nugget, Shasta Gold 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 Gamtoos River Valley. A range of new mandarin hybrids had been added to this area. The following varieties were evaluated: Saint Andre, Etna, Sirio, Nova ARC, Tasty 1, Tango, Tahoe Gold, 2PH Phoenix, Yosemite Gold, Mor 26, IRM 2, IRM 1, Gold Nugget, Shasta Gold and Tanor Late.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.18.1 List of experimental mandarin hybrid selections evaluated in the Loerie (N. Ferreira) region of the Gamtoos River Valley during the 2017 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Saint Andre	Carrizo	2012
Tasty 1	Carrizo	2012
Phoenix	Carrizo	2012
Nova ARC	Carrizo	2012
Tanor Late	Carrizo	2012
Gold Nugget	Carrizo	2012
Shasta Gold	Carrizo	2012
Tahoe Gold	Carrizo	2012
Tango	Carrizo	2012
Yosemite Gold	Carrizo	2012
Mor 26	Carrizo	2012
IRM 1	Carrizo	2012
IRM 2	Carrizo	2012

Results and discussion

Gold Nugget

Gold Nugget matured slightly later this season compared to the previous season. The fruit size for Gold Nugget ranged between 1 – 1xx. Fruit for this selection is pebbly and peelability is easy with low levels of rind oil. Before peak maturity was reached the external colour reach T1 on the colour plate. For this selection it is a yellow orange colour. There are thorns on the bearing branches, but they get smaller as the tree gets older. Gold Nugget is a seedless variety. At peak maturity the internal juice percentages were just below 55%. Internal quality for the selection is good with high sugars and good acids above 1%, this also contributes to the good flavour of Gold Nugget.

Shasta Gold

Shasta Gold ended off the UCR 5 mandarin selection season and was also the second last selection to reach peak maturity. The fruit was seedless. Shasta Gold had excellent colour development (colour plate T1) long before peak maturity. Shasta Gold developed a very good juice percentage, well over 60%. The selection had extra large fruit size and peaked at count 1xxx. Shasta Gold also had good Brix° (14°) and acid (1.38%) ratios towards peak maturity.

Tahoe Gold

Tahoe Gold were first to reach peak maturity for the UCR 5 selections. The fruit size was a count bigger this season and ranged between 1xx and 1xxx. Tahoe Gold had no problem with external colour break development as it was (T5) long before peak maturity. Tahoe Gold had some of the best juice levels of the

UCR 5 selections, with a juice percentage above 63%. Tahoe Gold is also a seedless variety. The selection normally have very high Brix and acids at peak maturity, but this season it was low.

Tango

Tango had a very good fruit size (count 1) and were seedless, as it is a seedless variety. The selection had good external colour development that ranged between T1 – T3 towards peak maturity. Tango rind and internal colour is a deep orange. The rind also has a natural shine and peels very easy, and the rind oil don't bother. Tango juice percentage was higher around 60% compared to last seasons 53%. Tango's Brix and acid ratios were good, indicating that the fruit will have a good shelf life and give Tango a good flavour.

Yosemite Gold

Yosemite Gold had big fruit this year and peaked at count 1xxx for the third year in a row. The juice levels were slightly lower this season but still good around 55% towards peak maturity. There were no seeds during evaluations. Yosemite Gold had no problem with colour development reaching T1 on the colour plate before peak maturity. External colour is deep orange and rind are smooth. Internal quality was still good but sugars can get higher.

Nova ARC

Nova ARC had a smaller fruit size count (1) this season compared to last years (1xx) count. Nova ARC had a seed count of 0.3 seeds per fruit, and only during one evaluation. Again this selection had no problem with external colour development, being T1 on the colour plate range by peak maturity. The juice % for Nova ARC at peak maturity was 53.7%. Internal quality was good for Nova ARC at peak maturity Brix 11° and acid 0.93%.

Saint Andre

Saint Andre were the first selection to reach peak maturity in this production region. Maturity was about a week later than the previous season. The fruit size count was between 1x and 1xx. The juice percentage this season was much higher compared to the previous seasons. This season's percentage was 67.2% the highest of all the selections. At peak maturity the the colour was T4 on the colour plate. The acids and sugars remained stable during the production season for Saint Andre. The highest seed count noted for the selection was 0.8 seeds per fruit.

Etna

The fruit size count for the Etna this season was smaller count 2 – 1, compared to last season 1x to 1xxx. The juice percentage for Etna was very good above 60 % juice at peak maturity and better compared to Sirio. The Brix and Acid levels of Etna were very much the same than Sirio towards peak maturity. Sirio had a slightly higher Brix and acid. Etna reached peak maturity before Sirio in the mandarin range of new experimental cultivars. Etna had a T1 on the colour plate range at peak maturity. On all three evaluations there was a seed count ranged from 1.2 – 2.3 seeds per fruit.

Sirio

Sirio had a good fruit size count and peaked at 1xx. Sirio developed a lower juice percentage than Etna at 61.3%. The juice percentage increased towards peak maturity. The Brix and Acid levels of Sirio were slightly higher than Etna at peak maturity. Sirio had no external colour development problems, being fully coloured before peak maturity. Sirio had the highest seed count and compared to Etna it was higher with a 3.5 – 4.8 seeds per fruit.

Tasty 1

Tasty 1 developed a large/xtra-large fruit size and peaked at count 1xxx. The external colour development was good with a T3 on the colour plate before peak maturity. The juice percentages were not good for Tasty 1 being below 50%. On 2 of the evaluations there was a seed count ranging between 0.3 and 1.8 seeds per fruit.

Phoenix

Phoenix bore a good fruit size on the trees with a 1 - 1xx count at peak maturity. The juice percentage of the Phoenix above 60 % and it had a T2 on the colour plate range towards peak maturity. The average seed count for Phoenix was 2.3 seeds per fruit. Peak maturity was reached at the beginning of August much later

compared to previous season. The acid percentage was still above 1% at peak maturity.

Tanor Late

Tanor Late is a late - ultra late maturing mandarin hybrid selection. This selection was last to reach peak maturity, being fully ripen end September, 3 – 4 weeks later than the previous season. The fruit size of Tanor Late was large and peaked at count 1xxx, with the correct rootstock you might get smaller fruit. The external colour development of this selection was very good, being a T1 on the colour plate long before peak maturity. Rind colour for Tanor Late is a deep orange. The fruit is firm and pebbly, but still peels easy. Tanor Late had a good juice percentage (around 56%). The seed count for this selection ranged between 0.3 – 1.4 seeds per fruit. The Brix and Acid ratio of Tanor Late was very good with an average Brix and Acid content being 12.3° and 1.04% (peak maturity). The fruit will have a good shelf life.

IRM 1

The IRM 1 selection reached peak maturity after the IRM 2. The IRM 1 fruit size count was 1xx, it was bigger than IRM 2 with count 1. IRM 1 had a very good juice content over 60%, it is slightly lower compared to IRM 2 juice content. Juice percentage increase towards peak maturity. Internal quality was excellent and very much the same between IRM1 and IRM 2. Brix was above 13° and acids above 1% at peak maturity. The selection was not seedless with seed counts between 2.7 and 3.6 seeds per fruit, basically the same as IRM 2. IRM 1 also had no problem with its external colour development reaching T1 colour, on the colour plate before peak maturity. Rind is thin and peelability easy. Fruit are prone to ribbing.

IRM 2

IRM 2 selection have coloured up completely at peak maturity (T1 on the colour plate). The selection matured before IRM 1. IRM 2 had a very good fruit size development and peaked at count 1. This was smaller compared with IRM 1. The juice levels of the IRM 2 increased with maturity up to 65.5% (peak maturity), slightly higher than IRM 1. IRM 2 had seeds with the evaluation ranging from 2.6 to 3.8 seeds per fruit. The Brix and acid levels for IRM was very good, Brix was above 13° and acids above 1% at peak maturity. IRM 2 have a very good flavour. The selection peelability is easy. IRM 2 have firm fruit and are prone to ribbing.

Mor 26

Size count for the selection was good with count 1 - 1xx. The juice percentage for Mor 26 was very good around 60% juice percentage at peak maturity. Rind colour development was excellent to reach T1 on the colour plate before peak maturity. The seed count was 0 – 2.3 seeds per fruit. Internal quality was good at peak maturity Brix° 13.5° and acid % was 1.11%. This good Brix: Acid ratio will contribute to good eating fruit with good flavour and shelf life.

Conclusion

Most of the selections (Nova ARC, Etna, IRM 1, IRM 2, Mor 26, Gold Nugget, Sirio, Shasta Gold, Tanor Late, Yosemite Gold) had a very good external colour development (T1) at peak maturity. The following selections had the largest fruit size (count 1xxx); Shasta Gold, Yosemite Gold, Tasty 1 and Tanor Late. Etna cropped the smallest fruit size (count 1 to 2). Most of the other selections were count 1. Phoenix, Etna, IRM1 and 2, Mor 26, Saint Andre, Sirio, Shasta Gold, Tahoe Gold and Tango developed juice percentages above 60%. Tasty 1 had juice percentages below 50%. IRM 1 and 2, Mor 26 and Shasta Gold had the highest Brix level above 13°. Tanor Late, Phoenix, IRM 1, IRM 2 and Mor 26 performed well with good Brix: acid ratios, improving shelf life and fruit quality. The selection with the highest seed count, Sirio; average 4.8 seeds per fruit. UCR 5 selections were seedless.

Table 4.6.18.2 Internal fruit quality data for experimental mandarin hybrid selections from the Loerie (N. Ferreira) region of the Gamtoos River Valley region during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-07-18	Phoenix	CC	1xx	53,5	10,6	1,10	9,6	2,3	T4

2017-08-02	Phoenix	CC	1	60,6	11,7	1,03	11,4	0,0	T2
2017-05-25	ARC Nova	CC	1	53,7	11,0	0,93	11,8	0,0	T1
2017-06-07	ARC Nova	CC	1	60,8	11,4	0,88	13,0	0,3	T1
2017-04-03	Etna	CC	2	62,4	9,9	1,28	7,7	1,2	T7
2017-05-18	Etna	CC	2	62,2	10,5	1,03	10,2	2,3	T4
2017-05-25	Etna	CC	1	62,0	10,4	0,86	12,1	1,6	T1
2017-08-29	IRM 1	CC	1xx	61,2	13,6	1,22	11,1	3,6	T1
2017-09-11	IRM 1	CC	1xx	64,6	13,4	1,00	13,4	2,7	T1
2017-07-18	IRM 2	CC	1	62,5	12,1	1,17	10,3	2,6	T2
2017-08-29	IRM 2	CC	1	65,5	13,7	1,15	11,9	3,8	T1
2017-08-02	Mor 26	CC	1	64,1	13,0	1,25	10,4	2,2	T1
2017-08-29	Mor 26	CC	1xx	61,2	13,5	1,11	12,2	0,0	T1
2017-09-11	Mor 26	CC	1	56,5	14,8	1,08	13,7	2,3	T1
2017-08-29	Gold Nugget	CC	1	53,8	12,9	1,18	10,9	0,0	T1
2017-09-11	Gold Nugget	CC	1xx	55,5	13,0	0,88	14,8	0,0	T1
2017-05-11	Saint Andre	CC	1	67,2	11,4	0,92	12,4	0,8	T4
2017-05-18	Saint Andre	CC	1xx	61,6	11,4	0,88	13,0	0,7	T3
2017-05-25	Sirio	CC	1xx	56,7	10,8	1,07	10,1	3,5	T1
2017-06-07	Sirio	CC	1x	61,3	11,7	0,91	12,9	4,0	T1
2017-06-20	Sirio	CC	1xxx	59,5	11,2	0,81	13,8	4,8	T1
2017-08-29	Shasta Gold	CC	1xxx	61,0	12,2	1,44	8,5	0,0	T1
2017-09-11	Shasta Gold	CC	1xxx	60,3	14,0	1,38	10,1	0,0	T1
2017-07-04	Tahoe Gold	CC	1xx	65,1	9,5	0,98	9,7	0,0	T5
2017-08-02	Tahoe Gold	CC	1xxx	63,6	10,1	0,65	15,5	0,0	T1
2017-06-20	Tango	CC	1	59,7	10,8	0,99	10,9	0,0	T4
2017-07-04	Tango	CC	1	62,9	10,5	0,95	11,1	0,0	T3
2017-07-18	Tango	CC	1	59,1	10,9	0,84	13,0	0,0	T1
2017-09-11	Tanor Late	CC	1xxx	61,3	12,2	1,1	11,0	1,4	T1
2017-09-20	Tanor Late	CC	1xxx	57,0	10	0,89	11,2	0,4	T1
2017-09-27	Tanor Late	CC	1xxx	56,3	12,3	1,04	11,8	0,3	T1
2017-06-07	Tasty 1	CC	1xx	48,5	11,8	1,15	10,3	0,3	T5
2017-06-20	Tasty 1	CC	1xxx	47,8	10,8	0,96	11,3	0,0	T3
2017-07-04	Tasty 1	CC	1xx	50,7	11,8	0,81	14,6	1,8	T1
2017-08-02	Yosemite Gold	CC	1xxx	55,9	10,3	1,05	9,8	0,0	T1
2017-08-11	Yosemite Gold	CC	1xxx	56,9	11,4	0,87	13,1	0,0	T1

4.6.19 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (Western Cape)
Project 997D by W. Swiegers (CRI)

Summary

The trial site in Citrusdal consists of a variety block with a selection of all the new experimental cultivars from early maturing to late maturing selections. The cross pollination is high in this block due to all the different selections that are present. The season started with Tami 2/65 and then Michal6/47 followed, Michal 89/64, Phoenix, Edit x Nova, Nova ARC, IRM 2, Meirav 119, Etna, Meirav 63, Sirio, IRM 1, Furr, Samba, Tasty 1, Mor 26, Or, Tango, B24, ARC Nadorcott, B17, Tahoe Gold, Gold Nugget, Yosemite Gold, Nad LS, Shani SL, Tanor Late and Shasta Gold. At the Paarl site most of the new experimental selections were topworked there. Cross

pollination is also high in this site. Here the season started with Nova, Etna, Sirio, Tasty 1, Tango, Gold Nugget, Tahoe Gold, and ended with Yosemite Gold.

Opsomming

Die proef perseël in Citrusdal bevat meeste van die nuwe eksperimentele seleksies van vroeg tot laat rywordend. Die kruisbestuiwing in hierdie proef perseël is baie hoog weens al die verskillende seleksies teenwoordig. Die orde van rywording was as volg gewees Tami 2/65, Michal6/47, Michal 89/64, Phoenix, Edit x Nova, Nova ARC, IRM 2, Meirav 119, Etna, Meirav 63, Sirio, IRM 1, Furr, Samba, Tasty 1, Mor 26, Or, Tango, B24, ARC Nadorcott, B17, Tahoe Gold, Gold Nugget, Yosemite Gold, Nad LS, Shani SL, Tanor Late en Shasta Gold. By die Paarl perseel is die meeste van die nuwe seleksies nou oorgewerk. Kruisbestuiwing is ook baie hoog in die perseel. Hier het die seisoen begin met Nova, Etna, Sirio, Tasty 1, Tango, Gold Nugget, Tahoe Gold, en afgesluit met Yosemite Gold.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from Citrusdal and Paarl region of the Western Cape. The following selections were evaluated: Tami 2/65, Michal6/47, Nova, Michal 89/64, Phoenix, Edit x Nova, Nova ARC, IRM 2, Meirav 119, Etna, Meirav 63, Sirio, IRM 1, Furr, Samba, Tasty 1, Mor 26, Or, Tango, B24, ARC Nadorcott, B17, Tahoe Gold, Gold Nugget, Yosemite Gold, Nad LS, Shani SL, Tanor Late and Shasta Gold.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.19.1. List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2017 season.

Selection	Rootstock	Topwork	Planted
African Sunset (B24)	CC		2009
Furr (Clemcott)	CC	2011	
Gold Nugget	CC	2010	
Meirav 63	CC	2010	
Meirav 119	CC	2011	
ARC Nadorcott	CC	2010	
Nadorcott LS	CC	2010	
Edit x Nova	CC	2010	
Nova ARC	CC		Unsure
Tahoe Gold	CC	2010	
Tango	CC	2010	
Shani SL	CC	2010	
Tanor late	CC	2012	
Valley Gold (B17)	CC	2011	
Yosemite Gold	CC	2010	

Etna	CC	2012	
Samba	CC	2012	
Sirio	CC	2012	
Tasty 1	CC	2012	
IRM 1	CC		2009
IRM 2	CC	2010	
Michal 6/47	CC	2010	
Michal 89/64	CC	2010	
Mor 26	CC		Unsure
Or	CC		Unsure
Phoenix	CC	2010	
Shasta Gold	CC	2010	
Tami 2/65	CC	2010	

Table 4.6.19.2. List of experimental mandarin hybrid selections evaluated in the Paarl region (Babylonstoren) of the Western Cape during the 2017 season.

Selection	Rootstock	Planted
Etna	CC	2013
Gold Nugget	CC	2012
Nova	C35	2012
Shasta Gold	CC	2012
Sirio	CC	2013
Tahoe Gold	CC	2012
Tango	CC	2012
Tango	RL	2012
Tasty 1	CC	2013
Yosemite Gold	CC	2012

Results and discussion

African Sunset (B24)

African Sunset bore extra large fruit on the trees (count 1xxx). Colour development was very good (T1) before peak maturity. Rind colour is deep orange. When evaluated, the internal juice percentages towards peak maturity was low (36.5%), at peak maturity it was better but still low (45.7%) and when it was over mature it was very low again (38.5%). The selection was seedless during evaluations. Fruit was pebbly, and some fruit had a closed protruding navel where some of the fruit start to split. At peak maturity Brix° was 10.9° with an acid % of 0.90%.

Tami 2/65

Tami 2/65 was the first selection to reach peak maturity at the Citrusdal site. The fruit size for Tami 2/65 was very good with fruit size count 1 – 1x. Internal juice percentage is high (60.1%). Internal colour is a deep orange. The fruit peels easily. The selection was seedless. Rind colour development was not good with T6 on the colour plate at peak maturity. The selection doesn't have high acid and it drops quickly.

Furr (Clemcott)

Furr is used as a control for the mid-maturing mandarin selections. The juice content was below 55% (peak maturity). The fruit size count was 1xx. Furr peels easily and has a very good eating quality. Due to the high cross pollination in the mixed trial block, Furr produced the highest number of seeds per fruit; up to 18.3. Furr's external colour development was better compared to the previous 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.

Michal 6/47

Michal 6/47 is an early – mid experimental maturing mandarin hybrid. It reached peak maturity end April beginning of May, it was slightly earlier than Michal 89/64. The rind colour was not good at peak maturity with T5 – T6 at peak maturity. Michal 6/47 fruit size was small; count 4 – 3. It is 1 – 2 size counts smaller than Michal 89/64. The selection had a good juice % at peak maturity above 55%, and it is higher than Michal 89/64 juice %. The selection did have seeds with a maximum of 2.1 seeds per fruit, slightly lower than Michal 89/64. Michal 6/47 Brix° and acid % at peak maturity was slightly higher than Michal 89/64 Brix and acid.

Michal 89/64

Michal 89/64 is also an early – mid maturing experimental selection, but this selection reach peak maturity 1 week later than Michal 6/47. External colour development for Michal 89/64 at peak maturity was T4 – T6 on the colour plate. Fruit size for Michal 89/64 was also larger this season and also larger than Michal 6/47 with fruit size count 2 – 1x. The juice percentage for this selection was low 49.2%. Michal 89/64 seed count peaked at 2.3 seeds per fruit. Brix° for Michal 89/64 was below 10° at peak maturity. Acid percentage was also lower this season, around 0.80%.

Etna

Etna is an experimental early – mid maturing mandarin hybrid. Etna reached peak maturity at both regions before Sirio. Etna reached peak maturity first at the Paarl trial site compared to the Citrusdal site. Etna's fruit was larger compared to Sirio's fruit size. The fruit size count for the Etna this season at Paarl ranged between 1xx – 1xxx and at Citrusdal it ranged 1x – 1xx. At both sites Etna had a juice percentage below 50%. Sirio had a higher juice %. Sirio had a much better Brix: Acid ratio compared to Etna. In Citrusdal, Etna had a T1 on the colour plate well before peak maturity, in Paarl it was T4 on the colourplate at peak maturity. Sirio also had much better external colour development. Etna have a deep orange internal colour. The seed count for Etna in Citrusdal was 1.8 seeds per fruit and in Paarl it ranged between 1.3 – 8.5 seeds per fruit.

Sirio

Sirio is also an early – mid maturing experimental mandarin hybrid. Sirio reached peak maturity after Etna. Sirio was also earlier in Paarl vs Citrusdal. Fruit size count for Sirio was very good it peaked at 1x in Citrusdal and in Paarl it ranged 1 – 2 count. Sirio had a higher seed count compared to Etna; seed count in Paarl was between 6.1 – 10.8 seeds per fruit and in Citrusdal one count was seedless and the other one was 6.7 seeds per fruit. Sirio developed a higher juice percentage than Etna around 50%. The Brix and Acid levels of Sirio were slightly higher than Etna at peak maturity. Sirio had good Brix: Acid ratio; in Citrusdal Brix above 11 and acid 1.23% towards peak maturity and in Paarl Brix above 12 and acid 1.15 towards peak maturity. Sirio had no external colour development problems, being fully coloured before peak maturity. Sirio have internally as well externally deep orange colour and also have a good flavour.

Phoenix

Phoenix bore a very good fruit size on the trees with a count 1 at peak maturity. The juice percentage for Phoenix was around 50% at peak maturity and it had a T4 – T7 on the colour plate at peak maturity. Due to the acids that did not drop while the fruit was at peak maturity the fruit could hang to change colour. The average seed count for Phoenix was between 0 – 1.4 seeds per fruit. Sugars ranged between 10° - 11° at peak maturity.

Mor 26

Size count for the selection was very good with count 1. The juice percentage for Mor 26 was very low, below 50% juice percentage at peak maturity. Rind colour development was fair - good to reach T2 – T3 on the colour plate at peak maturity. The seed count was 1.4 – 1.8 seeds per fruit. Internal quality was good at peak maturity Brix° above 12.5° and acid % were above 1.00%. This good Brix: Acid ratio will contribute to good eating fruit with good flavour and shelf life.

Or

Or is a late maturing mandarin hybrid. It reached peak maturity in July. The size count for this selection was very good count 2 – 1. Fruit is round to oblate. Juice percentage increase towards peak maturity to just below 55%. Internal quality for Or is very good. Brix is high above 13° and the acids was still above 1.00% even when the fruit was overmature. 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 at peak maturity. Average seed count was 0.9 seeds per fruit.

Gold Nugget

Tree manipulation is necessary to control the strong vegetative and upright growth habit. Gold Nugget developed one of the best tasting fruit with a high Brix: acid ratio. The fruit peaked internally with Brix of 14°. 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 1 at peak maturity and fully coloured (T1). The juice percentage for this selection was below 50%. Gold Nugget is seedless. There was no big difference between Gold Nugget in Paarl and Citrusdal internally of with colour development. The only difference was when peak maturity was reached. Paarl was about 2 weeks earlier.

Nadorcott ARC & Nadorcott LS

Nadorcott ARC and Nadorcott LS are induced Nadorcott selections to minimise the average seeds per fruit. Both selections have the same growth habit and characteristics as the Nadorcott. Fruit size for these selections peaked at count 1. The internal juice percentages were below 50% for ARC Nadorcott and Nadorcott LS towards and at peak maturity. The two Nadorcott selections developed good Brix above 12° with acids of 1.00%, ensuring a good balance and eating quality. Nadorcott LS had slightly higher Brix and acids. The fruit was fully coloured (T1) before peak maturity. The highest seed count during the three evaluations was 0.5 seeds per fruit for Nadorcott LS. This is in a high cross pollination trial site.

Nova ARC

Nova ARC is a selection from the ARC that was irradiated to improve the selection (completely seedless). The selection had a low seed count 0.8 seeds per fruit this is due to the high cross pollination trial site. Nova ARC is also an early to mid maturing experimental mandarin hybrid. The fruit had a good external colour development (T2) before peak maturity was reached and fruit size for Nova ARC was medium to large (count 1 – 1x). The internal juice percentages were good, above 50%. Nova ARC had a high Brix level of 13° and good acid % of 1.16%. This give Nova ARC its good flavour. To compare the Nova on C35 from Paarl with Nova ARC on CC from Citrusdal, the differences was that the Nova ARC had much better internal quality, higher sugars and acids. The other difference was the seed count, Nova had an average seed count of 22 seeds per fruit and Nova ARC had an average seed count of 0.8 seeds per fruit.

Nova

Nova is an early maturing mandarin hybrid. The fruit size for Nova was large to extra large with 1x – 1xx count. Nova's fruit is flat to round and does not peel easy and is oily. The juice percentage for Nova was good around 55% at peak maturity. Brix for Nova at peak maturity was around 10° the acids did drop quickly for Nova from peak maturity (0.86%) to overmature (0.81%), meaning the fruit will not hang well on the trees. External colour ranged between T1 – T4 on the colour plate at peak maturity. The seed count for Nova was high 22 – 26.3 seeds per fruit. This could be due to the high cross pollination.

Edit x Nova

Edit x Nova cropped a medium fruit size on the trees (count 1) with a good juice percentage of 57.4% close to peak maturity. Degreening might be necessary for Edit x Nova because of its external colour delay; T4 on the colour plate range close to peak maturity. The average seed count for Edit x Nova was 1.2 seeds per fruit. Edit x Nova had a good crop on the trees. Brix: Acid ratio is also very good for Edit x Nova close to peak maturity and it give Edit x Nova its good flavour.

Samba

Samba is an early – mid maturing experimental low seeded mandarin hybrid, with seed count that peaked at 1.4 seeds per fruit. Peak maturity was late this season. Samba have a favourable fruit size count that range between counts 3 – 1. Fruit is round to oblate with halo. Peelability is easy for Samba and can be oily. Long before peak maturity was achieved, Samba was a T 1 on the colour plate. Samba have an exceptional deep orange external colour and a very deep internal colour. At peak maturity; Samba have high Brix and good acids and this give Samba its unique and excellent flavour. The juice percentage at peak maturity for this selection was 54.8%.

Tasty 1

Tasty 1 developed a large/xtra-large fruit size and peaked at count 1xx for both regions. The external colour development was good at both sites with a T1 on the colour plate at peak maturity. The juice percentages were not good for Tasty 1 being below 50% in Paarl and Citrusdal. In the Paarl trial site the seed count were higher compared to Citrusdal. In Paarl seed count peaked (6.6 seeds per fruit) and Citrusdal peaked (4.1 seeds per fruit). Brix: Acid ratio was slightly better in Citrusdal. Paarl matured 3 weeks earlier than Citrusdal. In Paarl fruit size was medium/xtra large fruit count 2 – 1xx vs Citrusdal with xtra large fruit size, count 1xxx. Citrusdal matured a little bit earlier than in Paarl, but the internal quality for Tahoe Gold was better in Paarl. Juice % at both regions were not high. External colour development was very good, at both sites the selection was fully coloured before peak maturity.

Yosemite Gold

Yosemite Gold had big fruit this year and peaked at count 1xxx at both trial sites. At the Paarl trial site peak maturity was reached later vs the Citrusdal trial site. Juice percentage was low at both sites, Paarl site had higher juice percentage Internal quality is good in both regions. Paarl does have slightly higher Brix and acids. At both sites the external colour is T 1 on the colour plate. Yosemite Gold is a seedless selection.

Shasta Gold

Shasta Gold ended off the UCR 5 mandarin selection season and was also the last selection to reach peak maturity. The fruit was seedless. Shasta Gold had excellent colour development (colour plate T1) long before peak maturity. Shasta Gold developed low juice percentage, below 50% towards peak maturity. The selection had extra large fruit size and peaked at count 1xx. Shasta Gold also had good Brix° and acid ratios towards peak maturity. Paarl region was about 3 weeks behind with maturity compared to Citrusdal.

Valley Gold (B17)

Valley Gold is a late maturing mandarin hybrid. Fruit size was large and ranged 1 – 1x count. At peak maturity juice percentage was around 50%. Internal quality is excellent with Brix above 14° and acid % at 1.10%. This give Valley Gold its great flavour. Seed count peaked at 2.2 seeds per fruit and the external colour development was excellent, T1 on the colour plate before peak maturity. The fruit of Valley Gold is flat with a thin slightly pebbly rind. Peelability is easy and externally as well as internally the colour is dark orange. A lot of the fruit split on the trees.

Meirav 63

Meirav 63 is a mid-maturing experimental mandarin hybrid. Meirav 63 reached peak maturity after Meirav 119. Meirav 63 bore a smaller fruit size on the trees (count 2). Meirav 119 had larger fruit size (count 2 – 1x). The juice percentage of Meirav 63 was low 47.1% towards peak maturity. Meirav 119 also had low juice percentage below 50%. External colour development for Meirav 63 was very good with a T1 colour on the colour plate before peak maturity. The seed count for Meirav 63 peaked 1.6 seeds per fruit vs Meirav 119 that peaked at 1 seed per fruit. Meirav 63 had a slightly better internal quality than Meirav 119, but both selections had a good internal quality.

Meirav 119

Meirav 119 is a mid-maturing experimental mandarin hybrid. Meirav 119 reached peak maturity before Meirav 63. It is a low seeded selection, seed count for this season ranged between 0 – 1 seed per fruit. Fruit size count was between counts 2 – 1x. The juice percentage was not good, 48.6% at peak maturity. Meirav 119 was a T1 on the colour plate before peak maturity. Sugar and acid percentage at peak maturity was 12.2° and 0.97% respectively, slightly higher than last season.

Tango

Tango in the Paarl trial site reached peak maturity 2 weeks after peak maturity was reached in Citrusdal. 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 for both regions. Tango was seedless at both trial sites. The fruit size peaked at count 1 in Citrusdal and in Paarl it peaked at count 2. Internally the

juice percentage for Tango was below 50%. At both trial sites the Brix: acid ratio was good, but in the Paarl trial site it was a little bit higher with (Brix 12.6°) and (acid 1.06%).

IRM 1

The IRM 1 is a late maturing experimental mandarin hybrid. The IRM 1 fruit size count was 1x, one count larger than IRM 2. IRM 1 had a low juice content. Internal quality for IRM 1 was better than that of IRM 2. IRM 1, Brix was 12.5° and acid was 1.12% towards peak maturity. Seed count peaked at 4.1 seeds per fruit. One evaluation was seedless. External colour development was good (T 2 on the colour plate) towards peak maturity. External colour development was better for IRM 1 vs IRM 2. IRM 1 is prone to ribbing.

IRM 2

IRM 2 is a mid to late maturing experimental mandarin hybrid. The juice percentage for this selection was low. IRM 2 had a slightly lower Brix: Acid ratio compared to IRM 1. Internal quality for IRM 2 towards peak maturity was (Brix 10.5° and acid 1.01%). The fruit size for IRM 2 is very favourable with count 1. There were some seeds (0.8 – 1.0 seed per fruit) in the selection. Degreening would have to be done as the selection was T6 on the colour plate (towards peak maturity) and T4 on the colour plate (fruit was overmature). The IRM 2 fruit is firm with good flavour.

Shani SL

Shani SL was one of the last selections to reach peak maturity. In this high pollination site Shani SL seed count peaked at 2.7 seeds per fruit. The fruit size was ideal and peaked at count 1. Brix and acid part of internal quality was good, Shani SL had one of the highest Brix: acid ratio; Brix of 13.8° and acid of 1.36% towards peak maturity at a 10.1 ratio. Juice percentage 42.7% was not good. The fruit was fully coloured before peak maturity.

Tanor late

Tanor late is an ultra-late experimental mandarin selection maturing end of August to middle September. The selection is very thorny but on the oldest trees in South Africa the thorns tend to disappear or become fairly small. The external colour development is very good (T1) well before peak maturity. Tanor late bore fruit with a juice content of 36% towards peak maturity. The variety peels easily and clean, the fruit size peaked at count 1xxx (extra large). The selection had a low seed count of 0.2 – 0.6 seeds per fruit. Towards peak maturity with ratio of 8.8 Brix was 11.5° and acid was 1.31%.

Conclusion

African Sunset, Tahoe Gold, Tanor late, Yosemite Gold and Etna had the largest fruit size (1xxx). Samba and Michal 6/47 had the smallest fruit size with a count 3 followed by Meirav 63 at a count 2. Nova had the most seeds per fruit on average (26.3 seeds per fruit) followed by Furr (18.3 seeds per fruit). UCR 5 selections, African Sunset and Tami 2/65 were the only selections which were completely seedless. Edit x Nova, Michal 6/47, Tami 2/65 and Sirio had a juice percentage over 55% at peak maturity. All the selections that were a T1 on the colour plate range (good colour development) before or at peak maturity was UCR 5 selections, African Sunset, Meirav 119, Meirav 63, Furr, ARC Nadorcott, Nadorcott LS, Shani SL, Tanor Late, Valley Gold, Etna, Samba, Sirio and Tasty 1.

Table 4.6.19.3. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2017 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-06-29	African Sunset (B24)	CC	1xxx	36,5	10,4	1,02	10,2	0,0	T1
2017-07-24	African Sunset (B24)	CC	1xxx	45,7	10,9	0,90	12,1	0,0	T1

2017-08-10	African Sunset (B24)	CC	1xxx	38,5	11.7	0.86	13.5	0.0	T1
2017-05-10	Edit x Nova	CC	1	58.2	11.6	1.09	10.6	1.1	T4
2017-05-26	Edit x Nova	CC	1	57.4	12.1	1.04	11.6	1.2	T4
2017-07-24	Gold Nugget	CC	1x	48,6	12,9	1,18	10,9	0,0	T2
2017-08-10	Gold Nugget	CC	1	45,1	13,9	1,17	11,9	0,0	T1
2017-06-29	IRM 1	CC	1x	39.9	12.5	1.12	11.2	0.0	T2
2017-07-24	IRM 1	CC	1x	58.1	14.6	1.02	14.3	4.1	T1
2017-05-26	IRM 2	CC	1	49.1	10.5	1.01	10.4	1.0	T6
2017-06-15	IRM 2	CC	1	50.3	11.1	0.85	13.0	0.8	T4
2017-06-15	Meirav 119	CC	2	49.6	12.3	1.09	11.3	0.9	T1
2017-06-29	Meirav 119	CC	1x	48.6	12.2	0.97	12.5	0.0	T1
2017-07-24	Meirav 119	CC	1x	52.4	12.5	0.80	15.7	1.0	T1
2017-06-15	Meirav 63	CC	2	47.9	12.2	1.17	10.4	1.6	T1
2017-06-29	Meirav 63	CC	2	47.1	12.7	1.14	11.1	0.0	T1
2017-07-24	Meirav 63	CC	1	55.2	14.1	1.01	14.0	1.5	T1
2017-04-28	Michal 6/47	CC	4	60.1	9.7	0.84	11.6	1.9	T6
2017-05-10	Michal 6/47	CC	3	57.2	10.6	0.86	12.4	1.8	T5
2017-05-26	Michal 6/47	CC	2	51.5	10.6	0.77	13.7	2.1	T5
2017-04-28	Michal 89/64	CC	2	63.8	9.8	0.84	11.7	2.1	T6
2017-05-26	Michal 89/64	CC	1x	49.2	9.6	0.76	12.6	2.3	T4
2017-06-29	Mor 26	CC	1	49.6	12.5	1.66	7.5	1.8	T3
2017-07-24	Mor 26	CC	2	48,4	14.3	1.04	13.7	1.4	T2
2017-06-29	Furr	CC	1xx	46.4	13.1	1.20	10.9	14.5	T1
2017-07-24	Furr	CC	1xx	53.3	13.7	1.01	13.6	18.3	T1
2017-06-29	ARC Nadorcott	CC	1	50.0	11.1	1.13	9.8	0.2	T4
2017-07-24	ARC Nadorcott	CC	1	47.7	12.2	1.01	12.1	0.3	T1
2017-08-10	ARC Nadorcott	CC	2	43.5	12.8	0.96	13.3	0.0	T1
2017-06-29	Nadorcott LS	CC	1	46.4	11.6	1.31	8.8	0.0	T3
2017-07-24	Nadorcott LS	CC	1	50.1	12.4	1.17	10.6	0.3	T1
2017-08-10	Nadorcott LS	CC	2	42.1	13.1	1.17	11.2	0.5	T1
2017-05-10	Nova ARC	CC	1x	58.5	12.9	1.11	11.6	0.8	T 4
2017-05-26	Nova ARC	CC	1	51.8	13.4	1.16	11.5	0.8	T2
2017-06-29	Or	CC	1	49.5	12.4	1.22	10.2	0.9	T2
2017-07-24	Or	CC	2	54.3	14.4	1.09	13.2	0.9	T1
2017-05-26	Phoenix	CC	1	50.9	10.3	0.85	12.1	0.7	T7
2017-06-15	Phoenix	CC	1	51.7	11.7	0.93	12.6	1.4	T4
2017-06-29	Phoenix	CC	2	52.5	12.4	0.94	13.2	0.0	T3
2017-07-24	Shani SL	CC	1	49.9	13.4	1.47	9.1	2.7	T1
2017-08-10	Shani SL	CC	1	42.7	13.8	1.36	10.1	0.5	T1
2017-06-29	Shasta Gold	CC	1xx	51,9	11,4	1,69	6,7	0,0	T4
2017-08-10	Shasta Gold	CC	1xx	48,1	12,2	1,43	8,5	0,0	T1
2017-06-29	Tahoe Gold	CC	1xxx	49,5	10	1,20	8,3	0,0	T5
2017-07-24	Tahoe Gold	CC	1xxx	50,7	10,8	1,09	9,9	0,0	T1
2017-04-28	Tami 2/65	CC	1	60.1	9.5	0.80	11.9	0.0	T6
2017-05-10	Tami 2/65	CC	1x	61.4	9.9	0.78	12.7	0.0	T6
2017-06-29	Tango	CC	1	51,5	10,7	0,94	11,4	0,0	T4

2017-07-24	Tango	CC	1	45,3	11,5	0,93	12,4	0,0	T1
2017-08-10	Tango	CC	2	41,6	12,2	0,95	12,9	0,0	T1
2017-07-24	Tanorlate	CC	1xxx	42,4	10,3	1,40	7,4	0,6	T1
2017-08-11	Tanorlate	CC	1xxx	36,0	11,5	1,31	8,8	0,2	T1
2017-06-29	Valley Gold (B17)	CC	1x	50,2	13,2	1,35	9,8	1,6	T1
2017-07-24	Valley Gold (B17)	CC	1x	51,3	14,0	1,23	11,4	2,2	T1
2017-08-10	Valley Gold (B17)	CC	1	41,6	14,4	1,10	13,0	0,5	T1
2017-07-24	Yosemite Gold	CC	1xxx	50,2	12,2	1,17	10,4	0,0	T1
2017-08-10	Yosemite Gold	CC	1xxx	36,8	12,3	1,03	11,9	0,0	T1
2017-05-10	Etna	CC	1x	55,8	8,6	0,98	8,8	1,8	T 6
2017-06-16	Etna	CC	1xx	46,3	9,2	0,85	10,8	1,8	T1
2017-06-16	Samba	CC	1	52,8	12,2	1,12	10,9	1,4	T1
2017-07-05	Samba	CC	3	54,8	12,8	1,02	12,5	1,0	T1
2017-07-17	Samba	CC	2	54,1	13,4	0,98	13,7	0,0	T1
2017-05-10	Sirio	CC	1	59,7	10,7	1,39	7,7	6,7	T4
2017-06-16	Sirio	CC	1x	50,5	11	1,23	8,9	0,0	T1
2017-06-16	Tasty 1	CC	1xx	44,4	11,8	1,24	9,5	4,1	T2
2017-07-05	Tasty 1	CC	1x	44,1	12,5	1,15	10,9	2,9	T2
2017-07-17	Tasty 1	CC	1xx	44,9	12,4	1,02	12,1	2,8	T1

Table 4.6.19.4. Internal fruit quality data for experimental mandarin hybrid selections from the Paarl region (Babylonstoren) of the Western Cape during the 2017 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-04-18	Etna	CC	1xx	51,6	7,8	0,90	8,7	8,5	T6
2017-05-08	Etna	CC	1xxx	58,8	7,9	0,72	10,9	1,4	T5
2017-05-22	Etna	CC	1xxx	46,4	8,3	0,69	12,1	1,3	T4
2017-06-28	Gold Nugget	CC	1	47,8	12,5	1,47	8,5	0,0	T2
2017-07-27	Gold Nugget	CC	1	48,6	13,0	1,10	11,8	0,0	T1
2017-04-18	Nova	C35	1xx	54,2	9,3	0,91	10,2	26,3	T6
2017-05-08	Nova	C35	1x	55,1	10,1	0,86	11,7	22,6	T4
2017-05-22	Nova	C35	1x	54,4	10,5	0,81	12,9	22,0	T1
2017-08-28	Shasta Gold	CC	1xx	46,8	13,1	1,55	8,5	0,0	T2
2017-04-18	Sirio	CC	1	59,5	12,5	1,64	7,6	10,0	T1
2017-05-08	Sirio	CC	2	55,1	12,1	1,26	9,6	6,1	T1
2017-05-22	Sirio	CC	1	50,0	12,2	1,15	10,6	10,8	T1
2017-07-27	Tahoe Gold	CC	1xx	55,3	12,3	1,32	9,3	0,0	T1
2017-08-07	Tahoe Gold	CC	2	48,7	12,5	1,22	10,2	0,0	T1
2017-07-27	Tango	CC	2	47,8	12,6	1,33	9,5	0,0	T1
2017-08-07	Tango	CC	5	43,7	12,6	1,06	11,9	0,0	T1
2017-05-22	Tasty 1	CC	1x	46,6	9,8	1,08	9,1	6,6	T4
2017-06-26	Tasty 1	CC	1xx	45,5	11,2	0,93	12,0	5,5	T1
2017-07-27	Tasty 1	CC	1xx	46,3	12,4	0,96	12,9	1,4	T1
2017-07-27	Yosemite Gold	CC	1xxx	41,4	13	1,67	7,8	0,0	T1
2017-08-28	Yosemite Gold	CC	1xxx	50,3	13,0	1,39	9,4	0,0	T2

4.6.20 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (South West Cape)

Project 997E by W. Swiegers (CRI)

Opsomming

Dit is 'n nuwe proef perseël in die Suid Wes Kaap. 2017 Seisoen was die eerste vrugte op die proef 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 'n perseël bykom wat ook van die nuutste seleksies sal bevat. Die orde van rypwording was as volg gewees: Michal 6/47 het die seisoen begin, gevolg deur Michal 89/64, Tami 2/65, Meirav 119, Meirav 63, Edit x Nova, Taylor Lee LS, Phoenix, African Sunset, IRM 2, IRM 1, Valley Gold, Shani SL en Mor 26.

Summary

This is a new trial site in South West Cape. The trial trees had their first crop during the 2017 season. It's a variety block with a selection of all the new experimental cultivars from early maturing to late maturing selections. South West Cape is well suited for soft citrus. There is cross pollination in this block due to all the different selections that are present. A new site is going to be added to this site to cover more of the new selections. Michal 6/47 was first to reach peak maturity, followed by Michal 89/64, Tami 2/65, Meirav 119, Meirav 63, Edit x Nova, Taylor Lee LS, Phoenix, African Sunset, IRM 2, IRM 1, Valley Gold, Shani SL and Mor 26.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from Buffeljagsrivier region of the South West Cape. The following selections were evaluated: Michal 6/47, Michal 89/64, Tami 2/65, Meirav 119, Meirav 63, Edit x Nova, Taylor Lee LS, Phoenix, African Sunset, IRM 2, IRM 1, Valley Gold, Shani SL and Mor 26.

A ratio of 11:1 for mandarin hybrids is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.20.1 List of experimental mandarin hybrid selections evaluated in the Buffeljagsrivier region of the South West Cape during the 2017 season.

Selection	Rootstock	Topwork
African Sunset	CC	2014
Edit x Nova	CC	2014
IRM 1	CC	2014
IRM 2	CC	2014
Meirav 119	CC	2014
Meirav 63	CC	2014

Michal 6/47	CC	2014
Michal 89/64	CC	2014
Mor 26	CC	2014
Phoenix	CC	2014
Shani SL	CC	2014
Tami 2/65	CC	2014
Taylor Lee LS	CC	2014
Valley Gold		2014

Results and discussion

Tami 2/65

Tami 2/65 is an early maturing experimental mandarin hybrid. Fruit size for Tami 2/65 was large with fruit size count 1x. Internal juice percentage at peak maturity is high above (60%). Rind is smooth and the colour is a deep orange. The fruit peels easy. The selection was low seeded and peaked 0.8 seeds per fruit. Rind colour development was delayed with T5 on the colour plate at peak maturity. The selection dont have a high acid to start with and it drops quickly.

African Sunset (B24)

African Sunset cropped an extra large fruit size on the trees (count 1xxx) with a low juice percentage of 41.3% at peak maturity. External colour development was very good (T1) before peak maturity. African Sunset was seedless. At peak maturity the young trees had Brix° of 11.1° and acid percentage of 0.88%.

Edit x Nova

Edit x Nova is an early to mid maturing experimental mandarin hybrid. Edit x Nova had excellent internal quality it was also the best of all the selections. At peak maturity: Juice percentage was 63%; Brix° was 14.4° and acid percentage was 1.23%. This give Edit x Nova its good flavour. The seed count for Edit x Nova was also very low and ranged 0 – 0.3 seeds per fruit. Edit x Nova was fully coloured up at peak maturity. Fruit size for Edit x Nova is also favourable with 2 – 1x count.

IRM 1

The IRM 1 is a late maturing experimental mandarin hybrid. The fruit size count for IRM 1 was very good (count 1). Internally the juice content decrease towards peak maturity to below 50%. Brix: Acid ratio was very good at ratio 11.5; Brix was high 14.6° and acid was good 1.26%. Seed count peaked at 2 seeds per fruit. IRM 1 was a T1 on the colour plate at peak maturity. The rind was smooth and peeability easy.

IRM 2

IRM 2 is a mid to late maturing experimental mandarin hybrid. The fruit on the trees was small – medium count 5 – 2. IRM 2 have firm fruit and the juice percentage was 48.8% at peak maturity. IRM 2 had a great taste and flavour at peak maturity with very high (Brix 18° and acid 1.49%). Seed for IRM 2 was 1.3 – 1.8 seeds per fruit. Long before peak maturity was reached IRM 2 already had a T1 colour on the colour plate.

Mor 26

Mor 26 fruit had a medium size count, with (count 2). Towards peak maturity Mor 26 had a low juice percentage. Mor 26 had high sugars and good acids. Towards peak maturity with ratio 10.5 the Brix was already at 16° and acid 1.52%. Average seed count for this selection is 2.5 seeds per fruit. Rind colour development was great to reach T1 colour on the colour plate towards peak maturity.

Valley Gold (B17)

Valley Gold is a late maturing mandarin hybrid with good colour development T1 colour on the colour plate before peak maturity. Valley Gold bore a very good fruit size count 2 – 1. The juice decreased below 50% towards peak maturity. Internal quality is very good with Brix above 14.2° and acid percentage of 1.28% close to peak maturity. The selection did have seeds that peaked at 1.3 seeds per fruit. There was splitting of fruit on the trees

Meirav 63

Meirav 63 fruit size peaked at count 1x at peak maturity, with a very good juice percentage of 59.4%. Internally the quality was good at peak maturity Brix 12.9° and 1.03% acid. The seed count peaked at 1 seed per fruit. Meirav 63 had good colour development with T1 colour on the colour plate.

Meirav 119

Meirav 119 fruit size count ranged between counts (2 – 1xx). The juice content increased towards peak maturity with a good juice percentage of above 55% at peak maturity. With the good juice percentage the Brix and acid was also good at peak maturity (Brix and acid % above 12° and 1.0%). The average seed count is about 0.2 seeds per fruit. Towards peak maturity (ratio 10.2) colour was T 4 on the colour plate and when the fruit was overmature it was T1 on the colour plate.

Michal 6/47

Michal 6/47 reached peak maturity early end April, with an external colour T3 on the colour plate. On the trees there were medium size fruit, with count 3. Fruits internal quality for the young trees was good at peak maturity with juice percentage 57.8% and Brix 11° with 0.91% acid. The seed count ranged between 1.8 – 2.4 seeds per fruit.

Michal 89/64

Michal 89/64 fruit size was count 2. Toward peak maturity the juice percentage was above 55% which is good and when the fruit was overmature the juice percentage was just below 55%; 54.8% to be precise. Brix and acids were good but not very high at peak maturity. Fruit size for this selection peaked at 3.9 seeds per fruit. Rind colour development was delayed and degreening have to be done with a T4 colour on the colour plate.

Phoenix

Phoenix cropped a medium fruit size on the trees with a count 2 – 3, and the juice percentage for Phoenix was below 55% at peak maturity. External colour development was T2 on the colour plate towards peak maturity and T1 on the colour plate when the fruit was over mature. The seed count was very low and peaked at 0.2 seeds per fruit. Sugars were above 14° at peak maturity and acid % above 1%.

Taylor Lee LS

Taylor Lee LS is a mid to late maturing experimental mandarin hybrid. The trees bore large fruit on the trees with count 1, and towards peak maturity the juice content was 51.4%. Brix: Acid ratio at 11.4 the selection had high Brix 15.9° and acid of 1.40%. The selection seed count were 10.3 seeds per fruit. Taylor Lee LS reached T1 colour on the colour plate before peak maturity.

Shani SL

Average seed count for Shani SL ranged between 0.5 – 0.7 seeds per fruit. Shani SL had a high Brix° and acid ratio towards peak maturity (ratio 10.7), the Brix was 15° and acid was 1.40%. The juice percentage for Shani SL was low towards peak maturity 44.2%, and the fruit was fully coloured up before peak maturity.

Conclusion

African Sunset had the largest fruit size (1xxx) and Michal 6/47 had the smallest fruit size (3). Most of the other selections fruit size ranged between counts 2 – 1. Taylor Lee LS had the most seeds per fruit on average (10.3). African Sunset was to only selection that was seedless. Tami 2/65 and Edit x Nova were the only selections with juice percentage over 60% at peak maturity. Meirav 119, Meirav 63, Michal 6/47 and Michal 89/64 were the only selections with juice percentage over 55% at peak maturity. All of the selections were a T 1 on the colour plate before or at peak maturity except Tami 2/65 with a T5, Michal 89/64 with T4 and Michal 6/47 with T3 on the colour plate at peak maturity. The selection with the best internal quality at peak maturity (high juice %, high Brix and good acid above 1%) were Edit x Nova. Selections that also had Brix above 14° towards peak maturity and at peak maturity were IRM 1 & 2, Mor 26, Phoenix, Shani SL, Taylor Lee LS and Valley Gold.

Table 4.6.20.2. Internal fruit quality data for experimental mandarin hybrid selections from the Buffeljagsrivier region of the Western Cape during the 2017 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-06-27	African Sunset (B24)	CC	1xxx	43.5	11.0	1.08	10.2	0.0	T1
2017-07-18	African Sunset (B24)	CC	1xxx	41.3	11.1	0.88	12.6	0.0	T1
2017-08-14	African Sunset (B24)	CC	2	56.5	11.6	0.79	14.7	0.0	T1
2017-05-09	Edit x Nova	CC	2	59.8	13.1	1.29	10.2	0.0	T3
2017-05-29	Edit x Nova	CC	1x	63.0	14.4	1.23	11.7	0.1	T1
2017-06-27	Edit x Nova	CC	2	62.1	15.1	1.15	13.1	0.3	T1
2017-06-27	IRM 1	CC	1	50.0	14.5	1.56	9.3	0.8	T2
2017-07-18	IRM 1	CC	1	47.1	14.6	1.26	11.5	2.0	T1
2017-07-18	IRM 2	CC	2	39.0	16.2	1.57	10.3	1.7	T1
2017-08-14	IRM 2	CC	5	48.8	18.0	1.49	12.1	1.8	T1
2017-05-09	Meirav 119	CC	2	57.9	11.5	1.13	10.2	0.2	T4
2017-05-29	Meirav 119	CC	1xx	59.4	13.2	0.97	13.6	0.2	T1
2017-05-09	Meirav 63	CC	1	53.4	11.1	1.20	9.2	1.0	T5
2017-05-29	Meirav 63	CC	1x	59.4	12.9	1.03	12.5	0.8	T1
2017-04-10	Michal 6/47	CC	4	58.0	10.4	1.06	9.8	2.4	T6
2017-04-26	Michal 6/47	CC	3	57.8	11.0	0.91	12.1	3.4	T3
2017-05-09	Michal 6/47	CC	3	51.7	11.5	0.79	14.5	1.8	T4
2017-04-26	Michal 89/64	CC	2	57.5	10.0	0.91	11.0	3.9	T4
2017-05-09	Michal 89/64	CC	2	54.8	10.8	0.82	13.2	2.6	T4
2017-06-27	Mor 26	CC	2	58.9	14.9	1.77	8.4	2.2	T2
2017-07-18	Mor 26	CC	2	37.7	16.0	1.52	10.5	2.5	T1
2017-06-27	Phoenix	CC	2	52.8	14.4	1.41	10.2	0.2	T2
2017-07-18	Phoenix	CC	3	48.7	15.7	0.99	15.8	0.0	T1
2017-06-27	Shani SL	CC	2	43.9	13.8	1.50	9.2	0.5	T1
2017-07-18	Shani SL	CC	2	44.2	15.0	1.40	10.7	0.7	T1
2017-04-26	Tami 2/65	CC	1x	63.6	9.2	0.95	9.7	0.8	T5
2017-05-09	Tami 2/65	CC	1x	60.0	10.3	0.76	13.6	0.0	T5
2017-06-27	Taylor Lee LS	CC	1	51.4	15.9	1.40	11.4	10.3	T1
2017-06-27	Valley Gold (B17)	CC	2	52.5	13.2	1.44	9.1	0.9	T1
2017-07-18	Valley Gold (B17)	CC	1	42.3	14.2	1.28	11.1	1.3	T1

4.6.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)

Project 998B by W. Swiegers and Z. Zondi (CRI)

Summary

The early to late maturing navel selection trial is based in the Addo area of the Sundays River Valley. Most of the new selections were planted 2011 season. The older selections were topworked in 2007. The rootstocks Swingle citrumelo, Carrizo citrange and Troyer citrange were used for the trial. For the navel selections in this trial site the season started with Newhall, Fukumoto, Lina, Addo Early, Tule Gold, Dream, Glen Ora Late, Barnfield, Lane Late and Autumn Gold.

Opsomming

Die vroeë tot laat rypwording nawel seleksie proef is gevestig in die Addo area van die Sondagsrivier vallei. Die meeste van nuwe seleksies is in 2011 geplant. Die ouer seleksies is gedurende die 2007 seisoen getopwerk. Swingle citrumelo, Troyer citrange en Carrizo citrange word as onderstamme vir hierdie proef gebruik. Die volgorde van rypwording vir die nawel seleksie wat ge-evalueer was, is as volg: Newhall, Fukumoto, Lina, Addo Early, Tule Gold, Dream, Glen Ora Late, Barnfield, Lane Late en Autumn Gold.

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: Newhall, Fukumoto, Lina, Addo Early, Tule Gold, Dream, Glen Ora Late, Barnfield, Lane Late and Autumn Gold.

For navels, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 4.6.21.1. List of navel selections evaluated at Sundays River Valley (Penhill) during 2016.

Selection	Rootstock	Planted
Addo early	SC	2007
Dream	CC	2012
Lane Late	CC	2011
Newhall	TC	2007
Autumn Gold	CC	2012
Fukumoto	TC	2007
Barnfield Summer	CC	2012
Glen Ora Late	CC	2012
Lina	TC	2007
Tule Gold	TC	2007

Results and discussion

Addo Early

The selection had the same fruit size compared to 2016 being at count 56. Internal quality was good with a juice percentage between 51.4% and 54.2% and Brix above 10° and acid at 1%. Addo early's external colour development on the colour plate range was T2 at peak maturity. The selection developed a small protruding navel-end on the fruit. The fruit hang well on the trees due to the good acid.

Fukumoto

Fukumoto was one of the selection with the highest Brix for this trial site (above 11°). Fukumoto also had very good acid levels at peak maturity. The good Brix: Acid ratio give Fukumoto its good flavour. The colour development was delayed, with colour plate T6 close to peak maturity. The selection acids were stable and

the fruit hanged well and did manage to reach T1 on the colour plate just as the fruit was overmature. Fukumoto produced a good fruit size which peaked at count 56 a favorable size for navel production and export. The navel-end on the fruit was fairly open and protruding, one of the characteristics of the selection. The juice percentage of Fukumoto was below 50% close to peak maturity and below 55% just as the fruit was over mature. Fukumoto was second selection to reach peak maturity.

Lina

Lina were third to reach peak maturity. The selection had a delayed colour development with a colour plate T6 towards peak maturity and T3 colour plate when it was over mature. The selection had a very good fruit size and peaked at count 56. The fruit shape was more elongated with a large navel-end (fairly open). Lina developed a lower juice content (50%) compared to the 2016 season. Lina had good flavour.

Newhall

Newhall were the first selection to reach peak maturity for the second year in a row. The fruit size peaked at count 64 one size smaller than the 2016 season. Newhall had a delayed colour development (colour plate T6) when the fruit was over mature. The selection's juice percentage were 46.7% less than 2016 that was above 50%.

Autumn Gold

Autumn Gold was the last selction to reach peak maturity. The external colour development of the selection was one of the better ones (colour plate T1) just as the fruit was over mature (ratio 11.3). The rind colour was deep orange. The selection bore favorable fruit size fruit and peaked at count 56 with a high juice percentage of 55.8%. Internal quality was very good for Autumn Gold. The navel ends were small.

Tulegold

Tulegold had a delayed colour development with colour plate T3 at peak maturity. Tulegold had a large fruit size which peaked at count 56. The juice percentage increase towards peak maturity to 57% at peak maturity, one of the highest percentages. The Brix for this selection was below 10° with acids around 0.9% at peak maturity for the second year in a row. The fruit is round with smooth rind. Tulegold have very good flavour.

Barnfield Summer

Barnfield Summer had a very good and preferred fruit size (count 56) for export. The juice percentages were just below 55% for this selection. Barnfield Summer have a delayed external colour development, being a T5 at ratio 11. The acid remained fairly good up until peak maturity. The navel end was small.

Dream

Dream cropped a large fruit size on the trees with a count 56. The fruit shape was round with smooth rinds and the navel end was small. Dream had a delayed external colour development (T4 at peak maturity). Dream had the best internal quality at peak maturity the juice content was 52.2%, Brix was the highest 12.6° and acid was very good 1.23%. This will give Dream a good shelf life.

Glen Ora Late

The fruit size count of Glen Ora Late was good with a 56 count. It is a great size for navel production and export. The juice percentage for this selection was the highest 58.3%. The external colour development of Glen Ora late was also delayed like most of the other navel selections with a T5 on the colour plate. When the selection was just over mature (ratio 11.4) the Brix levels were good above 11° with high acids (0.99%), indicating that the fruit can hang slightly longer on the trees. The rind is smooth to slightly coarse with small to closed navel end.

Lane Late

Lane Late was the second last selection to reach peak maturity. Lane Late produced slightly smaller fruit in 2017 season count 56, compared to fruit size count of 48 in 2016 at peak maturity. Lane Late had some of the lowest juice percentage (49.9%) of all the navel selections at peak maturity. The external colour development was also delayed on Lane Late, (T5) by peak maturity. Lane Late also kept its acids quite well (good shelf life). The flavour was good. Lane Late had small protruding navel-end on the fruit.

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 Newhall peaking at count 64. All of the selections had delayed external colour development except Autumn Gold and Fukumoto both T1 on the colour plate. Glen Ora Late, Tulegold and Autumn Gold had the highest juice percentage at peak maturity with 58.6%, 57% and 55.8% respectively. Fukumoto, Glen Ora Late, Autumn Gold and Dream had the highest Brix above 11.0° for this trial at peak maturity. All the navel selections were seedless.

Table 4.6.21.2. Internal fruit quality data for early to late Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2017 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2017-05-10	Addo Early	SC	56	51,4	10,2	1,00	10,2	T2	0,0
2017-05-17	Addo Early	SC	56	54,2	10,3	0,91	11,3	T2	0,0
2017-05-17	Dream	CC	56	52,2	12,6	1,23	10,2	T4	0,0
2017-05-31	Lane Late	CC	56	49,9	9,5	0,89	10,7	T5	0,0
2017-06-19	Autumn Gold	CC	56	55,8	10,8	0,96	11,3	T 1	0,0
2017-05-31	Barnfield	CC	56	54,2	9,4	0,85	11,0	T 5	0,0
2017-04-04	Fukumoto	TC	56	47,9	11,1	1,15	9,7	T6	0,0
2017-05-17	Fukumoto	TC	56	54,9	11,4	1,01	11,3	T1	0,0
2017-05-31	Glen Ora Late	CC	56	58,6	11,3	0,99	11,4	T 5	0,0
2017-04-04	Lina	TC	56	47,0	9,9	1,13	8,8	T6	0,0
2017-05-10	Lina	TC	56	54,2	11,5	0,81	14,2	T3	0,0
2017-04-04	Newhall	TC	64	46,7	10,4	0,93	11,2	T6	0,0
2017-04-04	Tulegold	TC	56	56,6	9,3	1,10	8,5	T6	0,0
2017-05-17	Tulegold	TC	56	57,0	9,6	0,90	10,7	T3	0,0

4.6.22 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)

Project 1001B by W. Swiegers and Z. Zondi (CRI)

Summary

The trial consists of a few experimental early, mid and late navel selections. Washington was used as a control at the trial site. Painter Early 2 and Early Lina started the season as the two early navel selections for evaluation. De Wet 1 is a mid-maturing navel producing a round fruit shape. The fruit developed a closed navel end. The mid navel selections that was evaluated with they order of ripening was as follows: Washington, De Wet 1 and Caloma. The late maturing selection evaluated consist of Gloudi and Witkrans.

Opsomming

Hierdie proef bestaan uit 'n paar eksperimentele vroeë-, middel- en laat nawel seleksies. Washington is as kontrole gebruik in die proefperseël. Early Lina en Painter Early 2 is die 2 vroeë seleksies wat geëvalueer was. De Wet 1 is 'n mid-rypwordende nawel met 'n ronde vrugvorm. Die vrugte het 'n toe nawel-ent. Die ander mid-rypwordende kultivars wat ge-evalueer was bestaan uit Caloma en Washington. Die laat nawel seleksie wat ge-evalueer was bestaan uit Gloudi en Witkrans. Die volgorde van rypwording was as volg: Painter Early 2 was eerste gevolg deur Early Lina, Washington, De Wet 1 en Caloma wat baie dieselfde was, gevolg deur Gloudi en Witkrans.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from regions of the Gamtoos River Valley. The following selections were evaluated: Early Lina, De Wet 1, Gloudi, Caloma, Painter Early 2, Witkrans and Washington as a control.

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 4.6.22.1. List of navel selections evaluated at Loerie site in the Gamtoos River Valley, Eastern Cape during the 2017 season.

Selection	Rootstock	Topworked
De Wet 1	Carrizo	2012
Gloudi	Carrizo	2012
Caloma	Carrizo	2012
Early Lina	Carrizo	2012
Painter Early 2	Carrizo	2012
Witkrans	Carrizo	2012
Washington	Carrizo	2012

Results and discussion

Gloudi

Gloudi is a late navel selection with very high juice content. At peak maturity the juice content was 55.8%. It was the second last selection to reach peak maturity. The fruit shape was round and the fruit was firm with a small navel end. Gloudi had a medium to large fruit size that ranged between counts 64 - 56. The selection had a delayed colour development being at colour plate T6 at peak maturity. At peak maturity the Brix was 9.9° with acids above 0.96%. Even when the fruit was over mature the acid was at 0.80 (good shelf life).

De Wet 1

De Wet 1 is a mid-maturing navel that has produced a good crop consistently every year. Manipulation is necessary to control fruit size because over cropping results in smaller fruit. The selection developed a fairly soft rind that is slightly pebbly, one of the characteristics of the De Wet selection. De Wet 1 had a closed navel end on the fruit without having to spray 2,4-D; and developed a small internal navel. Less sanitation is necessary due to less fruit drop. The selection had good fruit size and peaked at count 56; perfect for navel production and export. Fruit shape was round. The internal quality was good with high juice content between 53.6% - 57.3%. At peak maturity, the external colour peaked at colour plate T6. The Brix remained around 10° and acid percentage around 1 %.

Caloma

Caloma bore fruit with a large fruit size and peaked at count 56. The fruit shape for Caloma is round and the fruit is firm. Caloma's internal quality was excellent and one of the best in the trial site. The selection had a

high juice percentage of 61.7%, high Brix 10.9° and a good acid around 1% at peak maturity. The external colour development of Caloma was delayed with a T6 on the colour plate at ratio 11. Caloma's rind is smooth and the navel ends are small to close.

Early Lina

Early Lina was the second selection at peak maturity in this trial site. This selection had a good fruit size count that peaked at count 56 at peak maturity. Towards peak maturity they were a count 48. The juice percentage for Early Lina was high 55.6% (peak maturity). The external colour development range was delayed T6 (colour plate). Early Lina had a good Brix and acid at peak maturity.

Painter Early 2

Painter Early 2 was the earliest selection to mature for this navel trial. Medium to large size fruit were on the trees with count 64 - 56. Painter Early 2 were around T5 – T6 on the colour plate at peak maturity. The juice percentage of Painter Early 2 increase towards peak maturity to around 50%. Painter Early 2 performed well with very good Brix and acid level at peak maturity. Fruit shape is round with smooth rind and small navel ends. The flavour for this selection is very good.

Washington

Washington was used as a control. Washington is a mid maturing navel. The external colour development behind the internal quality of the fruit (T6) on the colour plate even when the fruit was over mature. Washington fruit size count peaked at count 56. The juice content of Washington tested around 52% juice. High Brix levels above 11° with acids of 0.97%, assured good tasting fruit with good flavour. Navel ends were medium for Washington.

Witkrans 3

Witkrans 3 was one of the late maturing navel selections evaluated, with external colour development between T5 and T6. It was also the last selection to reach peak maturity. Fruit size for Witkrans 3 was medium (count 64) to Large (count 56). Witkrans 3 was also one of the selections with the best internal quality. The selection produced the highest juice percentage of 62% juice this season. High Brix levels of nearly 10.1° with acids of 1.00% assure a good internal quality. The navel ends were small to closed.

Conclusion

The fruit size of all the navel selections peaked at count 56 except Gloudi and Witkrans 3 that had a fruit size count of 64 as well at peak maturity. The Navel selection with the highest juice percentage was Witkrans 3 (62.8%) and Caloma at (61.7%). All of the selections had a delayed external colour development T6 on the colour plate at peak maturity. Washington, Caloma and Painter Early 2 respectively developed the highest Brix values for this trial.

Table 4.6.22.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2017 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2017-05-18	De Wet 1	CC	56	54,3	9,2	1,44	6,4	T7	0,0
2017-06-01	De Wet 1	CC	56	53,6	10,1	0,93	10,9	T6	0,0
2017-06-12	De Wet 1	CC	56	57,3	9,4	0,87	10,8	T6	0,0
2017-05-11	Early Lina	CC	48	53,7	9,3	0,95	9,8	T6	0,0
2017-05-18	Early Lina	CC	56	55,6	9,6	0,91	10,5	T6	0,0
2017-06-01	Early Lina	CC	56	53,0	9,6	0,75	12,8	T6	0,0
2017-06-12	Gloudi	CC	64	55,8	9,9	0,96	10,3	T 6	0,0
2017-07-12	Gloudi	CC	56	56,9	10,0	0,80	12,5	T 5	0,0
2017-06-01	Caloma	CC	56	56,8	10,9	1,20	9,1	T7	0,0

2017-06-12	Caloma	CC	56	61,7	10,9	0,99	11,0	T6	0,0
2017-04-03	Painter Early 2	CC	64	45,9	9,2	1,31	7,0	T6	0,0
2017-05-11	Painter Early 2	CC	56	52,2	10,8	0,93	11,6	T5	0,0
2017-06-01	Washington	CC	56	52,2	11,2	0,97	11,5	T6	0,0
2017-06-12	Witkrans 3	CC	64	62,0	10,1	1,00	10,1	T 6	0,0
2017-06-27	Witkrans 3	CC	56	62,8	10,5	0,92	11,4	T 5	0,0

4.6.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Western Cape)

Project 998D by W. Swiegers (CRI)

Opsomming

Citrusdal is seker een van die beste streke in die land vir Navel. Die proef het die meeste van die nuwe seleksies. Daar gaan nog uitgebrei word op hulle. Hierdie spesifieke proef bestaan uit 'n paar eksperimentele vroeë-, middel- en laat navel seleksies in 2 proef persele. Fischer is as kontrole gebruik vir die vroeë seleksies, Washington word as kontrole gebruik vir die middel seleksies en Lane Late dien as kontrole vir die laat navel seleksies. Die meeste van die bome is al ouer en die bome het 'n groot boom volume. Die orde van rypwording was as volg, beginnende met die vroeë seleksies Lina, Gerhard Early, Fischer, Fukumoto, Painter Early gevolg deur die middel seleksies se volgorde, Cara Cara, Washington, Early Lina, De Wet 1, Kirkwood Red, Dream en die seisoen was afgesluit met die laat seleksies se orde van rypwording Gloudi, Robyn 2, Lane Late, Glen Ora Late en Lazyboy.

Summary

Citrusdal is probably one of the best regions to farm Navels in the country. The trial consists of most of the new selections and a few newer ones will be added. The trial consists of a few experimental early, mid and late navel selections in 2 trial sites. Fischer was used as control for the early selections, Washington was used as control for the mid maturing navel selections and Late late was the control for the late navel selections. Most of the trees are older and have big tree volumes. Lina, Gerhard Early, Fischer, Fukumoto and Painter 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, Early Lina, De Wet 1, Kirkwood Red and Dream. The late selections that were evaluated and were last to reach peak maturity were Gloudi, Robyn 2, Lane Late, Glen Ora Late and Lazyboy.

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: Cara Cara, Dream, Fischer, Fukumoto, Gerhard Early, Glen Ora Late, Gloudi, Lane Late, Kirkwood Red, Washington, DeWet 1, Early Lina, Lazyboy, Lina, Painter Early and Robyn 2.

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately

three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 4.6.23.1. List of navel selections evaluated at various sites in the Citrusdal, Western Cape during the 2017 season.

Selection	Rootstock	Planted
Cara Cara	Carrizo	2009
Dream	Carrizo	2009
Fischer	Carrizo	2009
Fukumoto	Carrizo	2009
Gerhard Early	Carrizo	2009
Glen Ora Late	Carrizo	2009
Gloudi	Carrizo	2009
Kirkwood Red	Carrizo	2009
Lane Late	Carrizo	2009
Washington	Carrizo	2009
De Wet 1	Carrizo	2012
Early Lina	Carrizo	2012
Lazy Boy	Carrizo	2012
Lina	Carrizo	2012
Painter Early	Carrizo	2012
Robyn 2	Carrizo	2011

Results and discussion

Fukumoto

Fukumoto was the selection with the highest Brix for this trial site (above 14.4°). Fukumoto also had very good acid levels (1.44%) at peak maturity. The good Brix: Acid ratio give Fukumoto its great flavour. It is also a great eating fruit. The colour development was good, with colour plate T2 at peak maturity. The acids remain stable even when the fruit was over mature (above 1% for 4 weeks) fruit will hang well and have a good shelf life. Fukumoto produced a medium size fruit which peaked at count 72. The navel-end on the fruit was medium open and protruding, one of the characteristics of the selection. The juice percentage of Fukumoto started well and decreased as maturity went on.

Lina

Lina was the first selection to reach peak maturity. The selection had a delayed colour development T5 colour plate when it was over mature. The selection had a very good fruit size that ranged between counts 56 - 48. The fruit shape was elongated with a large navel-end (fairly open). Lina developed a low juice content below (50%). Lina had good flavour.

Gerhard Early

Gerhard Early was the second selection to reach peak maturity at the trial site. Gerhard Early tree bore medium size fruit that peaked at count 72. Gerhard Early had a fair colour development (colour plate T3) when the fruit was at peak maturity. The selection's juice percentage increased towards peak maturity to a high of 60.1%. Sugars and acids were also good at peak maturity Brix 12.9° and acid 1.22%.

Lazyboy

Lazyboy was the last selection to reach peak maturity. The external colour development of the selection was slightly delayed (colour plate T3) at peak maturity. The selection fruit size is favourable and peaked at count 56 with a low juice percentage (below 45 %). Brix and Acid were good at peak maturity. The selection had medium to small open navel end.

Fischer

Fischer (control) had a delayed colour development with colour plate T6 at peak maturity. Fischer had a good fruit size which peaked at count 64. Fischer internal quality was very good at peak maturity, juice content was 60%, Brix 10.6° and acid around 1.05%. The flavour was very good. The navels end for Fischer was small to closed and the fruit had a smooth rind.

Robyn 2

Robyn 2 had a very good and preferred fruit size (count 56) for export. The juice percentages were not very good 41.2% (peak maturity). Robyn 2 have a delayed external colour development, being a T5 at peak maturity. The acid remained fairly good even when the selection reached ratio 11 the acid was still 0.94% (good shelf life).

Dream

Dream cropped a medium - large fruit size on the trees with a counts 64 - 56. The fruit shape was round with smooth rinds and small open navel ends. Dream had a delayed external colour development (T5 at peak maturity) but as the fruit were left to hang Dream reached (T1 on the colour plate) still at peak maturity. Internal quality for the selection at peak maturity; the juice percentage was around 50%, Brix between 11 - 12° and acid was very good above 1.00%. This will give Dream its good flavour.

Glen Ora Late

Glen Ora Late was the second last selection to reach peak maturity. The fruit size count of Glen Ora Late peaked at 48 count. The juice percentage for this selection was low 41% at peak maturity. The external colour development for Glen Ora Late was delayed (T4 on the plate) as peak maturity was reached. Glen Ora Late acids stayed stable and the fruit did manage to reach (T1 on the colour plate) just as the fruit reached (ratio 11.1). Along with the good and stable acids the Brix was also good above 10°. The rind is smooth to slightly coarse with small to close navel end.

Lane Late

Lane Late (control) was the third last selection to reach peak maturity. Lane Late trees produced large fruit, (fruit size counts 56 – 48) at peak maturity. Lane Late juice percentage decrease with maturity 53% to 44 % while still at peak maturity. The external colour development was also delayed on Lane Late, (T3 – T5) by peak maturity. Lane Late also kept its acids quite well (good shelf life). The flavour was good. Lane Late had small protruding navel-end on the fruit.

Gloudi

Gloudi is a late navel selection with a good juice content. The juice content for this selection decrease towards peak maturity and at over maturity the juice content was 50.6%. 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. The selection had a delayed colour development being at colour plate T4 at peak maturity, degreening would have to be done. Gloudi Brix was high 11.2° with acids around 1.00%. Even when the fruit was well over mature the acid was at 0.85 (good shelf life).

De Wet 1

De Wet 1 is a mid-maturing navel that has produced a good crop consistently every year. 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 medium fruit size and peaked at count 64. Fruit shape was round. The internal quality for De Wet 1 is good. At over maturity, the external colour peaked at colour plate T5.

Early Lina

Early Lina reached peak maturity slightly later and even behind Lina. This selection had a good fruit size count that peaked at count 64 at peak maturity. The juice percentage for Early Lina was low 42.5% (peak maturity). The external colour development range was delayed T4 (colour plate). Early Lina had a very good Brix and acid at peak maturity, 11.4° and 1.17% respectively. Early Lina fruit was elongated with medium open protruding navel end. The flavour was good.

Painter Early 2

Painter Early 2 was the last of the early selections to mature for this navel trial site. Fruit size ranged quite a bit from small to large fruit with counts 88 - 56. Painter Early 2 was T6 on the colour plate at peak maturity. The juice percentage of Painter Early 2 increase towards peak maturity to around 50%. Painter Early 2 compared to the other selections had the lowest Brix and acid level at peak maturity. Fruit shape is round with smooth rind and small navel ends.

Washington

Washington was used as control. Washington is a mid maturing navel, but matured early in the 2017 season. The external colour development was behind the internal quality of the fruit (T5) on the colour plate even when the fruit was over mature. Washington fruit size count peaked at count 64. The juice content of Washington tested around 44.9% juice. High Brix levels above 11° with acids of 0.93%, assured good tasting fruit with good flavour. Navel ends were medium for Washington.

Cara Cara

Cara Cara was also one of the selections to reach peak maturity earlier in 2017 season. Cara Cara is a mid maturing pigmented navel. The selection had the most delayed colour development compared to the other selections in the trial site T7 on the colour plate at over maturity. The fruit size was very good perfect for export with count 56. Fruit shape was round with smooth rind. The Navel ends were small. The flavour was good due to the good internal quality. Internal colour was an intermediate red in the beginning of the season and as the season went on the red flesh became a bit deeper in red.

Kirkwood Red

Kirkwood Red is a mid maturing pigmented navel. Peak maturity was reached later than Cara Cara navel. Juice content for Kirkwood Red was around 50% at peak maturity and had a higher juice content compared to Cara Cara. The colour development for Kirkwood red was also delayed T6 on the colour plate. As the fruit was overmature Kirkwood Red still had an acid of 1% and did manage to get a T3 on the colour plate, better than Cara Cara. Internal quality for Kirkwood Red was preferred with slightly higher acid and Brix. The flavour was very good. Fruit size for Kirkwood Red was medium (count 64) to Large (count 56). Flesh colour was deep red, even the fruit stem was red.

Conclusion

The following selections (Lane Late, Gloudi, Glen Ora Late, Dream, Cara Cara and Kirkwood Red) were the only selections that peaked at fruit size count 56. The Navel selection with the highest juice percentage were Fukumoto 63.8%, Gerhard Early 60.1% and Fischer 60%. The selections were seedless. The best colour development were Dream T1 - and Fukumoto T2 on the colour plate. The selections with the highest Brix were Fukumoto and Gerhard Early respectively.

Table 4.6.23.2. Internal fruit quality data for Experimental Navel selections from the Citrusdal region of the Western Cape during the 2017 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2017-05-10	Cara Cara	CC	56	40,1	10,6	0,87	12,2	T7	0,0
2017-05-10	Dream	CC	56	44,1	10,2	1,12	9,1	T7	0,0
2017-05-26	Dream	CC	56	50,3	10,8	1,01	10,7	T5	0,0
2017-06-15	Dream	CC	64	48,6	11,9	1,14	10,4	T1	0,0
2017-04-28	Fischer	CC	64	60,0	10,6	1,05	10,1	T6	0,0
2017-05-10	Fischer	CC	64	45,3	11,5	1,02	11,3	T5	0,0
2017-04-28	Fukumoto	CC	72	63,8	14,4	1,44	10,0	T2	0,0
2017-05-10	Fukumoto	CC	72	33,4	14,3	1,38	10,4	T2	0,0

2017-05-26	Fukumoto	CC	72	45,7	15	1,21	12,4	T2	0,0
2017-04-11	Gerhard Early	CC	72	36.5	12.5	1.41	8.9	T5	0,0
2017-04-28	Gerhard Early	CC	72	60.1	12.9	1.22	10.6	T3	0,0
2017-05-10	Gerhard Early	CC	64	40.0	12.8	0.84	15.3	T4	0,0
2017-06-15	Glen Ora Late	CC	56	41.3	10.4	1.03	10.1	T4	0,0
2017-06-29	Glen Ora Late	CC	48	41.4	10.7	1.03	10.4	T4	0,0
2017-07-24	Glen Ora Late	CC	48	36.5	11.0	0.99	11.1	T1	0,0
2017-06-15	Gloudi	CC	56	50.6	11.2	1.00	11.2	T4	0,0
2017-06-29	Gloudi	CC	56	42,1	11.1	0.85	13.0	T4	0,0
2017-05-26	Kirkwood Red	CC	56	51.6	11.1	0.98	11.4	T6	0,0
2017-06-15	Kirkwood Red	CC	64	45.0	11.6	1.00	11.7	T3	0,0
2017-06-15	Lane Late	CC	48	53,0	9,7	0,93	10,5	T6	0,0
2017-06-29	Lane Late	CC	56	44,0	10,3	0,99	10,4	T3	0,0
2017-07-24	Lane Late	CC	48	38,7	10,4	0,89	11,6	T1	0,0
2017-05-10	Washington	CC	64	44,9	11,1	0,93	11,9	T5	0,0
2017-05-26	De Wet 1	CC	64	50,0	11,3	0,93	12,2	T5	0,0
2017-06-16	De Wet 1	CC	64	46,8	11,0	0,90	12,2	T1	0,0
2017-05-10	Early Lina	CC	64	42,5	11,4	1,17	9,7	T4	0,0
2017-05-26	Early Lina	CC	64	50,9	11,1	0,98	11,3	T4	0,0
2017-07-05	Lazyboy	CC	56	42,5	10,3	0,96	10,7	T3	0,0
2017-07-17	Lazyboy	CC	64	43,5	12,7	0,87	14,5	T1	0,0
2017-04-27	Lina	CC	56	46,0	8,3	0,73	11,3	T5	0,0
2017-05-10	Lina	CC	48	39,6	8,9	0,78	11,3	T5	0,0
2017-04-27	Painter Early	CC	56	51,8	8,2	0,84	9,8	T6	0,0
2017-05-10	Painter Early	CC	88	52,6	8,5	0,87	9,8	T6	0,0
2017-06-15	Robyn 2	CC	56	41,2	10,3	0,98	10,5	T5	0,0
2017-07-05	Robyn 2	CC	56	46,3	10,4	0,94	11,0	T1	0,0

4.6.24 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Sundays River Valley)
Project 1097A by W. Swiegers and Z. Zondi (CRI)

Summary

The Valencias discussed in this trial were top worked in the 2011 season. The trees produced their first crop in the 2015 season. There is a possibility to start a new trial site. The early maturing selection for the trial site is Bennie 2 with Midnight as control. The mid-maturing Valencia selections are Alpha, Gusocora, Henrietta, and Louisa. The late maturing Valencia selections will be McClean SL, Lavalley, Lavalley 2 and Ruby Red. At this trial site the season started with McClean SL, followed by Henrietta, Gusocora, Bennie 2, Ruby Val, Midnight, Louisa, Alpha, Lavalley and the season ended off with Lavalley 2. The differences in the maturing times of these Valencia selections could be due to the youth of the trees.

Opsomming

Die Valencias wat bespreek word in hierdie proef was in die 2011 seisoen getopwerk. Die bome het hulle eerste drag in die 2015 seisoen gehad. Daar is 'n moontlikheid om 'n perseel te begin. Die vroeë seleksie vir die proef perseel bestaan uit Bennie 2 en Midnight wat as kontrole dien. Die mid seleksies is Alpha, Gusocora, Henrietta, en Louisa. Die laat rypwordende Valencia seleksies was as volg; McClean SL, Lavalley, Lavalley 2 en Ruby Red. Die proef perseel se seisoen het begin met McClean SL, gevolg deur Henrietta, Gusocora, Bennie

2, Ruby Val, Midnight, Louisa, Alpha, Lavallo en die seisoen het geëindig Lavallo 2. Verskille in rypwording kan toegeskryf word aan die bome se jong ouderdom.

Objective

To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).

To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 2, Gusocora (G5), Henrietta, Lavallo, Lavallo 2, Louisa, McClean SL, Midnight (control), and Ruby Red.

Table 4.6.24.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 4.6.24.2. List of Valencia selections evaluated at Panzi (Kirkwood) during 2017 season.

Selection	Rootstock	Topwork
Alpha	CC	2011
Bennie 2	CC	2011
Gusocora G5	CC	2011
Henrietta	CC	2011
Lavallo	CC	2011
Lavallo 2	CC	2011
Louisa	CC	2011
McClean SL	CC	2011
Midnight	CC	2011
Ruby Red	CC	2011

Results and discussion

Alpha

Alpha bore medium size fruit this season on the trees, counts 72 - 64. The tree condition on Carrizo rootstock were good. Alpha Valencia were completely seedless and the fruit shape remained fairly round with slightly pebbly rind. The external colour development peaked at T1 – T3 with good internal quality by the time of maturity. Juice content decrease with maturity 59.1% close to peak maturity and 54.5% at peak maturity. Maturity was earlier 2017 season compared to 2016 season. Time of maturity seems to be beginning of July to end July.

Bennie 2

The fruit size peaked from count 56 this season, a good Valencia export fruit size. Bennie 2 have good acids for the fruit to hang on the trees longer to harvest at a later time, resulting in fewer rind problems (pitting). The selection was seedless. 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 excellent juice content around 62.7% at the beginning of peak maturity and it increase with maturity. Bennie 2 good internal quality give the selection its good flavour.

Gusocora

There was a slight delay in external colour development on the fruit (T3) just as the selection reached over maturity (ratio 11), with a Brix of 10.2 and acid of 0.93%. Gusocora was completely seedless and will be regarded as a seedless selection. The juice content of Gusocora was higher this season 58.9% and the fruit size ranged between counts 88 - 72. The fruit was firm with a round shape and a smooth rind.

Henrietta

The fruit shape of Henrietta remained round and the rind texture fairly smooth. Fruit peeled easily and contained a medium amount of rind oil. The seed count peaked at 2.3 seeds per fruit, with 1 evaluation being seedless. Fruit size was medium and one size smaller in the 2017 season with count 72. Henrietta colour development was delayed compared to the internal quality with T6 on the colour plate at over maturity. Henrietta produced a good juice content around 60% this season. Fruit was mostly round with fine coarse rind.

Lavalle 2

Lavalle 2 is known for its large fruit size, in 2016 fruit size peaked count 56 and in 2017 season fruit size ranged between counts 72 – 56. The higher acid level (1.10%) indicated that this selection was late maturing and ended of the Valencia season at this trial site for the second year in a row. Lavalle was completely seedless and the juice content of this selection increase towards peak maturity to a high of (63.8%) with a Brix: acid ratio of 9.5. Brix was also good. There was no problem with the external colour development when Lavalle 2 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.

Louisa

Two evaluations were completed for Louisa; the first evaluation had a seed count of 0.9 seeds per fruit and the second evaluation seed count was seedless. The fruit size ranged from count 72 to 64. The fruit shape was round with a fairly smooth rind texture and the fruit peeled easily. The juice content of Louisa stayed constant towards peak maturity around 56%. Louisa rind colour was slightly delayed at peak maturity with T3 on the colour plate.

McClellan SL

McClellan SL was about 6 weeks earlier to reach peak maturity first in this trial site of all the selections. Fruit shape for McClellan SL is fairly round fruit with a soft fibre strength that peels easy, containing low rind oil levels. All the fruit evaluated remained completely seedless. Many of the seedless selections have fruit set problems and bear poor crops, but this does not seem to be the case with this cultivar (good production). The trees bore small - medium fruit on the trees (count 88 to 72). The internal quality was good with high juice levels for this trial site (60%). There was no delay in external colour development being a T1 although the fruit have matured earlier.

Midnight

Midnight was used as control in this trial site. Midnight trees cropped medium fruit size with 72 count. The juice content of Midnight peaked at 51% to low for export but as the fruit hang the juice content increased enough before the acids were to low. The external colour development of Midnight was very good with a T1 on the colour plate range at peak maturity. Midnight internal quality was good and it gave Midnight its good flavour. The selection was seedless.

Lavalle

Lavalle was seedless during all three evaluations. Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The juice content decreased towards peak maturity, but still remained good for export standards at peak maturity (59 - 65%). The fruit size count for Lavalle ranged from counts 88 to 64. The external colour of this selection was T1 at peak maturity with a good internal quality.

Lavalle were also seedless during the evaluations. Lavalle flavour was good. Fruit shape was round with coarse rind. The rind colour was deep orange.

Ruby Red

Ruby Red bore small fruit that peaked at count 88. The selection tends to over crop and will lead to the smaller fruit size. The juice content of Ruby Red increased towards peak maturity to 62.5% at peak maturity, but decrease after peak maturity. At peak maturity Ruby's external colour development was T3 on the colour plate range and the fruit was completely seedless during two evaluations and had a seed count of 3.2 seeds per fruit during one evaluation. At peak maturity the internal quality was very good. The colour of the flesh was red, and the selection have a unique taste.

Conclusions

Most of the Valencia selections had no problem with external colour development, all of them reached T1 on the colour plate, except Henrietta T6, Gusocora T3, Louisa T3 and Ruby Red T3. All of the selection's internal and external qualities complied with the minimum export requirement for Valencia types. The following selections had a seed count; Ruby Red with the highest count of 3.2 seeds per fruit and Henrietta with a seed count of 2.3 seeds per fruit. Louisa had very low seed count (virtually seedless) and all the other selections were completely seedless. Most of the selections had a fruit size count of 72 - 64. Ruby Red size count was 88 the smallest and the best size count of 56 were Benny 2 and Lavalle 2. The following selections developed a juice content above 60% at peak maturity; Bennie 2, Lavalle 2 and Ruby Red.

Table 4.6.24.3. Internal fruit quality data for Valencia selections at Panzi (Sundays River Valley) during the 2017 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2017-06-28	Alpha	CC	72	59,1	10,0	1,05	9,5	T 3	0,0
2017-07-26	Alpha	CC	72	54,5	10,5	0,97	10,8	T 1	0,0
2017-08-23	Alpha	CC	64	51,0	10,8	0,82	13,1	T 1	0,0
2017-06-28	Benny 2	CC	56	62,7	10,3	1,04	9,9	T 1	0,0
2017-07-26	Benny 2	CC	56	68,7	10,1	0,83	12,2	T 1	0,0
2017-06-28	Gusocora (G5)	CC	88	58,9	10,2	0,93	11,0	T 3	0,0
2017-07-25	Gusocora (G5)	CC	72	59,9	10,6	0,80	13,3	T 1	0,0
2018-06-28	Henrietta	CC	72	59,7	9,4	0,83	11,3	T 6	2,3
2017-07-26	Henrietta	CC	72	62,5	10,5	0,79	13,3	T 1	0,0
2017-07-26	Lavalle	CC	64	65,0	10,7	1,08	9,9	T 3	0,0
2017-08-07	Lavalle	CC	72	59,0	10,3	1,05	9,8	T 1	0,0
2017-08-23	Lavalle	CC	88	51,0	10,3	0,75	13,7	T 1	0,0
2017-06-28	Lavalle 2	CC	72	60,8	10,3	1,33	7,7	T 4	0,0
2017-07-26	Lavalle 2	CC	56	63,8	10,5	1,10	9,5	T 1	0,0
2017-08-23	Lavalle 2	CC	72	47,0	9,9	0,65	15,2	T 1	0,0
2017-06-28	Louisa	CC	72	56,9	9,6	1,75	5,5	T 5	0,9
2017-07-26	Louisa	CC	64	56,8	9,7	0,85	11,4	T 3	0,0
2017-06-28	McClellan SL	CC	88	60,9	10,1	0,81	12,5	T 1	0,0
2017-07-26	McClellan SL	CC	72	59,4	9,8	0,68	14,4	T 1	0,0
2017-07-26	Midnight	CC	72	51,0	10,2	0,92	11,0	T 1	0,0
2017-08-07	Midnight	CC	72	60,7	10,1	0,86	11,7	T 1	0,0
2017-08-23	Midnight	CC	72	64,9	10,1	0,84	12,0	T 1	0,0
2017-06-28	Ruby Valencia	CC	88	62,5	10,1	1,04	9,7	T 3	3,2

2017-07-26	Ruby Valencia	CC	88	57,9	9,7	0,84	11,5	T 1	0,0
2017-08-23	Ruby Valencia	CC	88	48,0	10,5	0,92	11,4	T 1	0,0

4.6.25 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Citrusdal)

Project 1097B by W. Swiegers (CRI)

Opsomming

Die klimaat en die grond maak die streek geskik vir die verbouing van Valencias wat hoë suikers tot suur verhouding het. Dit maak goeie eet vrugte met goeie geure. Valencia's het ook nie 'n Navel ent nie wat sanitasie makliker maak. Die bome is in 2009 geplant en bestaan uit vroeë-, mid- en laat rypwordende seleksies. Die volgorde van rypwording was baie deurmekaar in die 2017 seisoen, dit is a.g.v. die sure wat baie stadig geval het. Van die seleksies was 4 – 6 weke laat omdat hulle sure te hoog was om te kon uitvoer. Die orde van rypwording was as volg, Turkey was eerste en McClean SL het hom opgevolg, gevolg deur Gusocora, Midnight, Alpha, Valencia Late, Delta, Bennie en die seisoen is afgesluit met Delta.

Summary

The climate and the soil make this region a good region to farm Valencias. The Valencias tend to get high sugars to acid ratio in this region. It gives fruit with great flavours and it is also good eating fruit. Valencias also don't have a navel end, and this make sanitation easier. 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 2017 season. Some of the selections were 4 – 6 weeks late in their acids reaching export levels. The order of ripening was as follow starting with Turkey, McClean SL, Gusocora, Midnight, Alpha, Valencia Late, Delta, Bennie and the season finished off with Henrietta.

Objective

- To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Benny, Delta, Gusocora, Henrietta, McClean SL, Midnight, Turkey and Valencia Late

Table 4.6.25.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 4.6.25.2. List of Valencia selections evaluated at Kweekkraal (Citrusdal) during 2017 season.

Selection	Rootstock	Topwork
Alpha	CC	2009

Bennie	CC	2009
Henrietta	CC	2010
Delta	CC	2009
Gusocora	CC	2009
Turkey	CC	2009
Valencia Late	CC	2009
McClellan SL	CC	2009
Midnight	CC	2009

Results and discussion

Alpha

The internal quality was good, juice levels peaked at 57.1%, Brix was between 11.5 – 12.5 and acids were fairly high at 1.5%. Fruit size varied from count 88 to 72, slightly on the smaller size but still good for Valencia production and export. External colour peaked from T1 to T2. In the beginning of July export standards were met but as the fruit hang on the trees the internal quality got better to export fruit of higher standards. During the 3 evaluations there was only one occasion that got a seed count of 0.1 seed per fruit.

Bennie

The fruit size count peaked at count 72 to 64, medium fruit size and a good Valencia export size. Bennie had a soft fibre strength compared to the other Valencia selections. Bennie an early maturing Valencia selection. But due to the high acid the fruit were left to hang so that the acids can come down and Brix up and also get better colour development. This made that the selection matured much later. It would have been best to harvest the selection in August for export. The selection had a much better internal quality. Seed count ranged between 0.5 – 1.8 seeds per fruit. There was no delay in external colour development (T1 to T3) before peak maturity. Bennie developed a juice content of 64.6% towards peak maturity.

Gusocora

In the beginning of July, the external colour development of Gusocora was T4 not good enough for export. The fruit were left on the trees to the beginning of August and the fruit coloured up completely to T1 on the colour plate. The Brix also went up and the acids also dropped to get a fruit that have very good export internal quality. The Brix was above 11 and acids were still good 1.38% with good juice content above 50%. Gusocora was completely seedless and will be regarded as a seedless selection for future plantings. Fruit size peaked at count 88 - 72.

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 4.7 seeds per fruit. Fruit size count range from (count 88 – 72). Henrietta had no problem with external colour development (T1) before peak maturity. Henrietta produced a good juice content around 55%. Harvest date for the best internal quality for export was at the end of August into September.

Valencia Late

The Valencia Late produced small size fruit at count 88. Acid levels were high close to peak maturity (1.54%) indicating the late maturity qualities of the selection. The juice content was low at 48.4% (just high enough for export) and Brix was above 12.5. The external colour development was good with T2 and seed counts was seedless. Maturity will be late in the season (September).

Delta

Delta, as control variety, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 125 and 88 with good internal quality (end of August), reaching juice levels of 50.8%, Brix of 12.3 and acid content of 1.56%. The external colour of the fruit when it met the export standards were between T2 and T1. Maturity is end of August into September. The fruit was round with a smooth rind and peeled fairly easy and also had a good flavour.

McClellan SL

McClellan SL tree bore small fruit with count 88. During one evaluation McClellanSL had a seed count of 2.3 seeds per fruit and with the last evaluation the selection was seedless. External colour varied from T1 – T3, Juice was above 51% and still increasing, Brix was good (above 10) and acids remain fairly high towards the end of the season, resulting a very good Brix: Acid ratio. The selection met the export standards in the beginning of July, but the fruit have good enough acids to hang. The fruit is firm with a round to elongated fruit shape with a smooth rind. Externally as well as internally the colour is deep orange.

Turkey

Fruit size was a very favourable for Valencia production and export, count ranged 72 – 56. During the season Turkey juice content was very low it stayed around 50%. Harvest time will be very important to meet the juice content standards for export. Brix was around 11 and acids around 1.3% this will meet the export standards as well as the external colour that range between T3 – T1 on the colour plate. There were seeds during the evaluation and it peaked at 1 seed per fruit. Fruit characteristics for Turkey was round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, easy fruit peeling. The internal colour was light yellow, and externally the fruit remained yellow. The selection has the qualities of a mid-season orange; for instance, the exceptionally soft fruit, and the soft rind that can result in rind problems if managed incorrectly.

Midnight

The fruit development peaked from count 88 to count 72. Midnight bore round fruit on the trees with a medium to coarse rind, fibre strength was fairly soft and the fruit peeled easy. The juice levels of Midnight was good and peaked at (55.5%); above the minimum export standard. Midnight were low seeded (0.1 seeds per fruit) making it virtually seedless. The colour development towards peak maturity was between T1 - T2 on the colour plate range. The trees had a good yield on them.

Conclusions

None of the selections had problems with their external colour development, that is due to the high acids so the fruit were able to hang longer on the trees to fully colour up to T1 on the colour plate. All the selections met the minimum export standards. The selections were harvested later than in a normal season due to the high acids that needed to drop. McClellan SL, Bennie and Henrietta were the selections on average with the highest number of seeds per fruit. Delta, Gusocora and Valencia Late were the only selections that were completely seedless. The Valencia selections with a juice content above 55% towards peak maturity or at peak maturity were Bennie, Henrietta, Alpha, McClellan SL and Midnight. The fruit size varied quite a lot between selections, but all of them were good enough for export. All the selections produced a Brix above 11 and acids content above 1%.

Table 4.6.25.3. Internal fruit quality data for Valencia selections at Kweekkraal (Citrusdal) during the 2017 season.

Date	Cultivar	Root stock	Count	Juice %	Brix °	Acid %	Ratio	Avg. Seed	Colour
2017-07-03	Alpha	CC	88	48.2	9.9	1.62	6.1	0.0	T1
2017-08-11	Alpha	CC	88	57.1	11.5	1.55	7.4	0.1	T1
2017-08-27	Alpha	CC	72	53,9	12,5	1,5	8,3	0,0	T2
2017-07-03	Bennie	CC	64	50,9	9,4	1,65	5,7	1.8	T3
2017-08-11	Bennie	CC	72	56,5	10,8	1,50	7,2	1.1	T1
2017-08-27	Bennie	CC	72	64,6	11,1	1,48	7,5	0,5	T2
2017-07-03	Delta	CC	88	47,9	9,9	1,67	5,9	0,0	T4
2017-08-11	Delta	CC	105	47,9	11,4	1,44	7,9	0,0	T1
2017-08-27	Delta	CC	125	50,8	12,3	1,56	7,9	0,0	T2

2017-07-03	Gusocora	CC	88	48.5	10.4	1.60	6.5	0.0	T4
2017-08-11	Gusocora	CC	88	52.2	11.2	1.38	8.1	0.0	T1
2017-08-27	Gusocora	CC	72	52,4	12,0	1,36	8,8	0,0	T2
2017-07-03	Henrietta	CC	72	50.4	9.6	1.72	5.6	0.6	T4
2017-08-11	Henrietta	CC	88	55.2	11.0	1.71	6.4	2.2	T1
2017-08-27	Henrietta	CC	88	55,4	11,8	1,66	7,1	4,7	T2
2017-07-03	McClellan SL	CC	88	51,5	10,4	1,47	7,1	2,3	T3
2017-08-11	McClellan SL	CC	88	56,4	11,7	1,30	9,0	0,0	T1
2017-07-03	Midnight	CC	72	52,7	10,6	1,58	6,7	0,1	T2
2017-08-11	Midnight	CC	88	55,5	11,9	1,44	8,2	0,1	T1
2017-08-27	Midnight	CC	88	54,4	12,7	1,47	8,6	0,0	T2
2017-06-15	Turkey	CC	64	52,7	11,4	1,57	7,2	1,0	T3
2017-07-03	Turkey	CC	56	49,7	10,7	1,32	8,1	0,0	T2
2017-07-24	Turkey	CC	64	49,4	11,5	1,38	8,4	0,8	T1
2017-08-11	Turkey	CC	72	52,3	12,4	1,28	9,7	0,1	T1
2017-08-27	Valencia Late	CC	88	48,4	12,5	1,54	8,1	0,0	T2

4.6.26 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)

Opsomming

Die seleksies wat geëvalueer was die seisoen was net oop seleksies. Hulle gaan dien as kontroles vir die nuwe seleksies. Vir die Sondags Rivier Vallei gaan daar 2 Clementine persele wees wat al die nuutste seleksies gaan bevat. Die seisoen het begin met Orogrande, gevolg deur Oronules, Nules en ge-eindig met Esbal. Esbal was later ryp as gewoonlik, dus was die rypwording van Nules en Esbal gelyk.

Summary

The selections that were evaluated were just some of the open selections. They will be used as controls for the new selections in the future. For the Sundays River Valley there will be two Clementine sites with all the newest selections. The season started with Orogrande, followed by Oronules, Nules and ended with Esbal. Esbal's peak maturity was delayed and reached peak maturity the same time as Nules.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Sundays River Valley region of the Eastern Cape; planted 2012. The following varieties were evaluated: Orogrande, Oronules, 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 4.6.26.1. List of Clementine selections evaluated at Invercloy (Kirkwood) during 2017.

Selection	Rootstock	Planted
Orogrande	Carrizo	2012
Oronules	Carrizo	2012
Esbal	Carrizo	2012
Nules	Carrizo	2012

Results and discussion

Orogrande

Orogrande was the first selection to reach peak maturity at the trial site. Fruit size for Orogrande ranged between fruit size count 1 – 1x. The juice percentages for Orogrande ranged between 53 – 57.3% (peak maturity). The differences in Brix: Acid ratio between selections were not significant, but Orogrande did have a good Brix and acid percentage around 0.90%. Seeds count was low 0 – 0.3 seeds per fruit. Rind colour development at peak maturity was delayed being T5 – T7 on the colour plate.

Oronules

Oronules was the second selection to reach peak maturity. Fruit size count for Oronules was 1. Towards peak maturity the juice percentage was around 55%. Brix was between (10 - 10.7°) and acid percentage was between (0.87 – 0.97%) towards peak maturity. Reaching peak maturity was also delayed as well as the external colour with T5 – T6 on the colour plate. The seed count for the selection was the highest 0 – 1.6 seeds per fruit.

Esbal

Esbal was the last selection to reach peak maturity along with Nules. Esbal normally mature earlier than Nules. Esbal's fruit was slightly smaller than Nules, with a fruit size count for Esbal 1 – 2 (count). Rind colour development for Esbal was the best, with T2 (colour plate) when the fruit was at peak maturity. Fruit was round to oblate with a smooth to pebbly rind. Seed count for Esbal was 0.2 – 1.2 seeds per fruit. Esbal had the highest percentage above 55%. Internal quality at peak maturity was Brix (10.5°) and acid (0.86%).

Nules

Nules were on time to reach peak maturity. The selection had a juice percentage that ranged between (51.4 – 55.8%) at peak maturity. Nules had the largest fruit size count 1x – 1xx. Internal quality for Nules at peak maturity were good, Brix (10.1°) and acid (0.83%). This contribute to Nules good flavour. Nules had a low seed count ranging from 0.0 – 0.3 seeds per fruit. Rind colour development was not good with a T5 –T6 on the colour plate at peak maturity. Peelability is easy and internal colour is orange.

Conclusion

Oronules had the highest seed count of all the selections that were evaluated, seed count of 1.6 seeds per fruit. Most of the selections had delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Esbal had the best rind colour development T2 on the colour plate. Oronules had the highest Brix (10.7°). Esbal had the smallest fruit size and peaked at count 1 - 2. All four selections had a very good juice percentage: all over 50%, with Esbal the highest (57.7%).

Table 4.6.26.2. Internal fruit quality data for Clementine selections in the Sundays River Valley region (Invercloy) of the Eastern Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
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2017-05-10	Esbal	CC	2	63,0	9,9	0,90	11,0	0,7	T6
2017-05-17	Esbal	CC	1	57,7	10,1	0,83	12,2	0,2	T5
2017-05-24	Esbal	CC	1	56,4	10,5	0,86	12,2	1,2	T2
2017-05-17	Nules	CC	1x	51,4	10,1	0,82	12,3	0,3	T6
2017-05-24	Nules	CC	1xx	55,8	10,1	0,83	12,2	0,0	T6
2017-05-10	Orogrande	CC	1	57,3	10,6	0,88	12,0	0,0	T7
2017-05-17	Orogrande	CC	1x	53,0	10,4	0,90	11,6	0,2	T5
2017-05-24	Orogrande	CC	1xx	50,5	11,3	0,83	13,6	0,3	T5
2017-05-10	Oronules	CC	1	57,7	9,9	0,87	11,4	0,0	T6
2017-05-17	Oronules	CC	1	53,8	10,0	0,89	11,2	1,2	T5
2017-05-24	Oronules	CC	1	54,5	10,7	0,97	11,0	1,6	T6

4.6.27 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)

Project 1000D by W. Swiegers (CRI)

Opsomming

Daar is 4 proef persele in 3 streke waar Clementines geëvalueer was die seisoen. Die 3 streke is Wellington, Paarl en die laaste streek en ook die grootste is Citrusdal. In Wellington het die seisoen begin met Clemenpons, gevolg deur Nules, Bonanules en ge-eindig met Marisol. In die Paarl was Clemenluz en Nules eerste ryp, en ook baie naby aan mekaar gevolg deur Esbal. 'n Vrug se Suiker: Suur verhouding van 12 word beskou as ryp. Op dieselfde datum was Clemenluz se Suiker: Suur verhouding 12.1 en Nules 11.9. Basol het die seisoen in Citrusdal laat begin, gevolg deur Basol op 'n tussenstam, Clemenluz, Clemenpons, Nules, Esbal, Orogrande en Saratoga het die seisoen afgesluit.

Summary

There are 4 trial sites in 3 regions where we evaluated Clementines this season. The 3 regions are Wellington, Paarl and the last one, also the biggest one, is Citrusdal. In Wellington the Season started with Clemenpons, followed by Nules, Bonanules and ended with Marisol. In the Paarl region the season started with Clemenluz and Nules, very close to each other followed by Esbal. Ratio of 12 is considered peak maturity for Clementines. On the same date the ratio for Clemenluz was 12.1 and Nules was 11.9. Basol started the season in Citrusdal followed by Basol on interstock, Clemenluz, Clemenpons, Nules, Esbal, Orogrande and Saratoga finished the season.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from various regions in the Western Cape. The following varieties were evaluated: Orogrande, Nules, Esbal, Clemenpons, Bonanules, Marisol, Clemenluz, Basol, Basol with interstock and Saratoga.

A ratio of 11:1 Clementines is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the

end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.27.1. List of Clementine selections evaluated at Bonathaba (Wellington) during 2017.

Selection	Rootstock	Planted
Bonanules	Troyer	2012
Clemenpons	Troyer	2012
Marisol	Troyer	2012
Nules	Troyer	2012

Table 4.6.27.2. List of Clementine selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2017.

Selection	Rootstock	Planted
Orogrande	Carrizo	2012
Clemenpons	Carrizo	2010
Saratoga	Carrizo	2012
Nules	Carrizo	2009
Basol	Carrizo	2010
Basol interstock	Carrizo	2011
Esbal	Carrizo	2009
Clemenluz	Carrizo	2009

Table 4.6.27.3. List of Clementine selections evaluated at Babylonstoren (Paarl) during 2017.

Selection	Rootstock	Planted
Clemenluz	Carrizo	2011
Esbal	Carrizo	2011
Nules	Carrizo	2011

Results and discussion

Bonanules

Bonanules together with Marisol showed the best external colour development of all the selections (colour plate T4) towards peak maturity in the Wellington trial site. The fruit has a thin rind and peels easily. Fruit size is medium with a 2 count. Bonanules have a very good juice percentage (58.1%) towards peak maturity. It was also the highest for the Wellington trial site. Internal quality was good for the selection towards peak maturity Brix (11.3°) and acid percentage (1.00%). The fruit was completely seedless, as expected when planted in a solid Clementine orchard.

Clemenpons

Clemenpons is early maturing Clementine selection. Citrusdal trial site was about 2 – 3 weeks behind Wellington to reach peak maturity. The juice for this selection was the lowest compared to the other selections at the Wellington trial site (47.2%). Juice was very good and much higher for the Citrusdal selection (60.1%). There was also a big difference in fruit size between the 2 sites. Fruit size count at Wellington (2) and at Citrusdal (1x). Sugars and acid percentage were much higher at Wellington trial site compared to Citrusdal. Wellington: Brix (12.4°) and acid (0.97%); Citrusdal: Brix (10.2°) and acid (0.88%). Colour development was delayed at both sites with a T5 on the colour plate. The Wellington selection was seedless because it is in a solid Clementine orchard. The seed count for Clemenpons at Citrusdal under heavy cross pollination the seed count was still low 0.3 seeds per fruit.

Marisol

Marisol and Nules are two older selections used as controls for Clementine trials. Marisol were seedless. This was the last selection to mature at the Wellington trial site. Fruit size was count 2. The juice percentage for the selection was very good above 55%. The colour development for this selections were the best for the Wellington trial site (colour plate T4). Marisol and Nules had the highest Brix (13.5°); (13.6°) respectively. Marisol still had an excellent acid (1.25%) towards peak maturity. Fruit shape is round and the rind is pebbly and oily when peeled.

Basol

Basol is the earliest maturing Clementine selection. Basol trees does get galls on the trunk. The Basol trees with the navel interstock does not have any galls on the trees. Fruit size on both Basol's was small to medium with count 2 - 3. There was a big difference in the juice percentages between the 2 Basol selections. Basol with interstock had a very good and high juice percentage (60.1%) while the selection without the interstock had a juice percentage of (53.3%). Basol on CC had a slightly higher Brix than the one on the interstock. Acid percentage for both selections was good above 1.00% at peak maturity. The selection on CC had a low seed count 0.2 seeds per fruit and Basol with interstock were seedless. External colour break was the same for both selections T6 on the colour plate, but the selection on the interstock was still on its way to peak maturity. The fruit peels easily. Basol have a very short harvest period before the fruit is over mature and start to granulate.

Esbal

Esbal was the last selection to reach peak maturity at Paarl and it also reached peak maturity later than in Citrusdal. Esbal normally mature earlier than Nules, but at both sites peak maturity was reached after Nules. Fruit were slightly smaller than Nules. Fruit size count for Esbal 3 - 4 (count) for Citrusdal and in Paarl it was count 4. Fruit is round to oblate and rind is smooth to pebbly. Peelability is easy but rind oil bother. Rind colour development was better than Nules. In Citrusdal the colour was (T5) on the colour plate and Paarl (T1) on the colour plate towards peak maturity. Juice percentage for Esbal was not good at the Paarl site below 50% while in Citrusdal the the juice percentage for the selection was very good above 60%. The sugars and acids were much higher for the Paarl site compared to Citrusdal, although both sites have good Brix: Acid ratio. Paarl Brix (14.1°) and acid (1.34%) and Citrusdal Brix (11.8°) and acid (1.06%) towards peak maturity. Seed count at Paarl was slightly higher for Esbal 7.3 – 9.7 seeds per fruit and at Citrusdal it was 4.8 – 7.7 seeds per fruit.

Nules

Nules is used as the control in all the sites. In Wellington Nules reached peak maturity quite early in mid April. It was then followed by the Paarl site a week later. In Citrusdal Nules matured on time early May. Its ratio holds well, it stayed at ratio of 12 for about 3 weeks. At all the sites the fruit size was the same count 2. In Citrusdal during one evaluation there were a count 1. At peak maturity the juice percentage was about 55% at Wellington and Citrusdal and in Paarl it was about 53%. Internal quality for Nules at peak maturity was the best in Wellington Brix (13.6°) and acid (1.11%) followed by Paarl Brix (12.2°) and acid (1.02%) and last for Citrusdal with Brix (11.7°) and acid (0.95%). At all 3 sites these internal quality is very good. Internal colour was orange. Those acids will give the fruit good shelf life and the high sugars with acids will give Nules its good flavour. The rind is smooth and thin and it peels easy. Nules in Wellington were seedless because it is in a solid Clementine orchard. In Citrusdal the seed count was 2.3 – 6.9 seeds per fruit. Paarl had the highest seed count ranging from 15.4 – 17 seeds per fruit. Rind colour development was not good for Nules with a T5 (Wellington), T5 – T6 (Citrusdal) and T7 (Paarl) on the colour plate at peak maturity. Yields were good for Nules at all the sites.

Orogrande

Orogrande was the second last selection to reach peak maturity at the trial site in Citrusdal. Maturity can be inconsistent for Orogrande. Fruit size for Orogrande ranged between fruit size count 2 – 1. The juice percentages for Orogrande decreased towards peak maturity 56.5% to 48.6%. Orogrande's peak maturity internal quality was Brix 11.1° with acid of 0.93%. The selection had seeds and the count ranged between 2.3 – 5.7 seeds per fruit. Due to the consistency of the acid the selection was able to reach T1 on the colour plate at peak maturity. One of only 3 selections from all the sites to reach T1 at peak maturity.

Saratoga

Saratoga reached peak maturity quite late this year. It could be that riper fruit were stolen. Fruit size for Saratoga was medium with a fruit size count of 2 – 3. The juice decreased towards peak maturity with a low juice percentage 47.9%. External colour development is very good for Saratoga T1 on the colour plate towards peak maturity. The good internal quality makes it possible for the fruit to hang to colour up complete to deep orange colour. Internally Saratoga had a good Brix: Acid ratio 11.4° and acid 1.04% (towards peak maturity). The selection did have seeds during the evaluations, 0.3 – 2.7 seeds per fruit. Crop on the trees were good for the selection.

Clemenluz

Clemenluz is early maturing Clementine selection. Nules is used as control for this selection. The Clemenluz in the Paarl trial site reached peak maturity first compared to the Citrusdal site. Citrusdal site was about 2 – 3 weeks later to reach peak maturity. Clemenluz at the Paarl site reached peak maturity 18 April 2017 with its ratio being 12.1 on the same date Nules ratio was 11.9, so Clemenluz was a little bit earlier in reaching peak maturity. In Citrusdal at the end of April, Clemenluz maturity was ahead of Nules, but in the middle of May Nules reached its peak maturity 12.3 (ratio) while Clemenluz was still on 11.6 (ratio). There was a difference in fruit size between the 2 regions. Paarl fruit size count was smaller count 3 and Citrusdal the count was 2 – 1. Compared to Nules in Paarl the fruit were one size smaller and in Citrusdal the fruit size was the same. Juice percentages for Clemenluz were higher in Paarl than in Citrusdal at peak maturity or towards peak maturity. The juice % was good for the Paarl region above 55% and it was higher than the juice percentage of Nules. At Citrusdal the juice % was not as good, and Nules had the higher juice percentage. Internal quality for Paarl region was much better than the Citrusdal region. In the Paarl trial site Clemenluz had excellent Brix and acids, higher than Nules. Clemenluz, Brix was 14.7° and acid 1.22% (peak maturity) and Nules, Brix was 12.2° and acid 1.02% (peak maturity). At the Citrusdal trial site there was no significance differences between the Brix: Acid ratios. In Paarl Clemenluz had a higher seed count compared to Citrusdal. In Paarl it was 11 – 12 seeds per fruit and in Citrusdal it was 2.4 – 6.1 seeds per fruit. Nules had the slightly higher seed count per fruit compared to Clemenluz. Rind colour development was more advanced in Paarl with a T4 on the colour plate at peak maturity compared to Nules T7 on the colour plate. In Citrusdal the Clemenluz had a T5 on the colour plate towards peak maturity and Nules had T5 at peak maturity. Rind colour is a yellow orange and the peelability of the fruit is easy.

Conclusion

All the selections (Bonanules, Clemenpons, Marisol and Nules) evaluated at Wellington were seedless, because they were planted in a solid Clementine orchard. Basol with the interstock were the only other selection that were seedless. Nules had the highest seed count 17 seeds per fruit. Most of selections had delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Orogrande, Saratoga and Esbal were the only selections to reach T1 on the colour plate at peak maturity. Clemenluz had the highest Brix (14.7°) and acid (1.22%) at peak maturity (Paarl). In Citrusdal Basol had the highest Brix (13.3°) and acid (1.12%) at peak maturity and in Wellington it was Nules that had the highest Brix (13.6°) and acid (1.11%) at peak maturity. Clemenluz (Paarl) and Basol (Citrusdal) had the smallest fruit size and peaked at count 3. Clemenpons had the largest fruit size count 1x in Citrusdal. The selections that had a very good juice percentage over 55% at peak maturity were Bonanules, Nules (Wellington) and Basol interstock, Clemenpons, Esbal and Nules (Citrusdal) and in Paarl it was Clemenluz.

Table 4.6.27.2. Internal fruit quality data for Clementine selections in the Wellington region (Bonathaba) of the Western Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-03-20	Bonanules	TC	2	54,7	11,8	1,09	10,8	0,0	T6
2017-04-12	Bonanules	TC	2	58,1	11,3	1,00	11,3	0,0	T4
2017-03-20	Clemenpons	TC	2	41.4	11.5	1.25	9.2	0.0	T7
2017-04-12	Clemenpons	TC	2	47.2	12.4	0.97	12.7	0.0	T5
2017-03-20	Marisol	TC	2	57,7	11	1,30	8,5	0,0	T6

2017-04-12	Marisol	TC	2	55,7	13,5	1,25	10,8	0,0	T4
2017-03-20	Nules	TC	2	54,5	11,9	1,52	7,8	0,0	T6
2017-04-12	Nules	TC	2	55,1	13,6	1,11	12,2	0,0	T5

Table 4.6.27.2. Internal fruit quality data for Clementine selections in the Citrusdal region (Kweekkraal and Stargrow) of the Western Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-03-17	Basol	CC	3	53.3	13.3	1.12	11.9	0.2	T6
2017-04-11	Basol	CC	2	54.2	14.5	0.78	18.6	0.0	T1
2017-03-17	Basol M7	CC	3	60.1	12.7	1.16	10.9	0.0	T6
2017-04-11	Basol M7	CC	2	56.2	13.7	0.84	16.3	0.0	T1
2017-04-28	Clemenpons	CC	1x	60.1	10.2	0.88	11.5	0.3	T5
2017-04-28	Esbal	CC	4	63,8	11	1,12	9,8	7,7	T6
2017-05-10	Esbal	CC	3	60,2	11,8	1,06	11,1	4,8	T5
2017-04-28	Nules	CC	2	63,8	10,8	0,98	11,1	6,8	T6
2017-05-10	Nules	CC	2	55,4	11,7	0,95	12,3	3,9	T5
2017-05-26	Nules	CC	1	53,1	11,1	0,91	12,2	6,9	T6
2017-06-15	Nules	CC	2	52,8	13	0,95	13,7	2,3	T1
2017-04-11	Clemenluz	CC	2	59,5	9,5	0,96	9,9	4,3	T6
2017-04-27	Clemenluz	CC	1	51,2	9,4	0,81	11,6	2,4	T6
2017-05-10	Clemenluz	CC	2	44,4	10,5	0,90	11,6	6,1	T5
2017-05-10	Orogrande	CC	1	56,5	10,2	0,91	11,2	3,4	T5
2017-05-26	Orogrande	CC	1	51,6	10,8	0,93	11,7	5,7	T6
2017-06-16	Orogrande	CC	2	48,6	11,1	0,93	11,9	2,3	T1
2017-05-10	Saratoga	CC	2	53,1	10,6	1,02	10,4	2,7	T5
2017-05-26	Saratoga	CC	2	48,1	10,5	0,96	10,9	1,8	T4
2017-06-16	Saratoga	CC	3	47,9	11,4	1,04	11,0	0,3	T1

Table 4.6.27.2. Internal fruit quality data for Clementine selections in the Paarl region (Babylonstoren) of the Western Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-04-18	Clemenluz	CC	3	55,6	14,7	1,22	12,1	11,0	T4
2017-05-08	Clemenluz	CC	3	54,0	14,9	1,16	12,9	12,0	T4
2017-05-08	Esbal	CC	4	51,4	13,7	1,38	10,0	7,3	T3
2017-05-22	Esbal	CC	4	48,2	14,1	1,34	10,5	9,7	T1
2017-04-18	Nules	CC	2	53,1	12,2	1,02	11,9	15,4	T7
2017-05-08	Nules	CC	2	51,4	13,1	1,01	13,0	17,0	T6

4.6.28 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (South West Cape)

Project 1000E by W. Swiegers (CRI)

Opsomming

Die proef perseel het nie op die oomblik 'n wye verskeidenheid van seleksies nie. Buffeljagsrivier area gaan in die toekoms een van die grootste Clementine proef persele word. Die seisoen het begin met Basol, gevolg deur Esbal en ge-eindig met Nules. Daar was 'n definitiewe vertraging in eksterne vrugkleur met 'n kleurplaat

T6 vir Nules. Esbal het 'n beter eksterne kleurontwikkeling met 'n kleurplaat T4 en Basol wat eerste was om ryp te word T1 op kleurplaat.

Summary

The trial site doesn't have a wide variety of selections at the moment. Buffeljagsrivier region will be one of the biggest Clementine trial sites in the future. The season started with Basol, followed by Esbal and ended with Nules. There was a clear indication of a delay in external fruit colour with a colour plate T6 for Nules. Esbal had a more promising colour development and peaked at colour plate T4 and Basol which matured first was a T1 on the colour plate.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Buffeljagsrivier region of the South West Cape; the planting date was 2014. The following varieties were evaluated: Basol, Esbal and Nules.

A ratio of 11:1 Clementines is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

Table 4.6.28.1. List of Clementine selections evaluated at Olivedale (Buffeljagsrivier) during 2017 season.

Selection	Rootstock	Planted
Basol	Carrizo	2014
Esbal	Carrizo	2014
Nules	Carrizo	2014

Results and discussion

Basol

Basol is an early maturing Clementine selection. Fruit size count for Basol was 1xx. Basol had a good juice percentage at peak maturity 57.5%. There were no seeds in this selection. This selection was the only one to reach T1 at peak maturity. Basol had the highest Brix (11.7°) and acid (0.93%) at peak maturity. The fruit peels easily. Basol's rind colour is deep orange. Basol have a very short harvest period before the fruit is over mature and start to granulate.

Esbal

Esbal was the second selection to reach peak maturity. Esbal mature earlier than Nules and its fruit is slightly smaller than Nules. Fruit size count for Esbal 2 – 3 (count). Rind colour development was delayed with T4 (colour plate) when the fruit was over mature. Fruit is round to oblate. Rind is smooth to pebbly. Peelability is easy but rind oil a bother. Seed count for Esbal were just below 4 seeds per fruit. Internal quality was good for Esbal, with high juice percentage.

Nules

Nules was the last selection to reach peak maturity. The selection had a high juice percentage (57.9%). Internal quality for Nules at peak maturity was good. Brix (11.2°) and acid (0.90%). This contributed to Nules good flavour. Nules had the highest seed count ranging from 3.2 – 9.8 seeds per fruit. Rind colour development was not good for Nules with a T6 on the colour plate at peak maturity. Nules had a good fruit size count 1 (peak maturity). Yields were good for Nules. Peelability is easy and internal colour is orange.

Conclusion

Basol was the only seedless selection and Nules had the highest seed count. Basol was the only selection to reach T1 on the colour plates. Degreening practices will be essential after harvesting to ensure optimal colour development for Esbal and Nules. Basol had the highest Brix (11.7°). Esbal had the smallest fruit size and peaked at count 2. All three selections had a very good juice percentage: all over 55%.

Table 4.6.28.2. Internal fruit quality data for Clementine selections in the Buffeljagsrivier region (Olivedale) of the South West Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2017-04-10	Basol	CC	1xx	57.5	11.7	0.93	12.5	0.0	T1
2017-04-26	Esbal	CC	3	60,7	10,6	1,11	9,6	3,8	T4
2017-05-09	Esbal	CC	2	53,1	11	0,76	14,6	3,7	T4
2017-04-10	Nules	CC	3	57,0	10,7	1,17	9,1	3,2	T6
2017-04-26	Nules	CC	1	57,9	11,2	0,90	12,5	6,8	T6
2017-05-09	Nules	CC	1x	53,7	11,4	0,86	13,2	9,8	T4

4.6.29 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape) Project 57D by W. Swiegers (CRI)

Opsomming

Die proef se ligging is goed geskik vir Satsuma produksie. Die meeste bome is ongeveer 5 jaar oud (geplant in 2012). Die 2017 seisoen was die eerste jaar van evaluasie. Die bome lyk goed met goeie boom volume. Die orde van rypwording was as volg: Miho Wase het die seisoen begin gevolg deur Sonet 2, Miyagawa Wase, Aoshima, Sugiyama, Ueno, Imamura en BelaBela 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.

Summary

The trial location is in an area well suited for Satsuma production. Most of the trees are 5 years old (planted in 2012). The 2017 season was the first year for evaluations. The trees look good with large tree canopies. The order of ripening was as follows; Miho Wase started the season, followed by Sonet 2, Miyagawa Wase, Aoshima, Sugiyama, Ueno, Imamura and BelaBela were the selection to finish the season.

Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).

- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Satsuma selections from the Citrusdal region of the Western Cape. The following selections were evaluated: Aoshima, Sonet 2, Imamura, Miho Wase, Miyagawa Wase, Sugiyama, Ueno, BelaBela.

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 4.6.29.1. List of Satsuma selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2017.

Selection	Rootstock	Planted
Aoshima	Carrizo	2013
Sonet 2	Carrizo	2011
Imamura	Carrizo	2012
Miho Wase	Carrizo	2011
Miyagawa Wase	Carrizo	2012
Sugiyama	Carrizo	2012
Ueno	Carrizo	2012
BelaBela	Carrizo	2012

Results and discussion

Aoshima

Aoshima is a mid – late maturing Satsuma. The selection reached peak maturity in the middle of all the selections. Fruit size count for Aoshima ranged between counts 1 - 1xxx. Aoshima was one of the selections with the biggest fruit size count as well as the highest juice percentage. Juice percentage for Aoshima was above 60% (peak maturity). For a satsuma selection the Brix was good (9.2°) as well as the acid percentage (0.85%). It gave Aoshima a good Brix: Acid ratio. There were some seeds in the fruit 0.4 -0.6 seeds per fruit. The external colour development of the Aoshima was not good at all, with a T7 on the colour plate. The fruit was pebbly had some damage due to sunburn.

Miho Wase

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 2, there was a few fruit with 1xxx count. The selection was seedless. Fruit colour on the colour plate was T5 at peak maturity. Fruit matured internally rind colour development was delayed. Miho Wase normally have a high juice percentage, this season the juice percentage was low (52.9%). Sugar at peak maturity was around 9.0° with a acid percentage around 0.80%.

Sonet 2

Sonet 2 is an early maturing Satsuma selections. Fruit size count for Sonet 2 ranged between counts 2 – 1x. Juice percentage for this selection was the highest from all the selections, with a very good juice percentage (64.9%). This selection had the highest sugars and the only selection to have Brix° above 10.0°. The acid percentage at peak maturity for this selection was the highest. Sonet 2 had the highest Brix: acid ratio of all the Satsuma selections that was evaluated. Good internal quality contributed to the flavour. The high Brix and relatively high acid would help the fruit to hang a bit longer. Seed count for Sonet 2 were 1.4 – 1.7 seeds per fruit. Colour on the colour plate was a T6, also delayed external colour development compared to the internal

maturing development. The advantage is the slightly higher acid to hang the fruit a little longer to get better colour. Just be careful for creasing.

Imamura

Imamura is a late maturing Satsuma. It was the second last selection to reach peak maturity. In this cold production region, it reached peak maturity mid May. The juice for Imamura was on the lower side with a juice percentage around 50.0% (peak maturity). For a Satsuma, Imamura had a good Brix: Acid ratio, 9.4° and 0.85 % respectively. Seed count was the highest (1 – 2.1) seeds per fruit. External colour development was T5 on the colour plate. Internal colour was deep orange.

BelaBela

BelaBela is a new late maturing Satsuma selection. This was also the last selection to reach peak maturity in June. The fruit is large with a fruit size count of 1x – 1xx. Fruit's rind was smooth to pebbly and peelability was easy. Internal colour is an excellent deep orange. Juice percentage for this selection at peak maturity was below 50%. At peak maturity the internal quality was good for BelaBela with Brix above 9° and acid above 0.80%. External colour development was delayed compared to the internal maturity with T6 on the colour plate. Seed count for the selection was 0.8 – 1 seeds per fruit.

Miyagawa Wase

The fruit size of Miyagawa Wase at peak maturity was count 1. The juice % at peak maturity was good around 55%. The Brix° and acid percentage were the lowest for Miyagawa Wase compared to the other selections at peak maturity. Brix was 8.2° and acid was 0.96%. Colour development was not good and delayed compared to the internal maturity. The colour on the colour plate at peak maturity was T6. Seed count for the selection was 0 – 0.8 seeds per fruit. Fruit was smooth and flat and the internal colour was deep orange.

Ueno

This selection is a mid to late maturing selection for this trial site. It reached peak maturity at the end of April beginning of May. Ueno had a large fruit size count with a 1xx count. Ueno juice percentage was good at peak maturity 54.5%. The Brix° and acid percentage for Ueno at peak maturity were 8.8° and 0.86% respectively. Ueno can have higher sugars and acids levels. There were 0.6 seeds per fruit and Ueno colour on the colour plate at peak maturity was T6. Fruit peeled easy.

Sugiyama

Sugiyama are mid to late maturing Satsuma. At this trial site it reached peak maturity at the end of April. Sugiyama had good fruit size count at 1xx one of the largest count compared to some of the other selections. The juice percentage for Sugiyama was 51.6% (peak maturity). The Brix° and acid percentage of Sugiyama were 8.2° and 0.77% respectively at peak maturity. One of the lowest acids at peak maturity compared to the other selections. Seed count for the selection was a very low count at 0.3 seeds per fruit. There was also a delay in colour development with a T6 on the colour plate.

Conclusion

Imamura, Sugiyama, Ueno and BelaBela had the largest fruit size (count 1xx) with Sonet 2 and Aoshima having the best juice percentages: 64.8% and 63.8% respectively. Sonet 2 had the highest Brix° of all the Satsuma selections (10.5°). Imamura had the highest seed count with 2.1 seeds per fruit. Rind colour development was not good T5 – T6 on the colour plate at peak maturity. BelaBela had the best internal colour.

Table 4.6.29.2 Internal fruit quality data for Satsuma selections in the Citrusdall region of the Western Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg, Seed	Colour
2017-04-28	Aoshima	CC	1	63,8	9,2	0,85	10,8	0,4	T7
2017-05-10	Aoshima	CC	1xxx	50,6	9,4	0,78	12,0	0,6	T6

2017-04-11	Sonet 2	CC	1x	64,8	10,5	0,92	11,5	1,7	T6
2017-04-28	Sonet 2	CC	2	60,1	11,1	0,79	14,1	1,4	T5
2017-04-27	Imamura	CC	1	53,0	9,4	1,11	8,5	2,1	T6
2017-05-26	Imamura	CC	1xx	48,7	9,4	0,85	11,0	1,0	T5
2017-03-17	Miho Wase	CC	1xxx	50,0	8,7	1,04	8,4	0,0	T6
2017-04-11	Miho Wase	CC	2	52,9	9,1	0,77	11,7	0,0	T5
2017-04-11	Miyagawa Wase	CC	1	54,2	8,2	0,77	10,7	0,8	T6
2017-04-27	Miyagawa Wase	CC	1xx	57,4	7,7	0,59	13,1	0,0	T6
2017-04-27	Sugiyama	CC	1xx	51,6	8,2	0,77	10,6	0,3	T6
2017-04-27	Ueno	CC	1xx	54,5	8,8	0,86	10,3	0,6	T6
2017-05-26	BelaBela	CC	1x	48,4	8,5	1,0	8,7	0,8	T7
2017-06-16	BelaBela	CC	1xx	44,0	9,5	0,8	11,4	0,9	T6
2017-07-05	BelaBela	CC	1xx	42,6	9,4	0,8	11,4	1	T5

4.6.30 Climatic Regions of Southern Africa and cultivars being evaluated

CLIMATIC REGION	AREA	PLACE	CULTIVARS
Hot-Dry	Limpopo	Tshipise	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Musina	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Letsitele	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Hoedspruit	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Hot-Humid	Mpumalanga	Malelane	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Komatipoort	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
	KwaZulu-Natal	Pongola	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Nkwaleni	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Swaziland	Lowveld	Grapefruit	
		Valencias	
		Mandarin Hybrids (Late)	

	Mozambique	Southern	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Intermediate	Limpopo	Tom Burke	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
		Letaba	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
		Levubu	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
	Marble Hall	Navels (Mid/Late)	
		Valencias	
		Mandarin Hybrids (Mid/Late)	
		Lemons	
	Mpumalanga	Nelspruit	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
		Karino	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
Hazyview		Navels (Mid/Late)	
		Valencias	
		Mandarin Hybrids (Mid/Late)	
		Lemons	
Schagen	Navels (Mid/Late)		
	Valencias		
	Mandarin Hybrids (Mid/Late)		
	Lemons		
Swaziland	Ngonini	Navels (Mid/Late)	
		Valencias	
		Mandarin Hybrids (Mid/Late)	
		Lemons	
Cold/Coastal	Eastern Cape	East Cape Midlands	Midseasons
			Navels/Valencias
			Mandarin Hybrids/Satsumas
	Gamtoos River Valley	Lemons	
		Mandarin Hybrids	
		Navels	
		Satsumas/Clementines	
	Sundays River Valley	Lemons	

			Mandarin Hybrids
			Navels/Valencias
	KwaZulu-Natal	Richmond	Lemons
			Navels
		Ixopo/Umzimkhulu	Lemons
			Navels
	Western Cape	Knysna	Lemons
			Mandarin Hybrids
		Heidelberg	Navels
			Mandarin Hybrids
			Lemons
		Paarl	Navels
			Mandarin Hybrids
			Satsumas/Clementines
		Wolseley	Navels
			Mandarin Hybrids
			Satsumas/Clementines
		Citrusdal	Navels/Valencias
			Mandarin Hybrids
			Lemons
		Clanwilliam	Navels/Valencias
			Mandarin Hybrids
			Lemons
		Swellendam	Navels/Valencias
	Mandarin Hybrids		
	Lemons		
	Satsumas		
	Robertson	Navels/Valencias	
		Mandarin Hybrids/Satsumas	
		Lemons	
Cool-Inland	North West	Rustenburg	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
	Limpopo	Zebediela	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
		Mokopane	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
		Burgersfort	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
	Ohrigstad	Navels (Mid)	
		Navels (Late)	
		Mandarin Hybrids	
	Mpumalanga	Ngodwana/Schoemanskloof	Navels (Mid)
			Navels (Late)

			Mandarin Hybrids
Semi-Desert	Northern Cape	Kakamas/Blouputs	Navels (Late)
			Valencias
			Grapefruit
			Mandarin Hybrids (Late)
		Groblershoop/Upington	Navels (Late)
			Valencias
			Grapefruit
			Mandarin Hybrids (Late)
		Vaalharts	Midseasons
			Navels (Late)
			Valencias
			Mandarin Hybrids (Late)

4.6.31 FINAL REPORT: Fine-tuning of molecular genotype reference database for mandarins, lemons and limes

Project ARC ref 000182-Y5 (2016/17) by E Hajari, D Nonyane, A Sippel (ARC-TSC) and G Barry (XLnT Citrus c.c.)

Summary

At present, cultivars entering the Citrus Improvement Scheme (CIS) via the two Virus Free Nucleus Blocks, i.e. at the Agricultural Research Councils' - Tropical and Subtropical Crops (ARC-TSC) and at Citrus Research International (CRI), are evaluated and identified based on agronomic and morphological characteristics. However, such characteristics can be influenced by environmental factors, amongst others. Therefore, a supportive tool is needed to confirm cultivar identification. This can be provided by the use of DNA molecular marker technology. However, molecular markers are not yet adequately developed to work all the time conclusively. A previously funded collaborative project between CRI and ARC-TSC investigated the establishment of a molecular genotype reference database for citrus cultivars in the CIS (project no 000182-01). This project was continued in order to verify the lemons, limes and mandarins (project 000182-Y5 in 2016-2017). The results suggest that the molecular markers tested to date are capable of distinguishing between groups, but are not sufficiently sensitive to conclusively discriminate amongst all cultivars within the same group. Nonetheless, the former observation will allow the genotype reference database to be used for applications including understanding the phylogenetic relationships in citrus and will also have some application (with certain limitations) in issues surrounding cultivar verification. It must be noted that there are reservations concerning the reproducibility of the database, as the methods used for its creation were not consistent. However, these concerns can be addressed by close evaluation of the database, generation of new genetic analyses and subsequent re-testing of samples that produce questionable results. This was tested by repeating the DNA analysis using fresh leaf samples for the lemons and limes. The new analysis was able to resolve some of the concerns identified with the previous analysis. This highlights the need to verify the results in the existing genotype reference database for all the other represented groups, by repeating the DNA analysis, where necessary.

Opsomming

Tans word kultivars in die Sitrus Verbetering Skema (SVS) via die twee Virusvrye Kernblokke, nl. Landbounavorsingsraad se Tropiese en Subtropiese Gewasse (LNR-TSG) en Citrus Research International (CRI), geëvalueer en geïdentifiseer gebaseer op agronomiese en morfologiese eienskappe. Sulke eienskappe kan egter beïnvloed word deur onder andere die omgewin. Daarom is 'n ondersteunende hulpmiddel nodig om kultivar-identifikasie te bevestig en kan moontlik deur die gebruik van DNA molekulêre merker tegnologie gedoen word. Molekulêre merkers is egter nog nie voldoende ontwikkel om altyd bo alle twyfel te werk nie. 'n Gesamentlike projek tussen CRI en LNR-TSG het die vestiging van 'n molekulêre genotipe verwysingsdatabasis vir sitrus kultivars in die SVS (projek 000182-01) ondersoek. Hierdie projek is voortgesit

om die data ten opsigte van suurlemoene, lemmetjies en sagtesitrus (projek 000182-Y5 in 2016-2017) te verifieer. Die resultate dui daarop dat molekulêre merkers kan onderskei tussen groepe, maar dat dit nie sensitief genoeg is om met alle sekerheid te onderskei tussen sekere kultivars binne dieselfde groep. Nietemin, die vorige observasies verskaf begrip van die filogenetiese verwantskappe in sitrus en kan aangewend word, met sekere beperkings, in kwessies rondom kultivar verifikasie. Daar is onsekerheid oor die herhaalbaarheid van die databasis as gevolg van die feit dat die metodiek gevolg vir die skep van die databasis nie altyd konsekwent was nie. Tog kan hierdie bekommernis aangespreek word deur in-diepte evaluering van die databasis, die daarstel van nuwe genetiese ontledings en die daaropvolgende hertoets van plant materiaal wat twyfelagtige resultate gelewer het. Gevolglik is 'n hertoets gedoen van die DNA ontledings met vars blaar monsters vir suurlemoene en lemmetjies. Die nuwe ontledings het sommige van die bekommernisse aangespreek wat voorheen ondervind is. Dit beklemtoon die noodsaaklikheid om alle resultate te verifieer in die huidige genotipe verwysingsdatabasis en al die ander sitrus-groepe in te sluit en die DNA ontledings te herhaal soos benodig.

Introduction

Technological advances in DNA molecular markers have made this technology more accessible to researchers in plant improvement and breeding programmes. Two applications of DNA molecular markers have been utilised in citrus, *viz.* as tools to verify cultivars and assess the amount of genetic diversity present in populations. A number of specific molecular biological techniques are available for this purpose and there are advantages and limitations associated with each of them. The selection of a particular technique will depend on the application required. Of the available marker systems, Simple Sequence Repeat (SSR) markers fulfill many of the required characteristics and are one of the most commonly used methods to determine the degree of genetic relatedness among cultivars. In citrus, SSR markers have been used in a number of studies, e.g. Corazza-Nunes *et al.* (2002); Novelli *et al.* (2006); Amar (2012); Shrestha *et al.* (2012); Kacar *et al.* (2013) and Nematollahi *et al.* (2013). Therefore, a previous study investigated the application of SSR markers in the establishment of a molecular genotype reference database for citrus cultivars in the CIS (project no 000182-01).

At the end of the above-mentioned project, a molecular genotype reference database was created for rootstocks, lemons, limes, grapefruits, pummelos, mandarins and oranges present within the Virus Free Nucleus Blocks and the Pre-Immunised Foundation Block of the CIS. The results indicated that it was possible to distinguish between the different citrus groups, however, difficulties were encountered when attempts were made to distinguish all cultivars within the same group. Furthermore, some concerns were noted following close examination of some of the results in that study, for example, the inability to replicate results (particularly for the sweet oranges). This could be ascribed to a number of reasons, including human error in the compilation of the database, changes in the methods employed, laboratory-based inconsistencies such as poor quality of the DNA template or insufficient amplification during PCR, failed runs during capillary electrophoresis, etc. In addition, the researcher responsible for compiling the database has resigned and cannot be contacted to address concerns. Nonetheless, the aim of the previous project was achieved, *viz.* the establishment of the molecular genotype reference database. The creation of the above-mentioned database represented a significant investment of time and resources over the three-year duration of that project. However, the issues raised cannot be ignored. It is therefore imperative to continue with verification of the database in order to address concerns and attempt to resolve issues noted with each cultivar group.

Objectives

The objectives were to:

- Analyse data for mandarins, lemons and limes
- Refine the gel alignment and analysis method
- Compile a new database for lemons and limes based on new analysis
- Conduct new genetic analysis with the revised database for lemons and limes and generate new dendrograms
- Repeat DNA analysis with the lemon and limes to verify results and attempt to resolve issues.

Materials and methods

DNA extraction

Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germany) as per the manufacturer's instructions. Cell lysis was performed using a Precellys homogeniser with zinc zirconium beads (Bertin Technologies, France).

Polymerase Chain Reaction

The SSR primers tested were as described by Barkley *et al.* (2006), Froelicher *et al.* (2008) and Ollitrault *et al.* (2010). Genomic DNA was amplified in a reaction volume of 15 µl containing 25 ng template DNA, TaKaRa EmeraldAmp Max HS PCR master mix (TaKaRa, Japan), 0.2 µM forward and 0.2 µM reverse primer. The SSR amplification reactions were performed using a G-Storm thermocycler with the reaction conditions as specified in the relevant references. The PCR products were visualised via capillary electrophoresis (Qiaxcel Advanced, Qiagen, Germany). All reactions were repeated to verify data.

Data analysis

The sizes of PCR products were determined using Screengel Software (Qiagen, Germany) and the data was used to compile a database. GenAlEx 6.3 was used to create a genetic distance matrix. Distance matrices were subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis. The cluster analysis was validated through bootstrapping and calculation of the co-phenetic correlation coefficient (CCC). The MEGA 5 software was used to draw the final tree.

Results and discussion

The current project (000182-Y5) was approved to fine-tune the molecular genotype reference database for lemon, lime and mandarin (to a limited extent). The initial budget requested for the project was significantly higher than the allocation that was approved. For this reason, it was not possible to analyse the mandarin to the same extent as the lemon and lime. Hence, a cursory analysis of the mandarin database was performed while a significantly more in-depth evaluation of the lemons and limes were undertaken.

The existing database for lemon, lime and mandarin was assessed to identify obvious discrepancies that could have arisen as a result of human error. The discrepancies noted in the database were corrected by examining the relevant gels and the band sizes were adjusted as necessary. Since these changes could potentially affect the genetic relationships as indicated in the dendrograms submitted as part of the termination report during the 2014-2015 financial year, new phylogenetic analyses were conducted in order to generate updated dendrograms. These were evaluated and it was determined that no major changes were evident. In order to fine-tune the database, it was necessary to evaluate the data analysis method to resolve inconsistencies and weaknesses. Therefore, new data analysis methods were investigated using the Qiagen Screengel Software to ensure that maximum efficiency was being gained from the software programs. This was an ongoing process that was done in consultation with an applications specialist with the aim being speeding up the analysis process and thereby increasing efficiency as well as reducing the labour cost related to the current project. Towards this end, the potential to automate part of the analysis component was investigated.

The new analysis method was developed and applied to the gels for the three fruit types. This allowed for partial automation of the process and included updated, defined experimental parameters, which served to minimise experimental error and human bias. From this work, it was evident that the new analysis resulted in less time being spent on this process. More importantly, it limited the variation observed between replicates, which was a significant problem encountered with the previous project. At this stage, it was decided that only the lemons and limes would be further critically evaluated as the allocated budget did not allow for a similar evaluation of the mandarins. Technical challenges regarding software incompatibility issues as a result of obtaining new computers were discussed in the relevant progress reports.

A new database was created for the lemons and limes using the improved analysis method and data. The database comprised a Microsoft Excel file containing band sizes as a result of the PCR amplification process. More differences were evident between the lemon and lime groups (in terms of presence and/or absence of bands) than within each group. Within the lemons, assessment of the database suggested difficulties in distinguishing cultivars within the group, except for Feminello SL. Differences were detected between Feminello SL and all the other tested cultivars with the following markers: GT03, TAA1, TAA15, TAA41, CiO, mCE, mCF and mCN. In addition, Lemox displayed a single polymorphism indicated by the presence of a band at approximately 216 bp with the mCN marker. This band was also present for Feminello SL. There were no polymorphisms detected between the lemons for all the other markers tested *viz.* CiB, CiC, CiL, CiM, CiP, CiR, CiU, mCA, mCK, mCR and SR6. Conclusions could not be drawn from six primers, i.e. mCQ, mCU, SR9, SR17, SR23 and SCM02 as this data was not verified. Within the lime group, polymorphisms were evident in certain cases, for example, for ITSG West Indian CSFRI (TAA15 and TAA41), Thai lime (CiL, CiM, GT03 and CiU), Bearss lime (CiO), Limequat (mCN) and West Indian Key (GT03). Similar to the lemons, the data were not verified for CiR, mCQ, mCU, SR9, SR17, SR23 and SCM02. All other tested markers displayed no discernible polymorphisms.

The above-mentioned data were used to generate new dendrograms for the lemons and limes (Figure 1). This genetic analysis indicated separation of the lemons and limes into two separate groups, with exceptions noted for Bearss lime and Lisbon Yen Ben, which grouped with the lemons and limes, respectively. In addition, Eureka SL was found to be more genetically similar to Genoa than Eureka and 2PHSLEurekaQDPI. Similarly, Limequat was more closely related to West Indian Key than ITSG West Indian CSFRI. Considering this result, fresh samples were obtained and the DNA analysis in the laboratory was repeated in an attempt to either address this concern and resolve it, or verify the result.

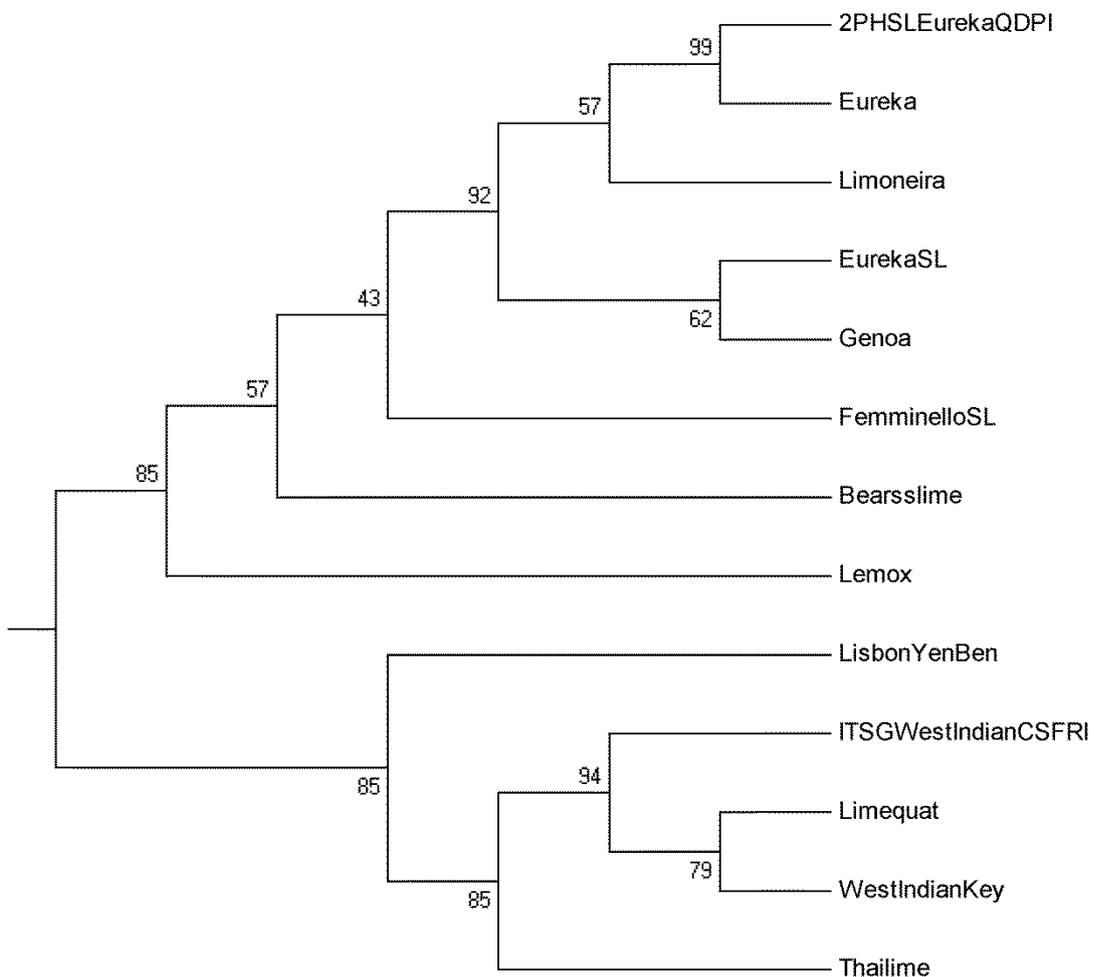


Figure 1: UPGMA dendrogram illustrating the genetic relationships between lemons and limes using data from initial genotype reference database (CCC = 0.994, Pearson).

After repeating the laboratory analysis with fresh material and subsequent processing of the data, a new dendrogram was generated (Figure 2). The high co-phenetic correlation coefficient (CCC) obtained (0.998) was indicative of a good fit between the data and analysis method. The results indicated separation of the cultivars into two groups, one comprised solely of limes and the other with all the lemons and Bearss lime (Figure 2). Within the lemon group, some rearrangements were apparent. For example, 2PHSLEurekaQDPI occurred in the same group as Lisbon Yen Ben and Feminello SL, however, in the previous analysis it grouped with Limoneira and Eureka. In the prior analysis, Eureka SL grouped with Genoa and in the current analysis it clustered with Limoneira. Therefore, the new analysis confirms the ability to distinguish between cultivars in different groups (in all but one case) and confirms the previously highlighted difficulty in distinguishing between cultivars within the same group. The new analysis was able to resolve some concerns identified with the previous analysis. For example, although Bearss lime occurred in the same group as the lemons, it now appeared as the most genetically distant cultivar whereas in the previous analysis, it grouped more closely within the lemon group. Furthermore, in the previous analysis, Lisbon Yen Ben occurred in the same group as the limes, while in the current dataset, it clustered with the lemons. The above-mentioned differences that were noted between the current and previous analysis highlights the need to verify the results in the existing genotype reference database for all the other represented groups by repeating the DNA analysis. The reasons for the differences noted above might be related to changes in methodology as new technologies were adopted during the duration of the first project, for example, 2-dimensional gel electrophoresis was replaced with high-throughput capillary electrophoresis.

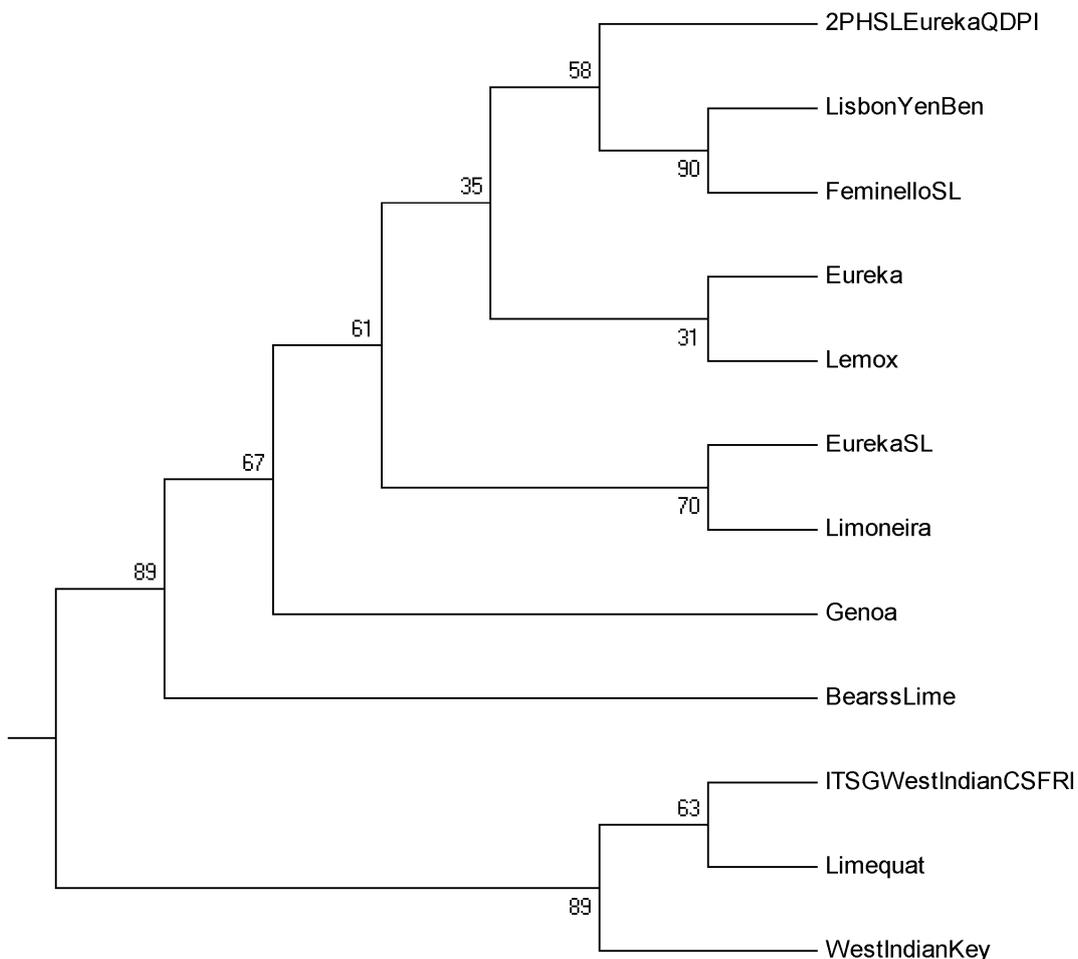


Figure 2: UPGMA dendrogram illustrating the genetic relationships between lemons and limes after repeating DNA analysis in the laboratory with new samples (CCC = 0.998, Pearson).

Conclusion

Examination of the results obtained to date suggests that the markers can discriminate between cultivars in different groups, however, difficulties were encountered in attempts to distinguish all cultivars within the same group. In this context, it was possible to distinguish between the lemons and limes, with a few exceptions that might be explained by cultivar phylogeny and limitations of the method. The strategy to re-test samples in the laboratory proved valuable in resolving some of the concerns highlighted with the original database.

Technology transfer

Poster presented at CRI Symposium 2016: Hajari E., Severn-Ellis A., Sippel A., Nonyane D., Combrink N., Cook G and du Toit T. 2016. Establishment of a molecular genotype reference database for citrus cultivars within the Citrus Improvement Scheme. CRI Symposium, Drakensberg.

Further research

It is proposed that additional research be undertaken to resolve inconsistencies in the existing molecular genotype reference database. It is suggested that the strategy followed in the current study be implemented for the remaining groups, i.e. mandarin, orange, grapefruit and rootstock.

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4.6.32 FINAL REPORT: Validation of the established citrus molecular genotype reference database for grapefruit, pummelo and rootstocks

Project 182-Y5 (September 2017-March 2018) by Elliosha Hajari, Dzunisani Nonyane, Arthur Sippel (Agricultural Research Council) and Graham Barry (XLnT Citrus)

Summary

At present, cultivars entering the Citrus Improvement Scheme (CIS) via the two Virus Free Nucleus Blocks, i.e. at the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) and at Citrus Research International (CRI), are identified based on morphological characteristics. However, such characteristics can be influenced by environmental and other factors. Therefore, a supportive tool is needed to confirm cultivar identification. This can be provided by the use of DNA molecular marker technology. However, molecular markers are not yet adequately developed to work all the time, conclusively. A previously funded collaborative project between CRI and ARC-TSC investigated the establishment of a molecular genotype reference database for citrus cultivars in the CIS (project no. 000182-01). This project was continued in order to verify the data collected, resolve inconsistencies (as a result of methodological issues) and to update the database using improved analysis methods. The current project involved verification of the grapefruits, pummelos and rootstocks (project 000182-Y5 in 2017-2018). In addition, the group of diverse citrus comprising non-commercial cultivars and ornamentals were added to ensure that all groups apart from the oranges were verified. The results suggest that the markers are capable of distinguishing between groups, but are not always sufficiently sensitive to conclusively discriminate amongst all cultivars/selections within the same group. This is especially evident in cases where selections arose as a result of small mutations and in groups with low genetic variability, e.g. grapefruit. Nonetheless, the limit of resolution of the markers have been determined and a molecular genotype reference database has been created. Despite the constraints mentioned above, the markers can be used for applications including understanding the phylogenetic relationships in citrus and will also have some application (with certain limitations) in issues surrounding cultivar verification. This latter application will have to be evaluated on a case-by-case basis.

Opsomming

Tans word kultivars in die Sitrus Verbetering Skema (SVS) via die twee Virusvrye Kernblokke, n.l. Landbounavorsingsraad se Tropiese en Subtropiese Gewasse (LNR-TSG) en Citrus Research International (CRI), geëvalueer en geïdentifiseer gebaseer op agronomiese en morfologiese eienskappe. Sulke eienskappe kan egter beïnvloed word deur onder andere die omgewin. Daarom is 'n ondersteunende hulpmiddel nodig om kultivar-identifikasie te bevestig en kan moontlik deur die gebruik van DNA molekulêre merker tegnologie gedoen word. Molekulêre merkers is egter nog nie voldoende ontwikkel om altyd bo alle twyfel te werk nie. 'n Gesamentlike projek tussen CRI en LNR-TSG het die vestiging van 'n molekulêre genotipe verwysingsdatabasis vir sitrus kultivars in die SVSSVS (projek 000182-01) ondersoek. Hierdie projek is voortgesit om die data, (wat ontstaan het a.g.v. teenstrydige data weens metodiek gevolg), ten opsigte van suurlemoene, lemmetjies en naartjies (projek 000182-Y5 in 2017-2018) te verifieer, asook om die databasis op te dateer. Addisioneel is nie-kommersieële kultivars en ornamentele sitrus bygevoeg om sodoende alle groepe, bo-en-behalwe lemoene, te verifieer. Die resultate dui daarop dat merkers in staat is om te onderskei tussen die groepe, maar dat dit nie altyd sensitief genoeg is om te onderskei tussen al die kultivars/seleksies binne dieselfde groep nie. Dit is veral so wanneer seleksies ontstaan het a.g.v. mutasies in groepe met min genetiese variasie, soos byvoorbeeld pomelo. Nietemin, die limiet van resolusie van die merkers is bepaal en 'n molekulêre genotipe verwysingsdatabasis is geskep. Ten spyte van die beperkinge bo genoem kan merkers gebruik word om byvoorbeeld die stamgeskiedenis verwantskappe in sitrus te verstaan, asook rondom kultivar verifikasie (met beperkinge). Laasgenoemde applikasie moet van geval-tot-geval gehanteer word.

Introduction

Technological advances in DNA molecular markers have made this technology more accessible to researchers in plant improvement and breeding programmes. Two applications of DNA molecular markers have been utilised in citrus, viz. as tools to verify cultivars and to assess the amount of genetic diversity present in populations. A number of specific molecular biological techniques are available for this purpose and there are advantages and limitations associated with each of them. The selection of a particular technique will depend on the application required. Of the available marker systems, Simple Sequence Repeat (SSR) markers fulfill many of the required characteristics and are one of the most commonly used methods to determine the degree of genetic relatedness among cultivars. In citrus, SSR markers have been used in a number of studies, e.g. Corazza-Nunes *et al.* (2002); Novelli *et al.* (2006); Amar (2012); Shrestha *et al.* (2012); Kacar *et al.* (2013) and Nematollahi *et al.* (2013). Therefore, a previous study investigated the application of SSR markers in the

establishment of a molecular genotype reference database for citrus cultivars in the CIS (project no. 000182-01).

At the end of the abovementioned project, a molecular genotype reference database was created for rootstocks, lemons, limes, grapefruits, pummelos, mandarins and oranges present within the Virus Free Nucleus Blocks and the Pre-Immunised Foundation Block of the CIS. Some concerns were noted following close examination of the results in that study, for example, the inability to replicate results. This could be ascribed to a number of reasons, including human error in the compilation of the database, changes in the methods employed, laboratory-based inconsistencies such as poor quality of the DNA template or insufficient amplification during PCR, failed runs during capillary electrophoresis, etc. In addition, the researcher responsible for compiling the database resigned and could not be contacted to address concerns. Nonetheless, the aim of the previous project was achieved, *viz.* the establishment of the molecular genotype reference database. The creation of the database represented a significant investment of time and resources over the three-year duration of that project. However, the issues mentioned above cannot be ignored. It was therefore imperative to continue with verification of the database in order to address concerns and attempt to resolve issues noted with each cultivar group.

In 2017-2018, a project was funded by CRI to verify the existing molecular genotype reference database for grapefruits, pummelos and rootstocks. Although not stated in the objectives below, the group of diverse citrus cultivars was included to ensure that data was verified for all groups except for the oranges.

The stated objectives as per the project proposal were as follows:

- Repeat DNA analysis with grapefruit, pummelo and rootstocks in order to verify results in the dendrograms and attempt to resolve issues
- Conduct data analysis for grapefruit, pummelo and rootstocks
- Compile a new database for grapefruit, pummelo and rootstocks and critically evaluate
- Conduct new genetic analysis with the revised database for grapefruit, pummelo and rootstocks and generate new dendrograms

Materials and methods

DNA extraction

Genomic DNA was extracted using the Macherey Nagel NucleoSpin Plant II Kit (GmbH and Company, Germany) as per the manufacturer's instructions. Cell lysis was performed using a Precellys homogeniser with zinc zirconium beads (Bertin Technologies, France).

Polymerase Chain Reaction

The SSR primers tested were as described by Barkley *et al.* (2006), Froelicher *et al.* (2008) and Ollitrault *et al.* (2010). Genomic DNA was amplified in a reaction volume of 15 µl containing 25 ng template DNA, TaKaRa EmeraldAmp Max HS PCR master mix (TaKaRa, Japan), 0.2 µM forward and 0.2 µM reverse primer. Cycling conditions consisted of an initial hot start at 98 °C for 1 min; 35 cycles of 98 °C for 15 s, 50-60 °C for 30 s, 72 °C for 1 min and a final extension step at 72 °C for 5 min. All reactions were repeated to verify data.

Visualisation of PCR products

The SSR-PCR amplification products were separated using the QIAxcel High Definition DNA Capillary Cartridge and OM800 running conditions to distinguish between fragment sizes of 100-500 bp with a resolution quality of 3-5 bp. A 15 bp-1 kb alignment marker and 50-800 bp size marker was used.

Data analysis

Analysis and fragment size annotation was carried out using the QIAxcel ScreenGel software (Qiagen, Germany) and the data was used to compile a database. GenAlEx 6.3 was used to create a genetic distance matrix. Distance matrices were subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Mean)

cluster analysis. The cluster analysis was validated through bootstrapping and calculation of the co-phenetic correlation coefficient (CCC). The MEGA 5 software was used to draw the final tree.

Results and discussion

Grapefruit

In the current study, the UPGMA method was used to generate all dendrograms while bootstrap analysis and the co-phenetic correlation coefficient (CCC) were used to illustrate the strength of relationships. It was noted that the CCC value obtained for all the tested groups (grapefruits, pummelos, rootstocks and diverse selections) were high (Figures 1-4: 0.955-0.999) which indicated a good fit between the data and analysis method (Benin *et al.*, 2012). The results for the grapefruit cultivars and selections tested in the present study are shown in Figure 1. In the current study, there was a grouping of the white fleshed grapefruits, *viz.* Marsh, Nartia and the Jackson selections. Certain relationships were expected, for example, Nelruby is a seedling of Ray Ruby and they occurred within the same group on the dendrogram. Similarly, Flame was reportedly selected from Henderson and these occurred together on the dendrogram. Also, all the Jackson selections clustered together.

Although grapefruits display a range of morphological variation in terms of rind and flesh colour, they are known to have a narrow genetic base as most selections have arisen through mutations (Novelli *et al.*, 2000). This high degree of genetic similarity amongst grapefruits was evident in the present study, as many selections could not be distinguished from each other. Similarly, Sharma *et al.* (2015) also reported on the low genetic variability using Inter Simple Sequence Repeat (ISSR) markers. In addition, Curk *et al.* (2014) confirmed the low heterozygosity of grapefruit cultivars using a more sensitive method, *i.e.* DNA sequencing. In recent times, Single Nucleotide Polymorphism (SNP) markers have become popular due to their widespread nature and potential direct link to genes associated with agronomic traits. In this context, Distefano *et al.* (2013) used SNP analysis with High Resolution Melting Curve (HRM) analysis for citrus genotyping. This analysis could distinguish between the different citrus species but could not differentiate between genotypes within the same species that arose from mutations. However, since then, more genotypes have been sequenced and more SNPs have been discovered.

Considering the above, it can be suggested that in general, the tested markers were not sufficiently sensitive to resolve the differences between many of the grapefruit cultivars. This is not surprising as Barkley *et al.* (2006) and Shahzadi *et al.* (2014) also noted that while SSR markers could distinguish between Citrus species, selections that arose by spontaneous mutations within groups were difficult to detect.

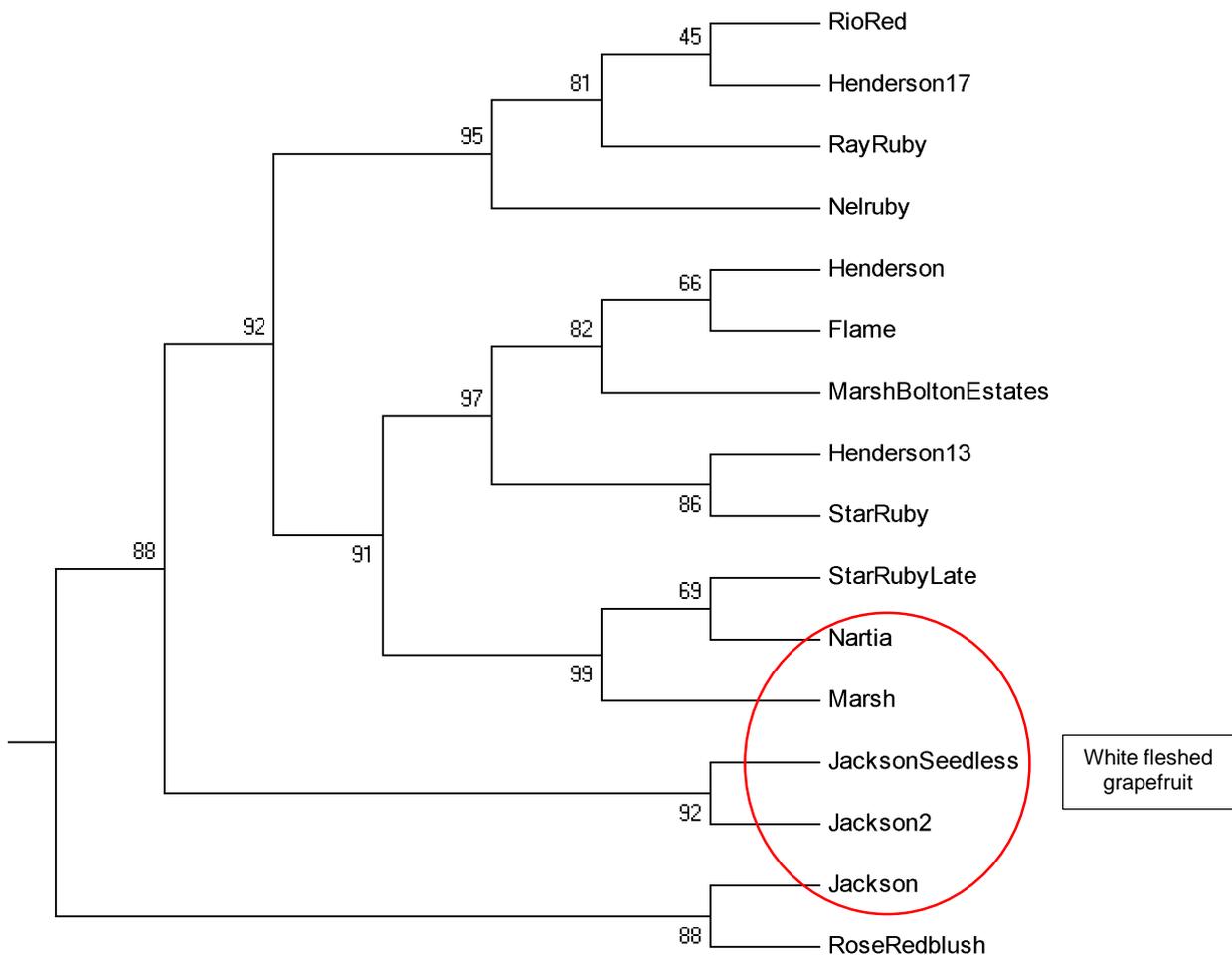


Figure 1: UPGMA dendrogram showing relationships between grapefruits (CCC = 0.955, Pearson).

Pummelos

The pummelos are a much older and larger group than the grapefruit (Hodgson, 1967). The pummelos investigated in the present study are shown in Figure 2. From the cluster analysis, it was evident that there were two major groups. The first group comprised Kukemoes, Tahiti and Pomelit 2 while all the other tested cultivars/selections clustered in the second group. The Cocktail Pummelo was found to be the most genetically different from the other tested pummelos in group 2. Similarly, Uzun *et al.* (2011) reported that Cocktail Pummelo separated from other tested selections when Sequence-Related Amplified Polymorphism (SRAP) markers were used and attributed this to the hybrid nature of the cultivar, i.e. that it originated as a hybrid between a *Frua* mandarin and a low acidity pummelo. In the current study, Melogold and Oroblanco displayed a high degree of genetic similarity as they occurred within the same subgroup. This is not surprising as both are reported to have similar origins. In this context, Melogold is reported to have resulted from a cross between a diploid acidless pummelo and a white seedy tetraploid grapefruit selection. In addition, both Melogold and Oroblanco are seedless and produce white flesh.

True pummelos are known to be monoembryonic therefore; each seedling that is produced would represent a different genotype (Hodgson 1967). Furthermore, the genetic diversity within pummelos is reported to be higher than that of grapefruit (Yong *et al.*, 2006). This was seen in the present study as the tested markers revealed higher levels of polymorphism within the pummelos than within grapefruit. For example, the primer GTO3 revealed a difference between Cocktail Pummelo and the other pummelos, CiC indicated a difference between Kukemoes, Tahiti and the other pummelos and CiM produced distinctive bands for Cocktail Pummelo and Oroblanco. From the results collected in the present study, it was evident that the tested markers revealed some polymorphisms between the tested pummelos. Differences between closely related selections were difficult to discern. In contrast to what was found with grapefruit by Curk *et al.* (2014), Wu *et al.* (2014) identified a set of 25 SNPs that can be used as reference genetic barcodes to identify pummelo cultivars. This panel of

SNPs was tested using 260 pummelo accessions and it was found to discriminate amongst all genotypes. Therefore, SNP markers provide a potential alternative tool for DNA fingerprinting of pummelos.

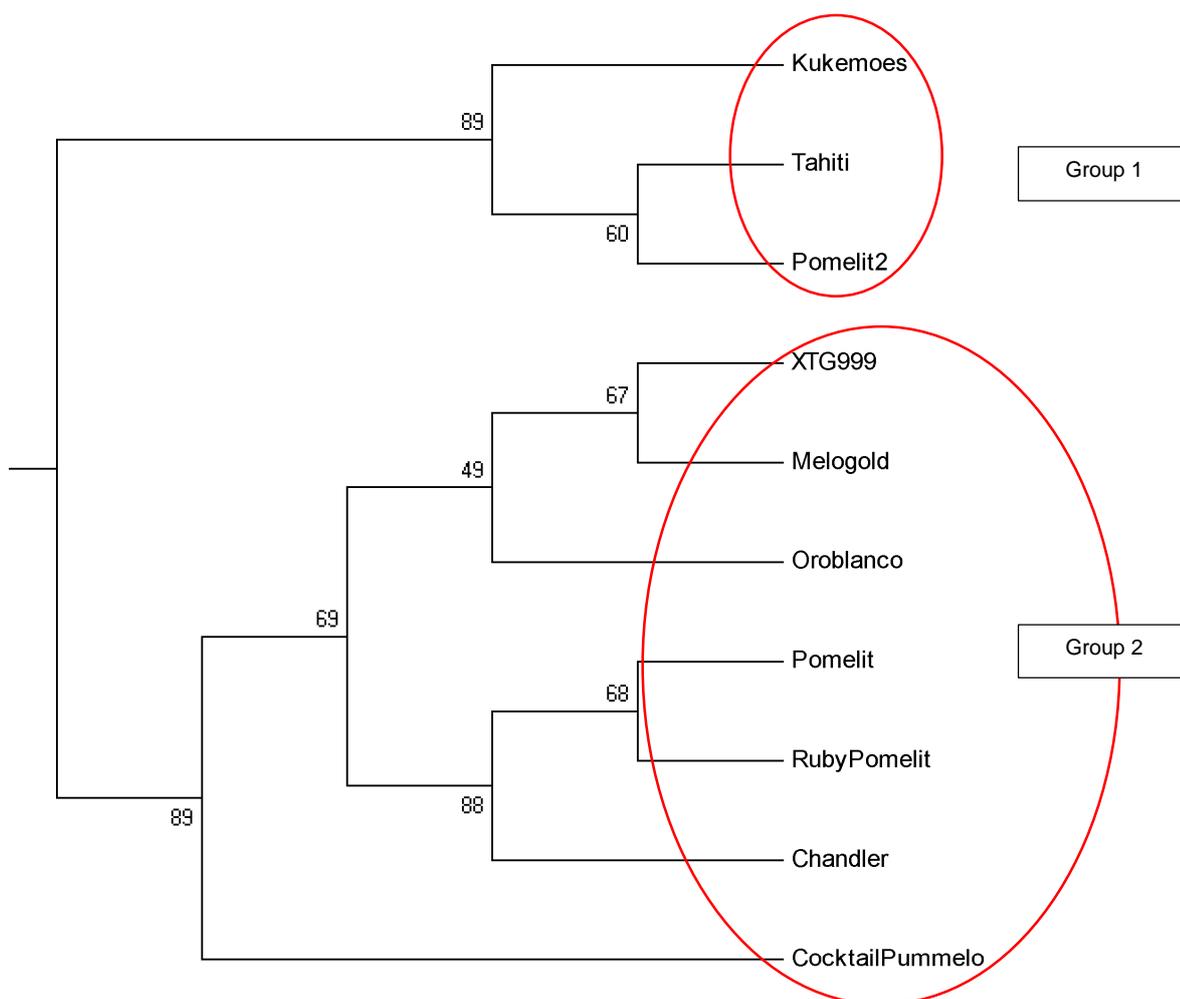


Figure 2: UPGMA dendrogram showing relationships between pummelos (CCC= 0.999, Pearson).

Rootstocks

The dendrogram illustrating the relationships between the tested rootstocks is shown in Figure 3. From this analysis, Macrophylla was found to be the most genetically distinct rootstock as it occurred in isolation on the dendrogram. Two major groups were evident from the cluster analysis. The first major grouping contained most of the trifoliolate hybrids with a sour orange hybrid being the most genetically distinct within that group. A clustering of citranges were apparent within group 1, which were genetically very similar. Citranges are hybrids of a sweet orange and a trifoliolate orange. It was observed that the first group was made up predominantly of sweet orange-type trifoliolate hybrids. Some exceptions were noted for example this group contained Swingle Citrumelo, which is a hybrid of Duncan grapefruit and *Poncirus trifoliata*. Close examination of the DNA fingerprints indicated that the Carrizo and Troyer citranges were indistinguishable using the tested markers. These were also reported to be genetically identical using Inter Simple Sequence Repeat (ISSR) markers (Fang and Roose 1997). It has been mentioned that Carrizo and Troyer may have originated from the same original selection (Savage and Gardner, 1965) as hybrids of Washington navel and *P. trifoliata*. They are also reported to be morphologically similar. The hybrid Poorman x Trifoliolate occurred on one end of the dendrogram in the vicinity of 530ARuby Grapefruit. This result is not surprising, as Poorman has been described as resembling a grapefruit (Hodgson, 1967). Also, the hybrid Sunki x Beneke occurred at the bottom end of group 1 close to the mandarin rootstocks in the next group. This is not unexpected as this is a hybrid of a mandarin and trifoliolate orange. In the current study, Rubidoux x Trifoliolate and Flying Dragon were found to be genetically

similar. Cristofani-Yaly *et al.* (2011) and Garcia-Lor *et al.* (2015) also found a high degree of genetic similarity between Rubidoux Trifoliolate and Flying Dragon using SSR markers and DNA sequencing, respectively.

The second major grouping was comprised of lemon-type hybrids with a clustering of three mandarin-type rootstocks (Cleopatra Mandarin, Sun Chu Sha and Changsa Mandarin) closest to group 1 (the sweet orange trifoliolate hybrids). Volkameriana was found to group with the rough lemons and both are known to display similar characteristics including general tree growth habit, tree vigour, foliar and other anatomical features (Lee *et al.*, 2009). Similar results were reported by El-Mouei *et al.* (2011) using Random Amplified Polymorphic DNA (RAPD) markers. Rangpur lime also occurred in close association with the Rough Lemon rootstocks and in the same subgroup as Volkameriana as similarly reported by El-Mouei *et al.* (2011). Lee *et al.* (2009) used vigour as a basis for categorisation of rootstocks. According to this criteria, the vigorous rootstocks are Rough Lemon, Volkameriana, Rangpur and Cleopatra mandarin. The intermediate rootstocks are the Carrizo and Troyer citranges, Swingle citrumelo and X639. The non-vigorous rootstocks are the trifoliolate orange selections such as Australian and Rubidoux trifoliolate. When this categorisation is compared with the results in the present study, it is apparent that the vigorous rootstocks occurred in group 2 on the dendrogram, the intermediate ones in group 1 and the non-vigorous ones at the basal end of the dendrogram. Lee *et al.* (2009) noted that although Cleopatra mandarin grouped with the vigorous rootstocks, it imparts fruit characteristics that are more similar to the intermediate rootstocks. In fact, in the present study, Cleopatra mandarin was the one rootstock from the vigorous category that was located closest to the group of non-vigorous rootstocks.

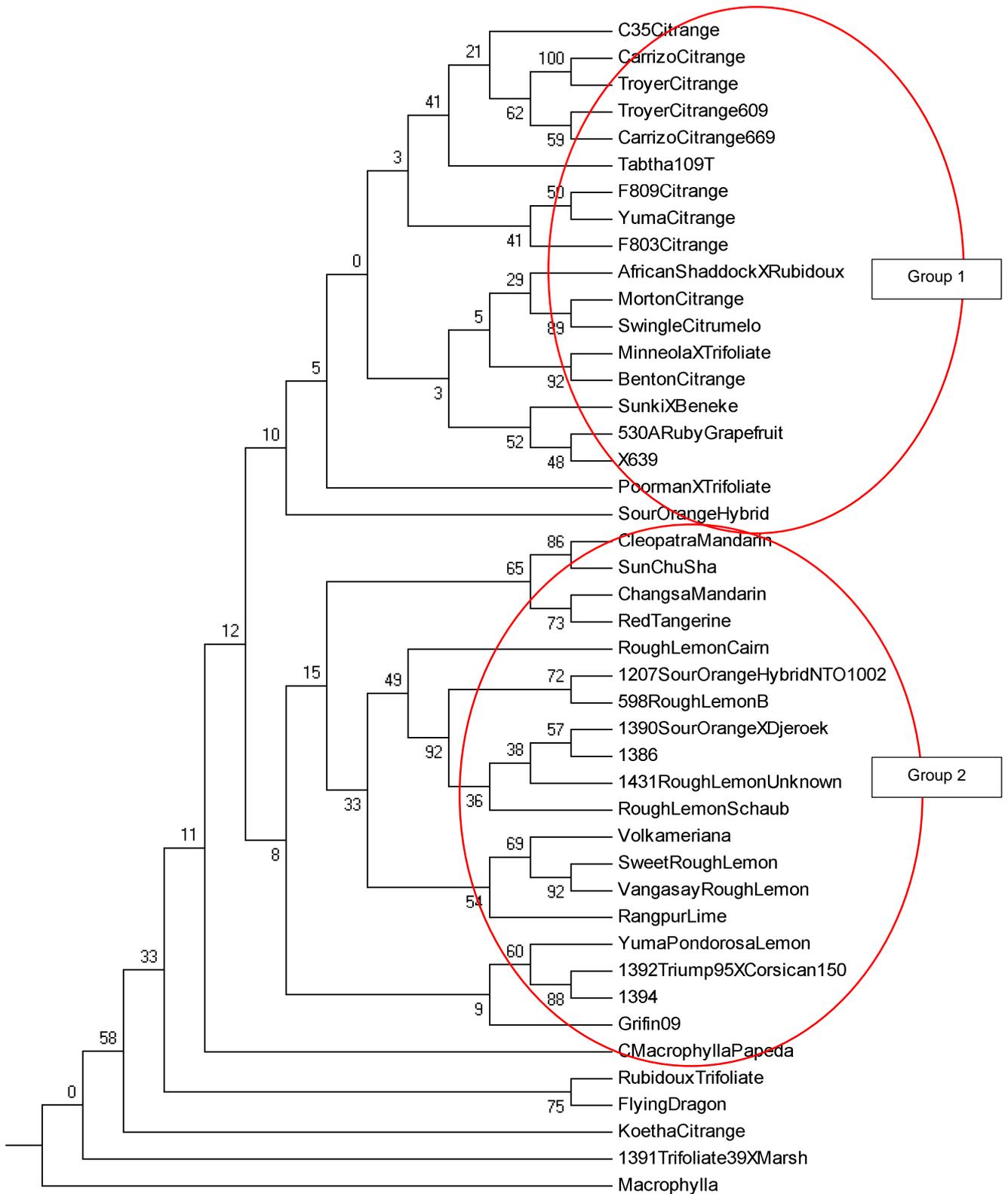


Figure 3: UPGMA dendrogram showing relationships between rootstocks (CCC = 0.983, Pearson).

Diverse selections

The diverse selections were included in the current investigation as this ensured that all groups within the original genotype reference database were verified except for the oranges. The diverse group consists of a range of citrus relatives and non-commercial/ornamental cultivars. The UPGMA dendrogram generated with

this group is shown in Figure 4. From the cluster analysis, two general groups are apparent. One is comprised of Nagami Kumquat, Fingered Citron and Macrophylla and the other group contains all the other cultivars. It was noted that the Nagami and Meiwa kumquats occurred in different groups. This is because differences in genetic profiles between the two were found for a number of primers, viz. CiM, Cil, TAA15, mCI and mCE. Shahzadi *et al.* (2014) also represented these two kumquats in separate groups when using SSR markers. In the past, the abovementioned kumquats have been classified as separate species, i.e. *Fortunella margarita* for Nagami and *F. crassifolia* for Meiwa (discussed by Shahzadi *et al.*, 2014).

The two Calamondins (Calamondin Green and Calamondin Variegated) were found to be genetically similar to each other and they were located in a group between the Nagami and Meiwa kumquats. This is not unexpected as the Calamondins are kumquat hybrids. It was also noted that although the papedas did not occur within the same group, they did occur in adjacent groups in close proximity. In a similar manner, Federici *et al.* (1998) also noted that the papedas are a very diverse group and that the tested papedas in that study did not cluster together when Restriction Fragment Length Polymorphism (RFLP) markers were tested. The subgroup at the top of the dendrogram was comprised of sour orange and lemon hybrids, a lime and kumquat. The two Bergamots from Addo and Messina displayed a high degree of genetic similarity.

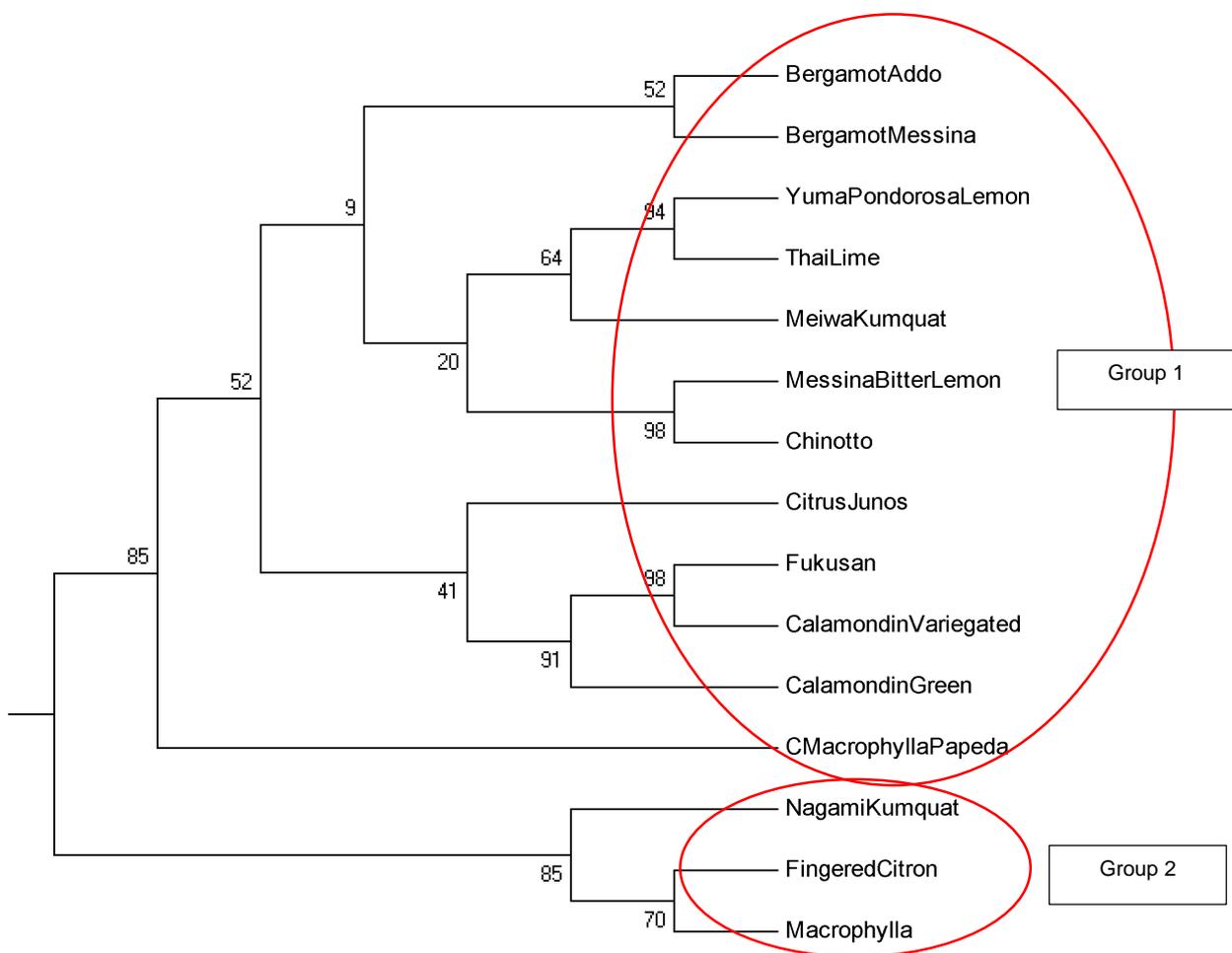


Figure 4: UPGMA dendrogram showing relationships between cultivars in the diverse group (CCC = 0.993, Pearson).

All cultivars combined

In the final analysis, all of the tested cultivars and selections were put together on a single dendrogram (Figure 5) in order to determine if the tested markers could separate the different citrus groups from each other. From the cluster analysis, it could be seen that the tested markers could separate the cultivars into the different groups with a few exceptions. The first cluster at the top of the dendrogram contained all of the pummelos except for Cocktail Pummelo. The adjacent cluster contained the grapefruits with Meiwa kumquat, Triumph95 x Corsican and 1394. As expected, the diverse selections occurred across a few groups, with most of them

occurring in a group adjacent to the grapefruit while a few grouped with the pummelos and rootstocks. Within the rootstocks, most of the citranges grouped together as did the rough lemon and mandarin types. Snoussi *et al.* (2012) also reported separation into varietal groups when 20 SSR markers were tested on rootstocks in Tunisia. Similar findings were reported by Barkley *et al.* (2006) and Baig *et al.* (2009) using SSR and RAPD markers, respectively.

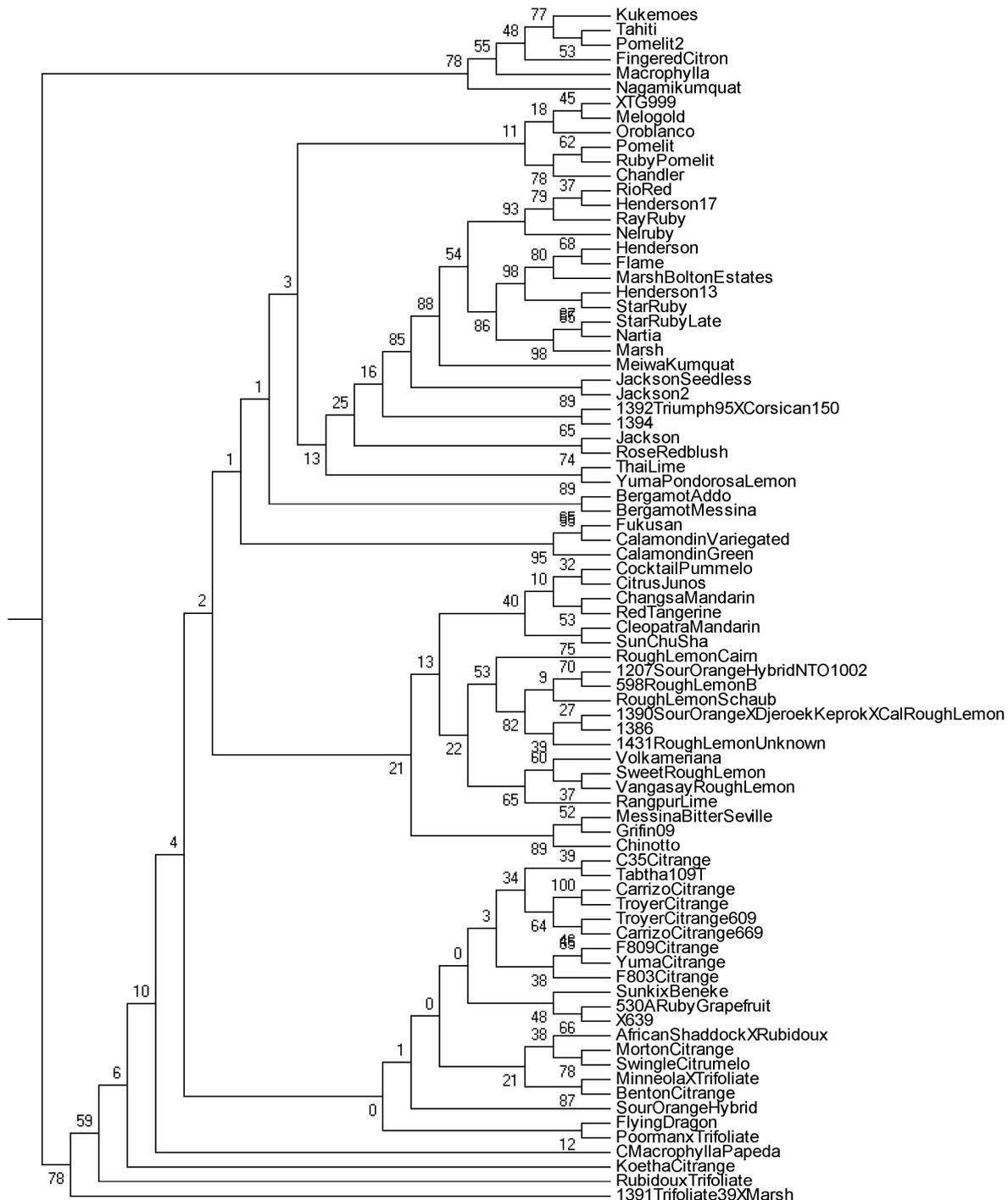


Figure 5: UPGMA dendrogram showing relationships between grapefruit, pummelos, rootstocks and diverse selections (CCC = 0.989, Pearson).

Conclusion

In the present study, a range of SSR markers were tested on grapefruits, pummelos, rootstocks and diverse selections. The tested SSR markers could distinguish between cultivars in the different citrus groups, however,

closely related selections within the same group could not always be distinguished. This study allowed for discrepancies in the previous database to be corrected and determined the limit of resolution of the tested markers. It is recommended that should the verified database be required for the purpose of cultivar identification; each request should be evaluated on a case by case basis to establish if the tested markers are suitable to distinguish any differences

Future research

The only group remaining which has not been verified are the oranges. However, considering the low genetic variability within this group, it is suggested that additional molecular tools be investigated for genotyping.

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5 CITRUS IMPROVEMENT SCHEME (CIS)

P.H. Fourie, J.B. Meyer, M.M.N. du Toit, M. le Roux, M. Ferreira, L. Olivier, J.H.J. Breytenbach, C Steyn and G. Cook (CRI)

Summary

The South African Citrus Improvement Scheme (CIS) strives to ensure a profitable citrus industry that is established with high quality citrus trees that are free from diseases and horticulturally true to type. Certified rootstock seed and budwood are supplied from the Citrus Foundation Block (CFB) outside Uitenhage. Following a record budwood supply year in 2016, which was dominated by lemon and mandarin supply, certified budwood supply in 2017/18 again exceeded previous records. A total of 7.15 million buds were supplied by the CFB or authorized for cutting in certified nurseries. Lemon demand declined from 33.1% to 14.8%, whilst mandarin supply increased from 37.5% to 43.6%, and Valencia from 10.1% to 18.3%. For the latter, 'Midknight' Valencia demand rose from 381 thousand to 700 thousand buds. A huge increase in 'Star Ruby' demand was also experienced, from 106 thousand to almost 300 thousand buds. Amidst the rise in budwood and rootstock seed demands, CFB's ability for primary supply in 2017/18 improved slightly from 66.5% in 2016/17 to 67.8% in 2017/18. Budwood stock of the 384 cultivars at CFB must be constantly managed to meet demand of sought-after varieties. In 2017/18, 25-thousand new multiplication trees were produced, 9-thousand redundant trees were removed, and plans are afoot to expand rootstock seed production by planting another 2.5 ha.

Opsomming

Die doelwit van die Suid-Afrikaanse Sitrus Verbeteringskema (SVS) is om die winsgewendheid van die suider-Afrikaanse sitrusbedryf te verbeter deur te verseker dat die industrie gevestig word met hoë kwaliteit,

siektevrige kwekerybome wat tuinboukundig tipe-eg is. Gesertifiseerde okuleerhout en saad word voorsien vanaf die in Sitrus Grondvesblok buite Uitenhage. Die rekord jaar in 2016, wat hoofsaaklik oorheers is deur aanvraag na suurlemoene en manderyne, is in 2017/18 oortref. 'n Totaal van 7.15 miljoen ogies is deur die Grondvesblok in samewerking met gesertifiseerde kwekerye verskaf. Die aanvraag na suurlemoene het van 33.1% tot 14.8% gedaal terwyl manderyne van 37.5% tot 43.6%, en Valencias van 10.1% tot 18.3%, gestyg het. Die vraag na 'Midnight' Valencia het van 381- tot 700-duisend ogies gestyg. Daar was ook 'n groot aanvraag na 'Star Ruby' waarvan die verskaffing van 106- tot byna 300-duisend ogies gestyg het. Te midde van die verhoogde aanvraag na okuleerhout en saad het die Grondvesblok die primêre verskaffing van 66.5% in 2016/17 tot 67.8% in 2017/18 verbeter. Okuleerhout voorraad van die 384 kultivars moet konstant bestuur word om soveel as moontlik van die hoë aanvraag kultivars te kan verskaf: in 2017/18 is 25-duisend nuwe vermeerderingsbome gemaak, 9-duisend onnodige bome verwyder en die vestiging van nog 2.5 ha onderstam boorde word beplan.

5.1 Introduction

The purpose of the CIS is to enhance the standard of the South African citrus industry by ensuring that only horticulturally superior plants, which are free of viruses, diseases and pests, are supplied to growers and certified. The Citrus Growers Association of southern Africa (CGA) is responsible for the CIS and delegated its authority to CRI. In order to achieve this objective, close co-operation is required between CRI, the Agricultural Research Council's Tropical and Subtropical Crops institute (ARC-TSC), DAFF's Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities. The organisations and committees, as well as all participating role players in the CIS are represented on the CIS Advisory Committee (CISAC), which advises CRI on the CIS operations as specified in its Procedural Guide.

The phytosanitary status of the CIS is ensured by virus-elimination and diagnostic services prior to CIS introduction and was again confirmed through routine re-indexing of mother trees as well as multiplication blocks.

5.2 Budwood

This report summarises the seasonal supply of budwood from 1 July 2017 to 30 June 2018. A record number of 7.15 million buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries (BCIN). This is 32.8% more buds than in the same period of 2015/16 and 6.0% more buds than in the same period of 2016/17. During this period 24 355 buds were exported to neighbouring countries.

Budwood demand generally increased in volume and was mostly from Limpopo (34.8%), Western Cape (29.7%), followed by the Eastern Cape (20.2%), Mpumalanga (6.4%) and the other provinces ranging from 3.9% to 0.7%.

Mandarin (43.6%) was the most popular citrus type, followed by Valencia (18.3%), lemon (14.8%), navel (11.1%), Clementine (6.4%) and grapefruit (4.7%); in 2016/17 this proportion was 37.5%, 10.1%, 33.1%, 6.8%, 8.5% and 2.1%, respectively (Tables 5.2.1 and 5.2.2). Valencia demand was stable in recent years with a 3-year average of 560 thousand buds. In the past season, we experienced an unexpected increase and 1.3 million buds were supplied in 2017/18, consequently surpassing the lemon demand. Whilst supply of lemon budwood decreased by 52% from 2016/17, it was still significant at 1.06 million buds supplied during 2017/18. Navel demand also increased by 72.6% to 793 thousand buds. In previous seasons, a huge increase in Clementine bud demand was observed (219.8%, 19.1% and 81.4% in 2014/15; 2015/16 and 2016/17, respectively), but a slight decrease of 19.8% from 2016/17 levels was observed in 2017/18 to a total of 459 thousand buds. The Clementine demand is still significant considering that the 10-year average in 2013/14 was 62 thousand buds. Grapefruit demand has been low for a number of years and increased from a low base of 45-, 77-, and 144 thousand buds in 2014/15, 2015/16 and 2016/17, respectively, to 336 thousand buds supplied in 2017/18. Demand for Star Ruby exceeded the availability of stock from CFB and BCIN. This increase of 136.2% is significant, but recent supply figures are still low when compared to the 10 year average of 535 thousand buds during the 1990's.

The top 30 varieties comprised 91.2% of total number of buds supplied. ARC Nadorcott LS (ARCCIT9) was the most popular cultivar, followed by Eureka, Midnight, Leanri, Or 4 and Nules (Table 5.1.3). ARC Nadorcott LS supply levels have increased from 29 160 to 267 422 to 513 582 to 1 080 328, and Leanri increased from 3 030 to 67 446 to 592 633 to 811 659 in 2014/15, 2015/16, 2016/17 and 2017/18, respectively. These two cultivars contributed 22% to the total budwood supply and the majority of these buds were BCIN supplied in 2017/18 (75.3% and 65.3%, respectively).

In general, the need for authorised cutting in nurseries has decreased from 40.0% in 2012/13 to 32.2% in 2017/18 (Figure 5.2.1), which is a 1.3% improvement on the previous season. BCIN proportion per cultivar type: mandarins (61.6% of which ARC Nadorcott LS (ARCCIT9) Leanri comprised 50% and Or4 a further 5%), Valencias (18.6% of which Midnight comprised 15%), Clementine (8.1% of which Nules comprised 7%), grapefruit (6.2% of which Star Ruby comprised 6%), navel (5.1%) and other (0.4%) (Figures 5.2.2-7).

Table 5.2.1. Buds supplied during the period July to June 2015/16-2017/18.

Area	2015/16	2016/17	2017/18
Local	4 801 933	6 719 186	7 131 525
Eastern Cape	1 075 103	1 535 698	1 448 102
Gauteng		96 400	120 267
KwaZulu Natal	37 530	101 300	47 600
Limpopo	1 168 611	1 899 713	2 491 852
Mpumalanga	294 165	267 210	460 315
North West Province	119 600	99 280	164 000
Northern Cape	333 100	488 866	275 950
Western Cape	1 773 824	2 230 719	2 123 439
International	9 490	6 400	24 355
Botswana		6 000	11 000
Congo	8 490		
Mozambique			3 965
Netherlands		400	
Nigeria			4 000
USA			40
Zimbabwe	1 000		5 350
Total	4 811 423	6 725 586	7 155 880

Table 5.2.2. Buds supplied during the period July to June 2015/16-2017/18.

Area	2015/16	Dist %	2016/17	Dist %	2017/18	Dist %
Local	4 801 933	99.8%	6719186	99.9%	7131525	99.7%
Eastern Cape	1 075 103	22.3%	1535698	22.8%	1448102	20.2%
Gauteng		0.0%	96400	1.4%	120267	1.7%
KwaZulu Natal	37 530	0.8%	101300	1.5%	47600	0.7%
Limpopo	1 168 611	24.3%	1899713	28.2%	2491852	34.8%
Mpumalanga	294 165	6.1%	267210	4.0%	460315	6.4%
North West Province	119 600	2.5%	99280	1.5%	164000	2.3%
Northern Cape	333 100	6.9%	488866	7.3%	275950	3.9%
Western Cape	1 773 824	36.9%	2230719	33.2%	2123439	29.7%
International	9 490	0.2%	6400	0.1%	24355	0.3%
Total	4 811 423	100.00%	6725586	100.0%	7155880	100.0%

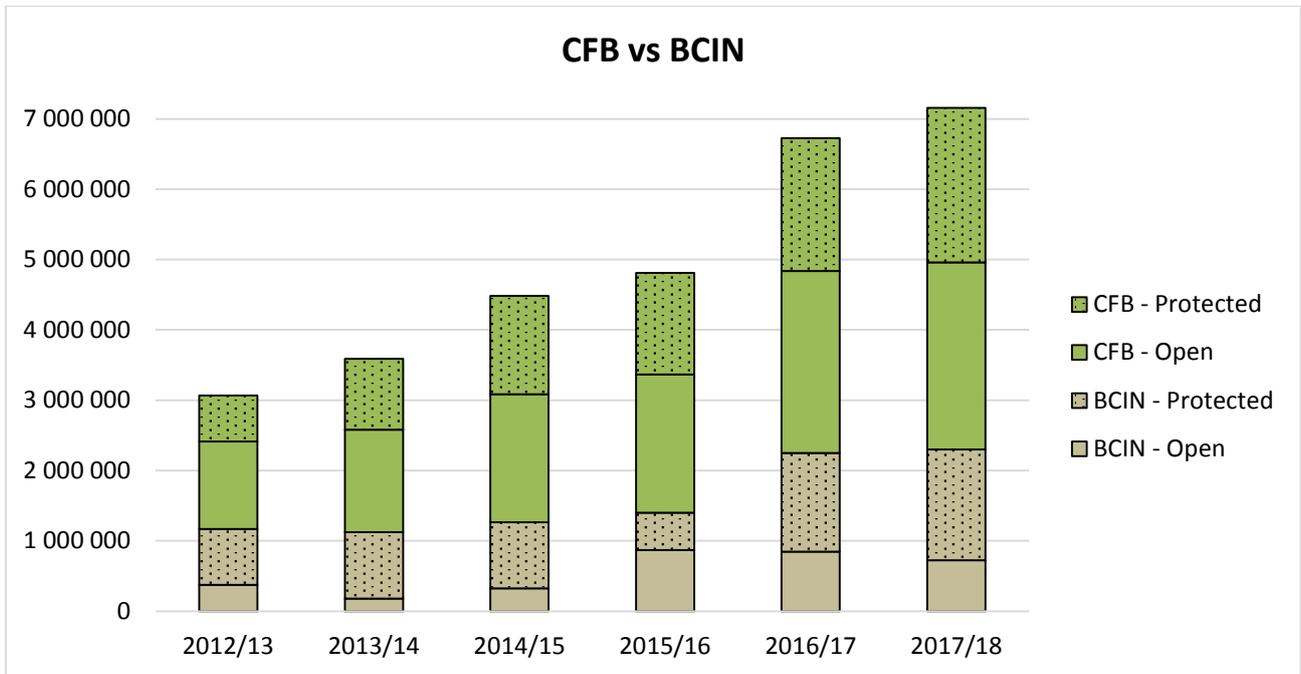


Figure 5.2.1. Budwood of open and protected cultivars (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2014/15-2017/18.

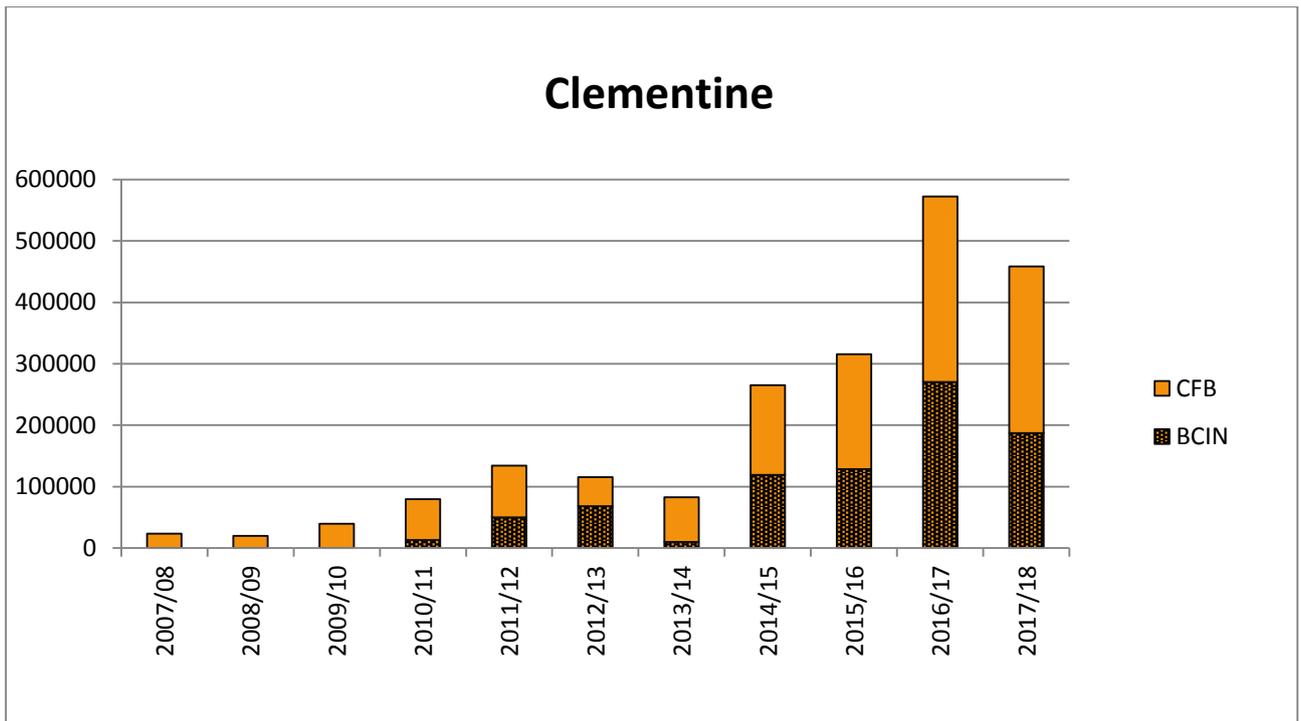


Figure 5.2.2. Clementine budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

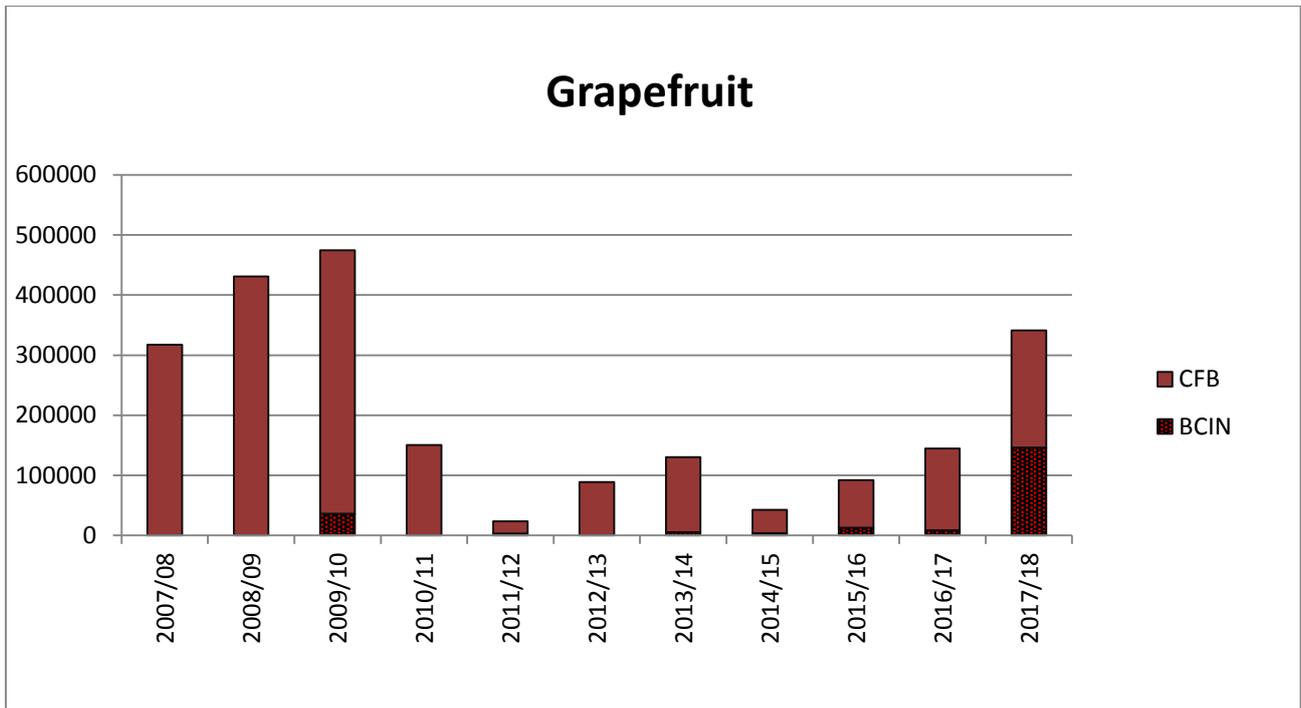


Figure 5.2.3. Grapefruit budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

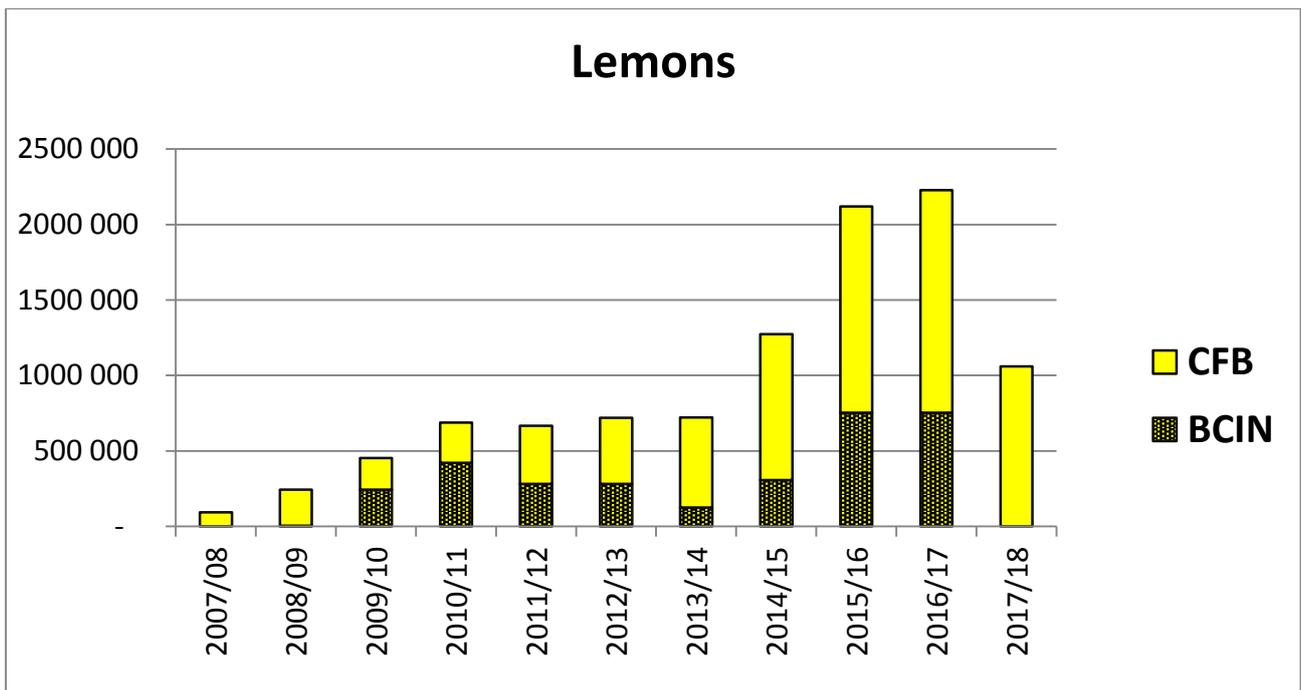


Figure 5.2.4. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

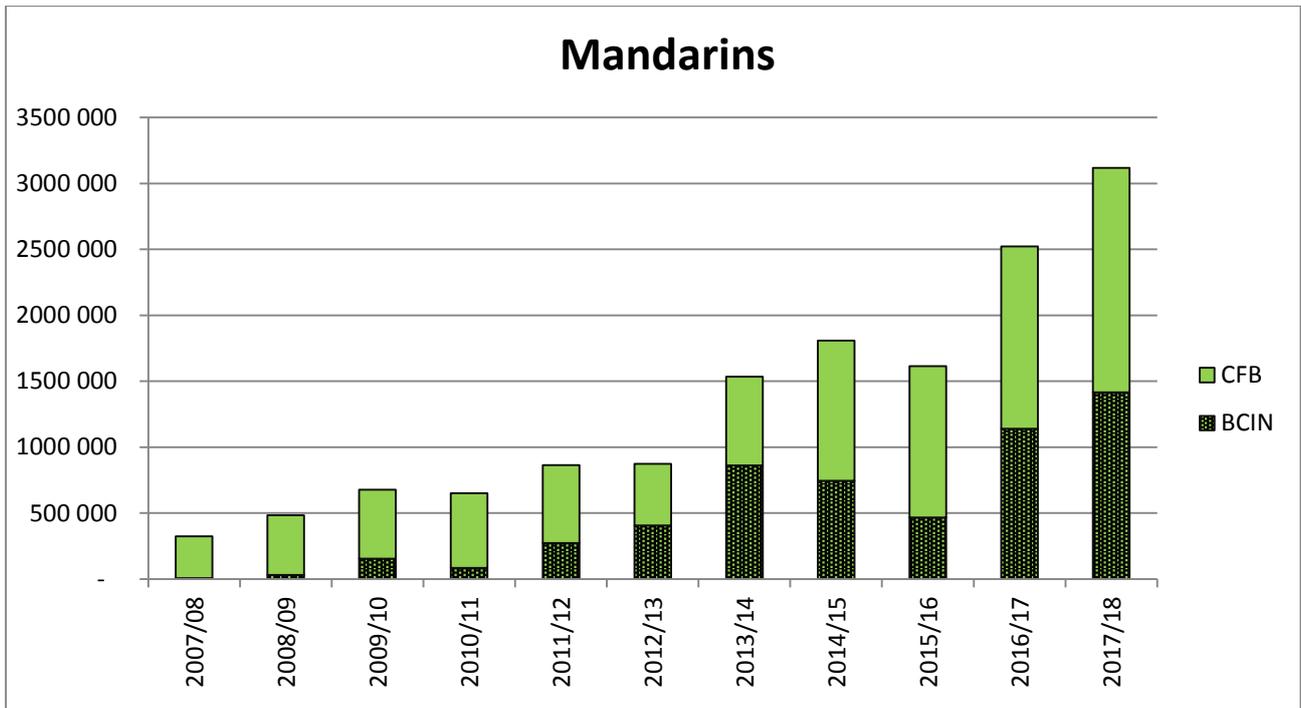


Figure 5.2.5. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

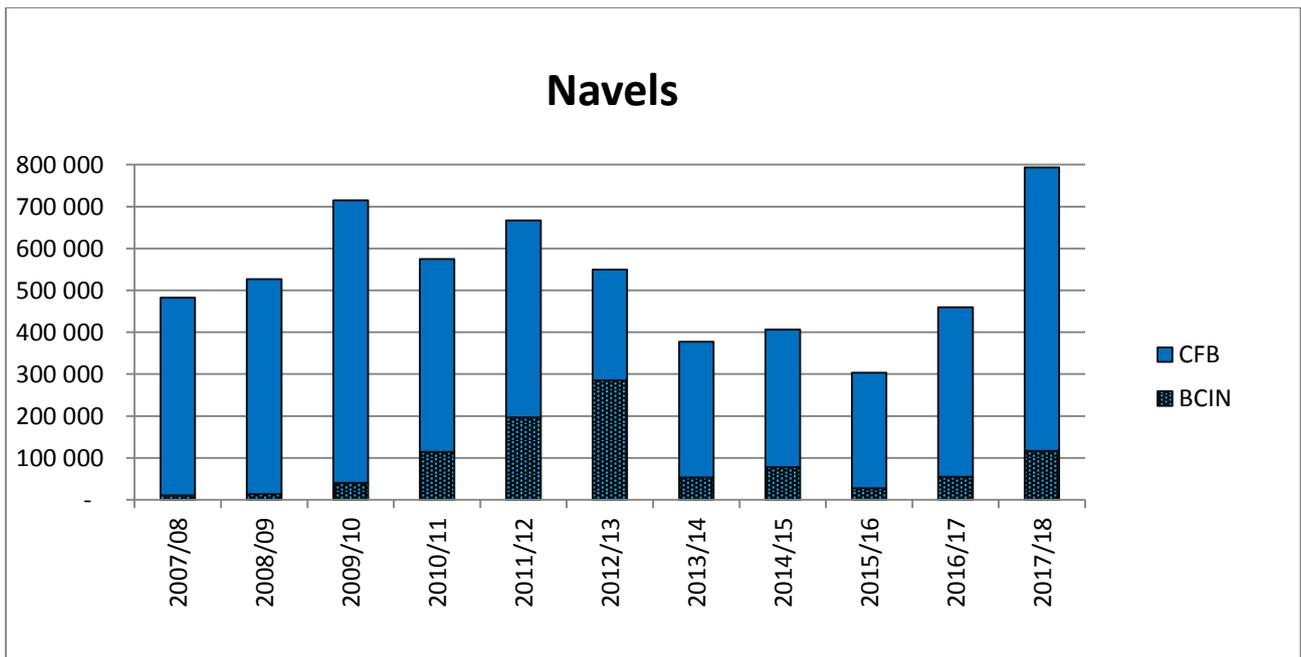


Figure 5.2.6. Navel budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

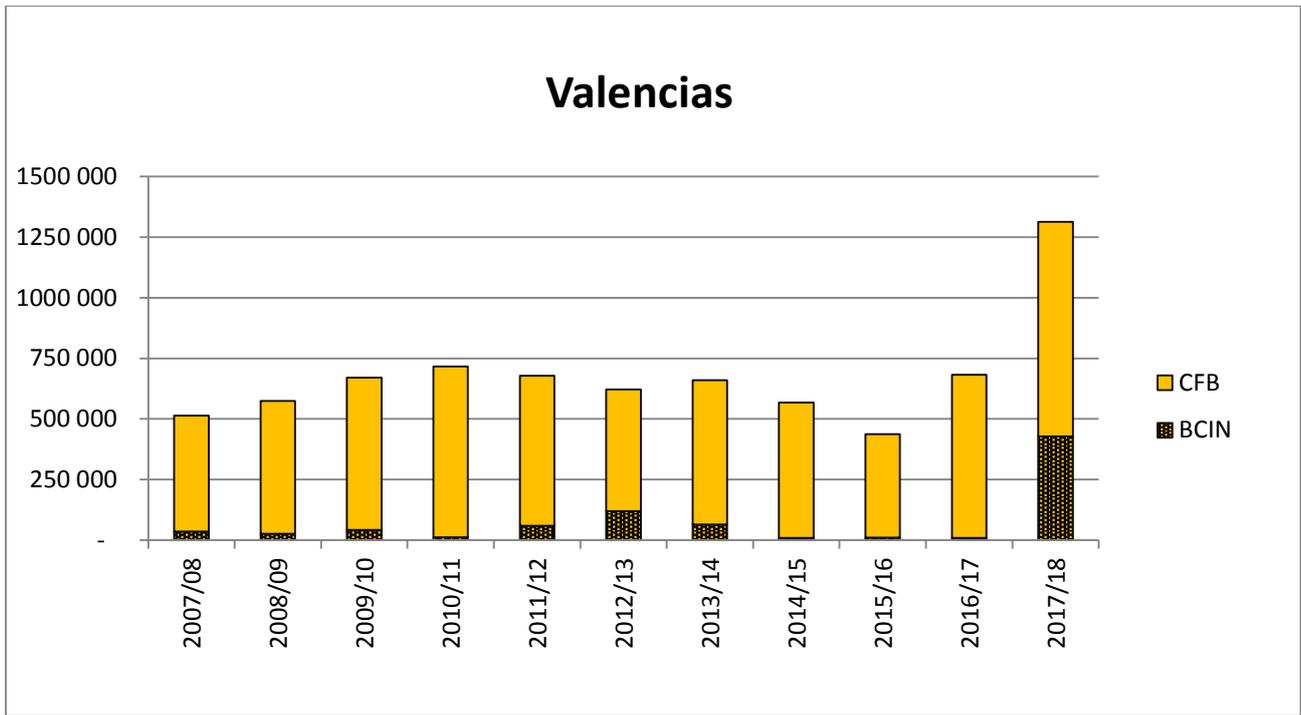


Figure 5.2.7. Valencia budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

Table 5.2.2. Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2015/16 – 2017/18.

Variety Type	Year	EC	GP	KZN	LIM	MPU	NWP	NC	WC	Local	International	Total
Clementine	2015/16	56 601			28 920	1 010	150	26 200	210 866	323 747	1 100	324 847
	2016/17	87 133	5 250		34 420	580	2 450	44 400	397 920	572 153		572 153
	2017/18	89 782	22 000		45 475	11 080	2 000	4 200	283 680	458 217	500	458 717
Diverse	2015/16	110			6 480	4 300	7 000		2 640	20 530	240	20 770
	2016/17	60		1 000	1 030	2 400	3 000		8 140	15 630	200	15 830
	2017/18		1 087		200		200		620	2 107		2 107
Grapefruit	2015/16	660		3 000	33 930	37 250			2 092	76 932	500	77 432
	2016/17	15 025	15 500	5 500	65 943	39 815			2 645	144 428		144 428
	2017/18	22 150	9 000	6 200	141 060	115 800	5 800	14 200	21 240	335 450	300	335 750
Kumquat	2015/16								200	200		200
	2016/17	70				1 020			2 950	4 040		4 040
	2017/18		880			1 200	2 000		4 220	8 300		8 300
Lemon	2015/16	673 981		16 000	490 566	118 335	59 000	181 000	500 305	2 039 187	1 500	2 040 687
	2016/17	778 248	100	44 000	571 694	89 750	22 700	202 000	520 621	2 229 113	200	2 229 313
	2017/18	189 239	22 500	18 400	515 050	68 265	40 700	35 800	166 949	1 056 903	3 000	1 059 903
Lime	2015/16	2 150		1 500	1 000	2 500			5 683	12 833	100	12 933
	2016/17	520		1 500	250	3 750			7 315	13 335		13 335
	2017/18	3 830	1 870		3 950	7 540	1 000	1 000	10 640	29 830		29 830
Mandarin	2015/16	226 126		2 500	230 270	64 630	17 300	93 100	912 201	1 546 127	750	1 546 877
	2016/17	388 259			903 871	85 710	30 580	213 466	902 562	2 524 448	1 000	2 525 448
	2017/18	471 630	2 105	3 000	1 285 141	152 825	33 190	125 550	1 041 127	3 114 568	3 650	3 118 218
Midseason	2015/16					15			3 368	3 383		3 383
	2016/17	50							8 794	8 844		8 844
	2017/18					60		600	820	1 480		1 480
Navel	2015/16	74 288		8 500	105 845	29 225	29 650	6 500	46 180	300 188	3 900	304 088
	2016/17	141 022	37 050	8 500	75 805	13 455	20 650	17 800	143 441	457 723	2 000	459 723
	2017/18	287 817	9 800	4 500	123 180	39 575	32 260	33 700	251 776	782 608	10 640	793 248
Pummelo	2015/16				-2640	15				-2 625		-2 625
	2017/18	970			4 350					5 320		5 320
Rootstock	2015/16					190				190		190
	2016/17	150							3 000	3 150		3 150
	2017/18	100				100				200		200
Satsuma	2015/16	6 852		3 000	800	700	500	1 400	34 976	48 228	1 400	49 628

	2016/17	12 234			10 250	1 155	500	4 700	37 744	66 583	1 000	67 583
	2017/18	19 475		3 000	2 000	35			5 330	29 840		29 840
Valencia	2015/16	34 335		3 030	273 440	35 995	6 000	24 900	55 313	433 013		433 013
	2016/17	112 927	38 500	40 800	236 450	29 575	19 400	6 500	195 587	679 739	2 000	681 739
	2017/18	363 109	51 025	12 500	371 446	63 835	46 850	60 900	337 037	1 306 702	6 265	1 312 967

Table 5.1.3. Top 30 cultivars based on total number of buds supplied for seasons July to June from 2015/16 – 2017/18.

	2015/16			2016/17			2017/18		
	Cultivar	BCIN	CFB	Cultivar	BCIN	CFB	Cultivar	BCIN	CFB
1	Eureka LEM	660 875	747 801	Eureka LEM	499 227	936 185	ARC Nadorcott LS MAN**	813 829	266 499
2	Tango MAN	131 000	214 930	Leanri MAN	509 643	82 990	Eureka LEM		814 031
3	Lisbon LEM	55 325	217 376	ARC Nadorcott LS MAN **	394 532	119 050	Midnight VAL	343 749	356 781
4	ARC Nadorcott LS MAN **	225 555	41 827	2PH Eureka SL LEM	250 755	138 482	Leanri MAN	334 150	177 509
5	Nadorcott 1 MAN	8 500	199 954	Midnight VAL	5 000	376 247	Or 4 MAN	108 360	266 852
6	Midnight VAL	10 000	196 662	Nules CLE	229 281	149 535	Nules CLE	157 661	209 063
7	Nules CLE	93 422	108 303	Nadorcott 1 MAN	26 000	278 555	Tango MAN	50 000	303 580
8	Or 4 MAN	36 600	126 541	Or 4 MAN	89 610	191 596	Nova MAN	37 300	294 375
9	Valley Gold MAN*		157 955	Nova MAN		266 245	Star Ruby GFT	136 550	162 610
10	2PH Eureka SL LEM	24 500	127 070	Lisbon LEM		209 289	Witkrans 3 NAV	40 950	156 240
11	Nova MAN		146 576	Tango MAN	26 791	160 320	Cambria 3 NAV	26 875	131 252
12	Limoneira 8A LEM	8 200	134 755	Andes 1 – Clemenluz CLE	20 244	127 320	Nadorcott 1 MAN	17 000	140 700
13	Witkrans 3 NAV	9 600	111 565	Cambria 3 NAV	9 200	133 291	Bennie 2 VAL		110 148
14	Andes 1 Clemenluz CLE	21 600	75 138	Valley Gold MAN*		109 484	Washington NAV		96 270
15	Late VAL		75 210	Star Ruby GFT	1 100	105 058	Turkey VAL	53 600	38 400
16	Leanri MAN	43 100	24 366	Witkrans 3 NAV		101 336	Late VAL		90 681
17	Genoa LEM	3 900	58 158	Limoneira 8A LEM		99 219	Bahianinha NAV		75 980
18	Gold Nugget MAN	4 100	43 904	IR M2 MAN	58 610	21 309	Delta VAL		75 203
19	Delta VAL		40 081	Cara Cara NAV	45 502	28 680	RHM MAN	20 000	52 359
20	Cara Cara NAV	13 800	25 615	Genoa LEM	4 300	68 281	Lisbon LEM		69 065
21	Cambria 3 NAV		29 418	Late VAL		67 365	2PH Eureka SL LEM		67 700
22	Mor 26 MAN		28 596	Queen MAN		64 240	Limoneira 8A LEM		63 470
23	Turkey VAL		28 415	Bennie 2 VAL		55 650	Cara Cara NAV	19 030	44 151
24	Star Ruby GFT		27 825	Turkey VAL		46 612	IR M2 MAN	18 300	33 899
25	FE 1 (Jackson 1) GFT		27 715	Tambor MAN	33 116	5 130	Gusocora (G5) VAL	2 120	49 635
26	Belabela SAT		26 360	Lavalle VAL		36 870	Rosalina NAV	23 100	23 330
27	Tanorlate MAN	700	23 940	Belabela SAT	7 415	27 779	Alpha VAL		39 520

28	Palmer NAV		24 276	Esbal CLE	20 700	8 362	Queen MAN		39 010
29	Bennie 2 VAL		21 925	Mor 26 MAN		27 248	Fischer NAV		37 801
30	Queen MAN		20 362	Washington NAV		27 006	Du Roi 2 VAL		37 780
	Top 30	1 350 777	3 132 619	Top 30	2 231 026	4 068 734	Top 30	2 202 574	4 323 894
	> Top	51 031	276 996	> Top	20 500	405 326	> Top	98 508	530 899
	Total	1 401 808	3 409 615	Total	2 251 526	4 474 060	Total	2 301 082	4 854 793
	%	29.1%	70.9%	%	33.5%	66.5%	%	32.2%	67.8%

* ARCCIT1614 (B17) (Valley Gold) MAN ** ARCCIT9 (ARC Nadorcott LS) MAN

5.3 Seed

During May 2017 to April 2018, 6735 litres of seed were supplied locally (Table 5.3.1) and 69 litres of seed were exported (Table 5.3.1). Carrizo Citrange remains the most popular rootstock (52.6%), followed by Swingle Citrumelo (13.7%), C35 Citrange (12.0%), Troyer citrange (6.1%), X639 (4.8%) and Rough Lemon (4.4%). The supply did not meet the extremely high demand for Eureka compatible rootstocks for a number of years (Table 5.3.1) and in 2015/16 and 2016/17 CRI imported Rough Lemon (RL) and Volckameriana (VA) on behalf of nurseries from USA. This seed was, however, defective and very poor germination was reported by nurseries (0% for VA and 19.3% for RL). In the order of 400 litres of seed were also imported directly by nurseries from USA (California) and Australia in 2016/17. We are not aware of any significant seed imports in 2017/18. The shift in demand towards mandarins, Valencias and navels has resulted in higher orders of trifoliate hybrid cultivars and the demand exceeded the supply in some cases (Figure 5.3.1).

Table 5.3.1. Seed (litres) supplied by the CFB during the periods May to April 2015/16 – 2017/18.

Area	2015/16	2016/17	2017/18
Local	4308	4749	6737
Eastern Cape	1229	613	958
Gauteng		22	131
KwaZulu Natal	36	27	70
Limpopo	1339	1559	2473
Mpumalanga	438	338	417
North West Province	91	179	179
Northern Cape	185	352	694
Western Cape	990	1660	1817
International	365	277	69
Botswana	2	2	4
Chile	105	201	
Congo	-13		
Egypt	9		
Mauritius			1
Mozambique	31	60	6
Portugal	213		
Reunion	10		
South America			12
Swaziland		7	32
Zambia	8	7	11
Zimbabwe			3
Total	4673	5026	6806

Table 5.3.2. Seed (litres) supplied by the CFB during the periods May to April 2015/16 – 2017/18.

Rootstock Cultivar	2015/16	2016/17	2017/18
CFB	3487	4887	6070
79AB		6	
79AC		6	0
BC	19	20	20
C35	561	644	808
CC	1403	2446	3105
CM			0
FD	213	25	
MXT	162	222	179
RL	334	483	282

SC	306	670	898
SXB	11	32	26
TC	13		347
VA	125	92	108
X639	339	227	280
YC		15	19
Imported	231	139	
SPIN*	955		736
Grand Total	4673	5026	6806

*Seed produced in nurseries

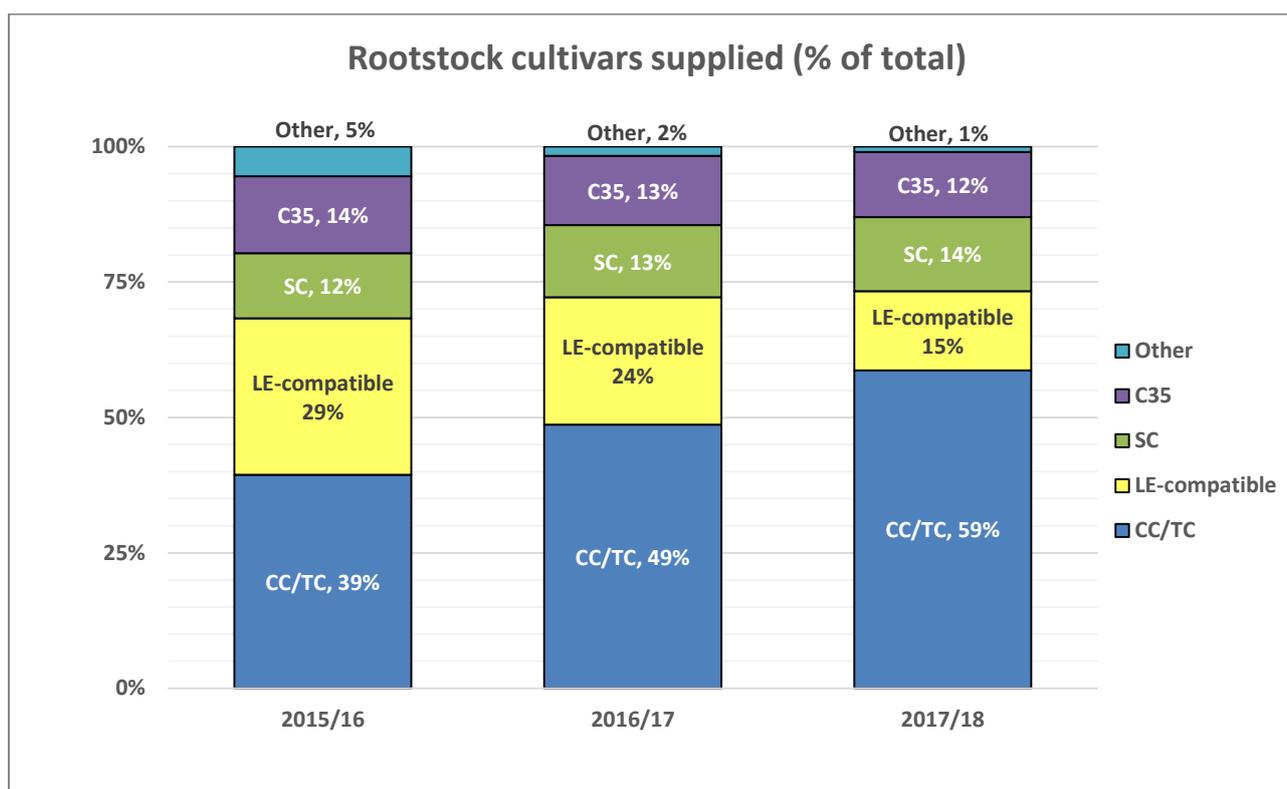


Figure 5.3.1. Rootstock cultivars grouped by type (percentage of total litres of seed per season) supplied by the CFB and seed produced by nurseries (SPIN) during the periods May to April from 2015/16-2017/18.

5.4 Production

Budwood: CFB presently maintains 135 290 multiplication trees of approximately 384 cultivar lines with a potential annual budwood stock of >10 million buds. The STG facilities in Nelspruit, CRI has released 7 new and 21 existing cultivars to the CFB. The ARC also introduced 14 new cultivars and re-introduced 15 existing cultivars to the CFB (Table 5.4.1). As the top 30 varieties comprise 91.2% of demand, multiplication tree stocks are being managed in order for CFB to be timeously able to supply demand of the sought-after varieties. During 2017/18 25 100 new multiplication trees were made in the rapid multiplication tunnels to address the shortage of high demand cultivars. Whilst CFB's multiplication system is addressing the budwood demand in the medium term (1-2 year lag phase), it was not sufficient to rapidly increase budwood stock to address short-term demand; for example, Nules Clementine, Leanri Mandarin and ARC Nadorcott LS Mandarin, for which the huge demand experienced in 2016/17, nearly doubled in 2017/18. CFB is investigating *in vitro* rapid multiplication systems as a potential solution, but concluded that the risk of inadvertently multiplying off-types outweighed the reward. To address space constraints, 9 thousand redundant multiplication trees and all the redundant mother trees in greenhouse 1 were removed.

Two trees of 311 cultivars were planted out during October 2016 in a newly established evaluation block at the CFB. These trees were successfully used for true-to-type evaluation in 2018. Newly released cultivars will be planted out during the following season.

Table 5.4.1. Cultivar introductions from 2014/15 – 2017/18.

Area	2014/15	2015/16	2016/17	2017/18
ARC: New introductions	17	15	7	14
ARC: Existing lines with new CTV Strain			7	15
CRI: New introductions	18		13	7
CRI: Reintroductions from the Nucleus Block			8	21
CFB: Re-multiplication of existing cultivar lines	13	58	35	84

Seed: In order to meet the growing demand for rootstock seed as well as a contingency and maintenance measure, the CFB seed source was expanded from 2420 trees by planting another 1362 trees in 2014/15. These trees were planted at high density and will significantly contribute to the supply in 2017/18 and 2018/19. Thereafter, every other tree will be removed or transplanted to new orchards. Another 318 rootstock trees of high demand and experimental cultivars were made and were planted in the spring of 2017. A reassessment will be made of CFB's seed orchards, particularly with regard the older orchards, more redundant cultivars, and also to make space for greenhouse expansion. Most of the previous orchard space will be prepared for new rootstock orchards in 2018/19; approximately 514 trees will be replanted and 729 new trees will be planted in these orchards. For older orchards replacement, topworking or heavy pruning options will be investigated.

5.5 Tree Certification

There were 3 827 466 trees certified during April to March 2017-18 (Table 5.5.1). A high number of applications were received and were processed. Of the applications received, the trees not meeting the certification requirement were 22 798, 72 753 and 69 886 for the last three consecutive years. This was mostly because of the Phytophthora status or tree age that exceeded 30 months after budding. The trees not meeting the certification requirements have decreased by 69% on the previous year (Table 5.5.2). Nurseries are required to apply for certification for all trees supplied to industry, and in future the percentage of trees certified as a proportion of the total number of buds received will be used as a nursery certification criterion.

Table 5.5.1. Trees certified during the period April to March from 2015/16 – 2017/18.

Variety Type	Year	EC	FS	GP	KZN	LIM	MP	NC	NW	WC	EXP	Total
Clementine	2015/16	18 626				10 215	8 280		1 700	56 739		95 560
	2016/17	40 579		1 688		12 160	10 470	3 163	1 700	56 233		125 993
	2017/18	68 354				14 900	12 677	1 238		137 085	550	234 804
Diverse	2015/16	1 355			200	7 640	1 441			200		10 836
	2016/17	1 000			1 140	6 650	1 600			1 886		12 276
	2017/18									1 700		1 700
Grapefruit	2015/16	2 300		12 735		28 134	18 168					61 337
	2016/17			6 762	5 000	15 581	9 850		1 500		1 000	39 693
	2017/18			11 948	9 000	50 758	8 690		400	1 600		82 396
Lemon	2015/16	296 675	1400	8 000	6 440	120 178	137 139		3 560	126 896		700 288
	2016/17	448 267			16 258	202 957	182 780		8 800	249 924	7 300	1 116 286
	2017/18	509 024		3 051	21 250	347 466	138 397		11 181	263 141	17 535	1 311 045
Mandarin	2015/16	189 758	1 900	1 000	1 020	128 142	253 329	7 470	13 380	390 347		986 346
	2016/17	314 776		7 400	610	104 128	217 192	4 150	39 655	455 916	1 784	1 145 611
	2017/18	385 070	351	28 205	2 000	384 525	317 089		33 003	530 299	700	1 681 242
Navel	2015/16	105 732			2 000	46 125	49 159	11 500	1 826	94 498		310 840
	2016/17	94 563	2 230	3 610		74 760	44 721	2 710	8 600	31 156	14 510	276 860
	2017/18	60 945		2 700		20 285	55 312	3 174	6 680	33 538	4 800	187 434
Satsuma	2015/16	7 952				2 744	4 160	10 000		19 602		44 458
	2016/17	2 823					1 400	10 000		18 040		32 263
	2017/18	1 661				300				12 162	3 650	17 773
Valencia	2015/16	69 906		12 735	800	247 291	76 944		2 450	25 980		436 106
	2016/17	45 547	2 581		10 764	233 558	55 779		4 300	26 060	3 370	381 959
	2017/18	40 955		10 620	1 250	168 319	26 000		500	63 264		310 908
Total		2 705 868	8 462	110 454	77 732	2 226 816	1 630 577	53 405	139 235	2 596 266	55 199	9 604 014

Table 5.5.2. Trees not meeting the certification criteria during the period April to March from 2015/16 - 2017/18

Tree Certification	Year	EC	FS	GP	KZN	LIM	MP	NW	NC	WC	EXP	Total
Not Certified	2015/16	38 753						2 745	4 729	23 659		69 886
	2016/17	16 232		563		42 310	3 000	2	499	10 097	50	72 753
	2017/18	11 249					177		120	11 252		22 798
Certified	2015/16	692 304	3 300	34 470	10 460	590 469	548 620	22 916	28 970	714 262		2 645 771
	2016/17	947 555	4 811	19 460	33 772	649 794	523 792	64 555	20 023	839 215	27 964	3 130 941
	2017/18	1 066 009	351	56 524	33 500	986 553	558 165	51 764	4 412	1 042 953	27 235	3 827 466

5.6 Nursery Certification

Twenty-nine (29) nurseries were visited during the May 2017 audits. Twenty-two nurseries retained their certification status, while four nurseries were provisionally certified and another three new nurseries were provisionally certified. One nursery has delayed their inspection, while another is relocating to new premises. Upon completion of the outstanding requirements, the provisionally certified nursery may be fully certified. 97 samples were submitted to the diagnostic centre, of which 28 tested positive (28.8%).

Thirty-five (35) nurseries were visited during November-December 2017 audits (Table 5.6.1). Twenty-eight were certified, three were provisionally certified and three new nurseries were certified. Of the 99 samples submitted to the diagnostic centre for *Phytophthora* analysis, 10 tested positive (10.1%).

Table 5.6.1. CIS Certified Nurseries in November 2017

Nursery	Town / Province		Contact Person	Tel	Cell	Email
Apapanzi Kwekery	Kirkwood	EC	Nellis Meiring	042 230 1483	082 550 6210	nellis@srvalley.co.za
Atwell Citrus Nursery	Kirkwood	EC	Wayne Attwell	042 230 1560	072 463 7118	attwellcitrus@srvalley.co.za
Augsburg Kwekery	Clanwilliam	WC	Alta Laing	082 952 8127	079 527 0316	admin@augsburnursery.co.za
BF Joubert Kwekery	Kirkwood	EC	Francois Joubert	042 230 0309	084 951 1922	bfjkweek@srvalley.co.za
Casmar Kwekery	Mooinooi	NW	Neville Wenhold	014 574 3152	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	Citrusdal	WC	Patricia Willemse	022 921 3526	076 622 7007	info@cederbergreenursery.co.za
Du Roi Kwekery	Letsitele	LP	Mariska Benn	015 345 1650	072 475 5568	mariska@duroi.co.za
Esselen Kwekery	Malelane	MPU	Leon Esselen	013 790 0160	083 325 0565	esselenk@mweb.co.za
Gamtoos Kwekery	Patensie	EC	Keuler Engela	042 283 0506	072 260 9813	keuler@rikusld.co.za
H J Joubert Kwekery	Montagu	WC	Herman Joubert	023 614 2237	082 578 5747	hopewell@breede.co.za
Henley Citrus (Letsitele)	Letsitele	LP	La-Ruscha Strydom	015 386 0211	063 292 7109	larushca@bigday.co.za
Letsitele Kwekery	Letsitele	LP	Barend Vorster	015 345 1600	083 259 5590	barend@mahela.co.za
Mabu Zest **	Bapsfontein	GP	Dr. Linda Meyer		082 374 7707	linda@mabucasing.co.za
Mistkraal Nursery	Kirkwood	EC	Tyna Ferreira	042 230 0614	082 789 5150	beans@srvalley.co.za
Moorland Seedlings **	Loerie	EC	Rian Moore	042 286 0605	082 2860 604	info@moorland.co.za
Groot Patrysvlei Kwekery	Clanwilliam	WC	Helgard Smit	027 482 2619	084 524 7417	nursery@capspanfarms.co.za
Ngwenya Kwekery	Malelane	MPU	Milanie v/d Merwe	013 790 3004	082 418 7693	milanie@riversidefarm.co.za
Oase Sitrus Kwekery	Hartswater	NC	Gerrit Schlebusch	053 474 2080	082 907 1562	oasekwekery@lantic.net
Oranjerivier Sitrus Kwekery	Kakamas	NC	Blom Rossouw	054 441 0183	083 306 0622	osk@vodamail.co.za
Parma Kwekery **	Hoedspruit	LP	Albert Horn	087 806 5649	072 022 4356	parma@global.co.za
Paksaam Kwekery	Patensie	EC	Michael J. van Rensburg	042 283 0201	063 776 1347	paksaam@gamtoos.co.za
Rietvlei Kwekery	Tzaneen	LP	Lucas McLean	083 630 3236	083 630 3236	rietvlei@global.co.za
Sondagsrivier Hillside Kwekery	Kirkwood	EC	Willem Truter	042 230 0349	083 227 6655	willem@srvalley.co.za
Stargrow Kwekery	Citrusdal	WC	Hennie Prins	022 921 2232	084 563 4412	hennie@stargrow.co.za
Sundays' River Citrus Nursery**	Kirkwood	EC	Riaan Slabbert		072 184 8726	srcnursery@igen.co.za
Tulbagh Kwekery	Tulbagh	WC	Bredell Roux	023 230 0694	082 214 2520	admin@tulbaghnursery.co.za
Tweeling Kwekery	Kirkwood	EC	Jan Potgieter	042 230 1408	082 560 2179	tweeling@srvalley.co.za
Waterfall Nursery	Adelaide	EC	Rudi van der Meulen	046 684 0738	082 695 3433	waterfall@intekom.co.za
Witkrans Kwekery	Boshhoek	NW	Linda Grobler	014 573 3036	082 414 4739	Witkrans1@mweb.co.za

** Provisionally certified – New Nursery

5.7 Statutory Improvement Scheme

The statutory CIS proposal was extensively discussed and debated in meetings with all participating citrus nurseries, a retail nursery, cultivar management companies and growers. A status document stating the benefits and detriments of a voluntary or compulsory statutory improvement scheme, including summarised feedback and inputs from all stakeholders, was discussed at a public workshop facilitated by the NAMC on 9 April 2014. The workshop was attended by 38 persons representing stakeholders, including growers, SACNA, nurserymen, cultivar managers, CGA, CRI and DAFF representatives. The workshop debated matters arising from the consultation process on which more clarity or consensus was required. The NAMC meeting

concluded, as was reported in 2013/14, that a compulsory scheme offered the most advantages as well as protection from biosecurity risks for the citrus industry in South Africa, but that the needs of all role players including those not supportive of a compulsory scheme should be considered. Subsequently, meetings were also held with the Registrar-PIA, the ARC, as well as private cultivar managers and SACNA, of whom certain members opposed a compulsory scheme. A Memorandum of Understanding between the Minister of DAFF and the designated authority, CGA, has been drafted and will be discussed with stakeholders.

5.8 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5 km around the CFB as a citrus free area, was published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DAFF. Most residents have removed their citrus trees. DAFF has made several follow-up visits to owners refusing to remove trees. Two owners have agreed to either have their trees moved or destroyed; this has been done, and there is only one owner who still refuses to remove his citrus trees. DAFF is following up on this case as well as two other cases of reported transgressions.

5.9 PROGRESS REPORT: Citrus Improvement Scheme: Shoot tip grafting and diagnostic services

Project 1144 by J.H.J. Breytenbach, C Steyn and G. Cook (CRI)

Summary

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, their eradication and the maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before release to the Citrus Foundation Block (CFB) and introduction into the Nucleus Block (NB). Biological and molecular indexing is done on new introductions, prior to release to the CFB, as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents might have been inadvertently introduced. Mother trees maintained at the CFB are indexed every two years on a rotational basis to confirm the presence of Citrus tristeza virus (CTV) as introduced by the CTV pre-immunisation programme and also to monitor for the presence of citrus viroids. General diagnostics and investigations into *ad hoc* problems or outbreaks, potentially relating to graft transmissible diseases, are also conducted. The ongoing activities of these CIS functions are reported. Ten new selections were received for STG and 6 were released to the CFB and added to the gene source. The gene source maintained at CRI currently comprises 371 accessions. In the previous report cycle, nine cultivars in the gene source tested positive for GTD pathogens and are now included in the STG pipeline for reintroduction to the NB. The biological and molecular evaluation to assess CTV status of row ten to fourteen of the CFB mother trees was initiated. A new tunnel was erected for plant propagation and research trials and three tunnels required repair after a hailstorm. Additionally, seed transmission of *Citrus tatter leaf virus* in 'Meyer' lemon was tested, but could not be demonstrated.

Introduction

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria, phytoplasmas and unidentified pathogens. The overall objective of the southern African Citrus Improvement Scheme (CIS) is to enhance the productivity of the industry by ensuring supply of the highest quality propagation material. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees and are responsible for stunting, decline, small fruit and a range of other harmful effects. The framework of disease-free planting material is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and has been used in South Africa since 1977 (de Lange *et al.*, 1981). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). STG facilities at CRI are used to introduce new virus-free cultivars and selections which are added to the gene source after STG and indexing. Some cultivars and selections of the virus-free gene source, maintained at the ARC-ITSC, have been duplicated in part at CRI Nelspruit as back-up sources.

Indexing, or establishing whether GTD disease agents are present, is primarily done by inoculating indicator

host plants that are sensitive to various graft transmissible pathogens. Molecular and serological detection techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR), PCR and ELISA are used to confirm biological indexing results.

Since CTV and its vector, *Toxoptera citricidus*, is endemic in South Africa, virus-free material is pre-immunised with a suitable cross-protection source to mitigate the effects of severe CTV strains (Müller and Costa, 1987). Cross-protection is a function of the CIS, where specific 'pre-immunising' CTV sources are applied to all citrus cultivars apart from lemons and limes, before supply to the Citrus Foundation Block (CFB) at Uitenhage. Currently, three CTV sources are used for cross-protection in the CIS depending on the citrus type (von Broembsen and Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000) and pre-immunisation procedures have been adapted to 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 and that the CTV sources introduced, remain mild within these cultivars. Indexing for CTV and viroids are done biennially. Screening for other GTD such as *Citrus psorosis virus* (CPsV), *Citrus tatterleaf virus* (CTLV) and Citrus Impetratura disease (CID) are done every 10 years.

Objectives

- A. Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection, submission to the CFB and NB)
- B. Maintenance of the virus-free gene source
- C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB
- D. Collaboration and duplicate indexing with ARC-ITSC laboratory
- E. Ad hoc diagnostics for GTDs for growers and external institutions
- F. Ad hoc investigations as required by CIS
- G. Facility management

Materials and methods

A. Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection, submission to CFB and Nucleus Block)

In vitro cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange or Rough lemon seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962). Germination takes place in an incubator at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1; buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth and maturation (approximately 3 to 4 months) the source plant is defoliated by hand to induce flushing. Ten to 14 days later the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile distilled water.

Method 2; bud sticks from the source plant are cut in 50 mm lengths and surface sterilised by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile distilled water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand or agar medium. The cultures are incubated at 32°C and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG: The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth tip is placed on the horizontal cut of the incision on

the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light/day.

STG plant propagation. The shoot tip normally starts growing 3 to 4 weeks after STG. The growing shoot tip is micro-grafted with the seedling rootstock onto a vigorous-growing virus-free rootstock in the glasshouse. After micro-grafting, the graft is closed by a plastic bag for 8 days. Once the graft has sufficiently grown, buds for indexing are taken from this material.

Virus indexing. Elimination of graft transmissible pathogens is established by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter confirmed with molecular diagnostic techniques. RT-PCR is used to detect CVd, CTV, CPsV and CTLV. PCR is used to detect the bacterial pathogen causing citrus greening.

On average it takes 24 to 30 months to obtain a virus-free STG followed by the scheduled indexing to confirm the virus-free status of the cultivar. However, delays can occur with elimination of some pathogens. The reason for these “difficult to remove” cases is unknown.

B. Maintenance of the virus-free gene source

Virus-free STG plants are multiplied on virus-free rootstocks and maintained in an insect-free tunnel. Material derived from the gene source is multiplied and pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage. Two trees of each selection are maintained in the gene source and trees have to be re-budded to new rootstocks every five years as part of the routine maintenance. Photo records of fruit from each cultivar/selection are kept on the database to confirm cultivar identifications. The purpose is to ensure that the correct citrus fruit type is produced from each accession and that no mix-ups have occurred.

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The population structure of *Citrus tristeza virus* (CTV) sources used for pre-immunisation can change by the segregation of strains or by re-combination events. These events may be induced by environmental conditions such as high temperatures or other factors such as host influences. All trees are therefore re-indexed every second year to establish the severity status of the CTV present.

Citrus Viroids (CVd) are mechanically transmitted by grafting and contaminated cutting tools, but are not vectored by insects. Re-indexing for CVd follows the CTV re-indexing schedule and is done every second year.

All CFB mother trees and seed source trees are inspected annually for symptoms of citrus greening disease by TSC and CRI Virologists. PCR and/or biological indexing are conducted on plants showing suspicious symptoms.

Most other citrus viruses are transmitted by infected bud wood only, minimizing the infection potential at the CFB. Re-indexing is therefore done only every 10 years for CTLV and CPsV.

Screening of the multiplication blocks for the presence of viroids is done by direct RT-PCR of pooled samples of each cultivar. Each pooled sample consists of 20 leaves. A leaf is taken from every third tree and a sample is therefore representative of a block of 60 trees. Each cultivar is sampled separately and the number of sub-samples of a cultivar is proportional to the size of the block. Each sample is first tested with 2 group specific viroid primer sets, Apsca and non-Apsca. If a sample tests positive in either of these tests, viroid-specific tests are done to determine the specific viroid species present. If weak signals are obtained with the initial tests and are not confirmed by viroid specific tests, the specific accessions are resampled and retested.

D. Collaboration and duplicate indexing with ARC-TSC laboratory

Shoot tip grafting for the CIS is done at both the CRI and ARC-TSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is performed.

Cultivar identification of the NB accessions at the ARC are conducted with the assistance of the CRI cultivar evaluator and CIS-Nelspruit facility manager. The purpose is to ensure that the correct citrus fruit type is produced from each accession and that no mix-ups have occurred.

E. Ad hoc diagnostics for GTDs for growers and external institutions

Field material received for diagnostics is generally budded on 3 indicator host plants. The plants are cut back to force new growth and maintained in glasshouses at various temperatures required for symptom expression depending on the disease being indexed. The indicators are monitored for symptoms for a minimum of 3 months post inoculation. Molecular or serological tests are performed as a confirmation of any biological result. Direct molecular tests are also done, depending on the diagnostic requirement.

F. Ad hoc investigations as required by CIS

Problematic disorders of unknown aetiology or outbreaks of graft transmissible pathogens are occasionally encountered and require investigation. Investigations may include biological and molecular indexing for the presence of graft transmissible diseases, surveys, trials or other analyses.

G. Facility management

Maintenance and improvements at the CIS Nelspruit facilities.

Results and discussion

Objective / Milestone	Achievement
Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection and CFB and nucleus block submission)	Ongoing: - 34 accessions still in progress - 10 new selections received - 6 submissions released to the CFB
Maintain the virus-free gene source	Ongoing: 371 cultivars maintained
Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB	Biological CTV evaluation of rows 10 to 14 of mother trees initiated.
CIS collaboration with ARC-TSC	CFB releases were tested at both facilities for various pathogens. CRI personnel assisted in cultivar verification of fruit produced on accessions in the ARC NB. Kobus Breytenbach and Zama Theledi (ARC) were on a study tour to the USA. Various STG laboratory facilities were visited in Florida and California.
Requests from growers and institutions to diagnose suspected material for GTD	Approx. 170 samples analysed for various clients. Investigation conducted into the viroid incidence associated with a navel cultivar in the Sunday's River Valley.
Ad hoc investigations as required by CIS	Seed transmission of CTLV in Meyer lemon was tested. Investigation of CLaf in citrus roots (in collaboration with Texas A&M).
Infrastructure management	A new tunnel was erected. Three tunnels were repaired after hailstorm damage.

A. Cultivar introduction for STG

Introductions for STG and subsequent releases to the CFB from 2013 to date are summarised in Table 5.9.1. Ten new selections of four variety types were submitted for STG in the current year. At the end of this report period, 34 accessions are at various stages in the STG pipeline. A total of 263 STGs were done within this period, including failed grafts. Forty STGs were successfully micro-grafted. In the previous annual cycle, nine

cultivars in the NB tested positive for GTD pathogens and are now included in the STG pipeline for reintroduction.

To facilitate a faster turn-around with the STG process, new introductions are initially tested directly with PCR prior to STG to determine the original pathogen status and then again directly after STG as soon as sufficient material is available for testing. These additional steps allow quicker detection of pathogens not eliminated by the initial STG step. Re-STG can therefore commence quicker rather than waiting for completion of the biological indexing. This process does, however, not replace the final biological indexing and PCR to confirm the pathogen-free status prior to final release of the accession. These additional tests are routinely done and the number of tests conducted is not reported.

Biological indexing for nine successful STGs commenced for CTV, CTLV and CVd. Biological indexing for fourteen STGs also commenced for CPsV and Citrus Impiortatura Disease (CID). The results of these tests are pending until completion of the evaluation periods. Confirmation of the biological indexing will be done by PCR by both the CRI and ARC-ITSC laboratories prior to final release.

Table 5.9.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Variety type ²	STG introductions and releases 2012 to 2017 ¹															
	2013			2014			2015			2016			2017/8			Balance
	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	
C	5	1	0	6	0	3	3	0	1	2	0	1	1	0	1	0
G	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
L	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	1
Mi	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
Ma	0	4	0	4	0	0	4	1	1	4	2	5	1	3	3	1
N	34	2	4	32	0	13	19	2	2	19	1	4	16	3	1	18
V	6	0	2	4	1	0	5	1	0	6	2	1	7	2	1	8
Or	1	0	0	1	1	0	2	0	0	2	0	0	2	0	0	2
Rs	2	0	1	1	0	1	0	0	0	0	0	0	0	2	0	2
Total	49	7	7	49	3	17	35	4	4	35	6	11	30	10	6	34

¹ Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

² Variety type: C = Clementine; G = Grapefruit; L = Lemon; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

B. Maintenance of the virus-free gene source

The CRI gene source currently comprises 371 accessions and the number of selections per variety type is presented in Table 5.9.2. Two trees of each accession are maintained, each in a separate greenhouse structure.

Nine accessions tested positive for CVd and/or CTV in the last report period. These positive accessions were resubmitted for STG.

Photos of fruit are taken as fruit is produced on the NB trees and are maintained in the data-base to confirm cultivar identifications. The purpose of this process is to ensure that the correct citrus fruit type is produced from each accession and that no potential mix-ups have occurred. The number of accessions confirmed to be the correct variety type is presented in Table 5.9.2.

Table 5.9.2. The number of accessions per variety type maintained in the CRI Nucleus Block and the number of accessions confirmed to be the correct variety type based on fruit produced on the trees.

Variety Type	No. of accessions	Variety type confirmation
Clementine	31	9
Diverse (Citron, Sour orange, etc.)	2	1
Ellendale	4	0
Grapefruit	23	12
Kumquat	1	2
Lemon	23	19
Lime	4	2
Mandarin hybrid	65	21
Midseason	34	15
Navel	85	12
Ornamental	4	4
Pummelo	8	4
Rootstock	23	17
Satsuma	8	6
Valencia	56	15
Total	371	139

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The biological and molecular evaluation, to assess CTV status of row ten to fourteen of the CFB mother trees, was initiated and will be reported in the next annual report

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 on these accessions and the number of accessions tested for specific pathogens are presented in Table 5.9.3.

Table 5.9.3. Sample numbers of duplicate testing for various pathogens prior to final release to CFB.

Pathogen	ARC-TSC accessions	CRI accessions
CTV ³	39	18
CVd	18	7
CTLV	19	7
CPsV	28	-
'Ca' L. africanus	19	11

³ Includes testing to confirm CTV pre-immunization

Cultivar identification of the NB accessions at the ARC are conducted with the assistance of the CRI cultivar evaluator and CIS facility manager in Nelspruit. The purpose is to ensure that the correct citrus fruit type is produced from each accession and that no mix-ups have occurred.

Kobus Breytenbach (CRI) and Zama Theledi (ARC) went on a study tour visiting various STG laboratory facilities in the United States. In Florida they visited the Bureau of Citrus Budwood Registration facilities both Winterhaven and Lacrosse as well as the University of Florida (CREC) in Lake Alfred. In California, the Riverside Rubidoux Quarantine Facility, the USDA laboratories and the PCR laboratories of the University of California were visited.

E. Ad hoc diagnostics for GTDs for growers and external institutions

Suspect citrus greening samples are first tested with a non-specific real-time assay for universal detection of '*Candidatus Liberibacter*'. If a positive sample is detected, confirmation tests are done to determine whether '*Ca. L. africanus*' or possibly '*Ca. L. asiaticus*' is present. Most greening samples are brought in by the CRI extension team. Forty three suspect samples were received of which four tested positive for '*Ca. L. africanus*'.

Samples from Nkwaleni, showing typical fruit symptoms of citrus Impetratura disease, were inoculated to host plants to preserve the sources. As no disease agent has been linked to this disease, RNA was extracted for storage for future reference until the aetiology of the disease is identified.

A greater awareness of citrus viroids resulted in more samples being sent for analysis for these pathogens. Numerous samples of a navel cultivar received from various growers in the Sunday's River Valley were found to be infected with a combination of *Hop stunt viroid*, *Citrus dwarfing viroid* and *Citrus exocortis viroid*. The origin of the trees could be traced to a nursery that produced trees from non-certified budwood.

Approximately 170 samples were received from various clients for analysis in this report period.

F. Ad hoc investigations as required by CIS

A single report of seed transmission of *Citrus tatter leaf virus* (CTLV) in Eureka lemon has not been confirmed since the initial report. It is important to establish whether seed transmission of viruses occur in citrus, especially in rootstock varieties, as seed is often imported for tree production. Seed was obtained from CTLV positive Meyer lemon trees at the ARC premises at Addo. These trees were visually positive for CTLV and infection was confirmed with biological indexing and RT-PCR. Seeds were germinated and seedlings tested for the presence of CTLV with both endpoint and real-time PCR. A leaf of each of 18-27 plantlets were sampled and pooled as a single sample. There were 35 samples representing 893 seedlings. All samples tested negative for the presence of CTLV. Although Meyer lemon is not a rootstock, this was as an opportunity to test seed transmissibility of CTLV in citrus. Given the number of seedlings tested, a 0.1% seed transmission would have been detected.

A small collaborative project is underway with researchers at Texas A & M University. Their work focuses on detection of Huanglongbing in citrus roots and they would like to simultaneously investigate 'Ca. L. africanus' (CLaf) in citrus roots. We have supplied them with DNA samples extracted from both roots and leaves of field trees. All samples supplied were from orchards established on Rough lemon. Although leaf samples of the trees were positive, there was no detection of CLaf in roots. We have further sent DNA extracted from a Rough lemon plant inoculated with CLaf and maintained in a glasshouse. The plant was sectioned into 10 cm pieces from the roots upwards to the top of the plant. The CRI lab did not detect CLaf in the roots and the DNA was sent to the US laboratory. It will be of value to know the sensitivity of Rough lemon to CLaf.

G. Facility management

The rebuilding of the large tunnel used for seedling production and research trials was concluded. Repair work was done to 3 tunnels after a hail storm. Routine maintenance and internal audits were done on a weekly basis.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy budwood to the industry and is the primary objective of this project. Successful elimination of GTDs from new selections were achieved. New selections were added to the gene source and released to the CFB. Diagnostic services were provided and analysis of industry problems relating to graft transmissible diseases were addressed. Additionally seed transmission of CTLV in Meyer lemon was tested. Significant infrastructure improvements and repairs were concluded.

Technology transfer

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6 INTERNATIONAL VISITS

- 6.1 **Technical visit to citrus shoot tip grafting laboratories in Florida and California (USA)**
21 February – 2 March 2018
 Kobus Breytenbach (CRI) and Zama Theledi (ARC)

Summary

Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before introduction to the Nucleus Block and release to the Citrus Foundation Block. A study tour was undertaken to

visit STG facilities in the United States of America to learn from their approaches and experiences. We visited facilities of the United States Department of Agriculture (USDA) in Florida and California and also the University of California Riverside's Citrus Clonal Protection Program (CCPP) facility. The USDA laboratories process all foreign citrus introductions. We also had the opportunity to visit both HLB and citrus canker infected orchards in Florida and the Wonderful citrus nursery in California.

Possible changes to be investigated for implementation at CRI and ARC laboratories include:

- The use of thermotherapy
- Establish budsticks *in vitro* for quicker shoot development
- Different types of media use during the STG process
- Rootstock choice for STG
- Alternative STG incision techniques
- Hardening off STG's after micro-grafting
- Additional lighting in indexing rooms
- Improved pathogen detection
 - Establish tests for viruses, viroids and other pathogens not currently tested for, i.e. CVd-VI, CYVCV, CVEV, CSDaV, CYMV, SDV, *Phytoplasma* spp., ICRSV, CCDaV, CCGaV, CSPO, HLB, CSPO, Canker, CVC.

Alternative triangle cut for STG demonstrated in California has been implemented at the ARC and shows increased STG success rate.

Florida

We were met by Prof. Megan Dewdney and Dr. Evan Johnson from the University of Florida, stationed at the Citrus Research and Education Centre in Lake Alfred. They accompanied us to HLB and Citrus Canker infected orchards. Typical symptoms for these diseases were observed as well as *Diaphorina citri*, the vector for HLB. Public awareness campaign material used in Florida and California was shared, which are informative and similar formats can be used locally to disseminate information.



Citrus Research and Education Centre (Lake Alfred) and Prof. Megan Dewdney and Dr. Evan Johnson



8 year old orchard, 100% infection with HLB

Symptoms observed in the HLB infected orchard:

- Young leaves leathery
- Curling of leaves due to *Diaphorina citri* infestation
- Nutrient deficiency
- Leaf drop in winter
- Vein corking
- Asymmetrical fruit shape
- Fruit drop



Citrus Canker lesions on fruit and leaves

Florida Department of Agriculture and Consumer Services: Bureau of Citrus Budwood Registration – Winter Haven and La Crosse.

Winter Haven receives all domestic introductions and La Crosse all foreign introductions.

1. Winter Haven



Ben Rosson is now Chief of the Bureau of Citrus Budwood Registration and replaced Peggy Sieburth after her retirement.

Justin Ezell and various lab technicians took us through the facilities and shared their STG and indexing protocols. They index for a wide range of pathogens and their methodologies will be investigated and implemented in the post entry quarantine (PEQ) procedures for the South African citrus industry to prevent the introduction of these diseases to RSA.

Molecular detection protocols

- PCR detection is all probe-based (protocols obtained)

Shoot tip grafting procedures

Mention is made of procedures where they differ from those done locally at CRI or ARC.

Media and seedling preparation:

- Two types of media are used: Gelrite for STG and Agar for seed germination.
- *Poncirus trifoliata* is used as the preferred rootstock for STG.
- Only one seed is placed per test tube.
- STG success rate is much higher when fresh rootstocks, not older than one month, are used.



Poncirus trifoliata seedlings 2 weeks after planting

Establishment of parent budwood:

- Bud sticks are surface sterilized and placed *in vitro* to force shoot development. New shoots develop after 4-6 weeks.
- Back-up parent trees are budded and kept in the glasshouse.
- Pre-screening qPCR tests on all local pathogens are performed on parent material.
- Biological indexing was previously also done on original parent budwood, but this will no longer be done.



Bud stick set up *in vitro*



Shoots develop 7-10 days later

STG procedures:

- Collected shoot tips are kept on moist filter paper to prevent desiccation.
- A different grafting method is used to graft the meristem to the rootstock. A vertical incision is made and the shoot-tip placed between two flaps either side of the incision. The incision is also made higher up on the rootstock.
- Gelrite (solid medium) and not liquid media is used for STG plant development.
- After STG, plants are placed in the growth room with lower light intensity for the first 7 days to adapt to the growth room conditions.
- They aim for 3-6 successful STG's for one accession.



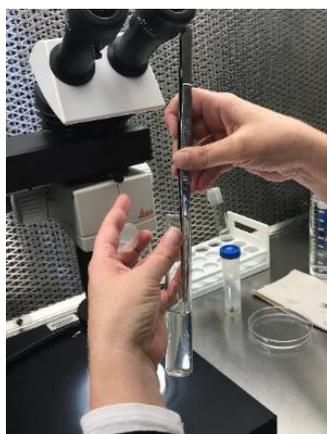
Preparation of rootstock



Collected shoots kept in filter paper



Preparation of shoot tip for grafting



Place STG plant into Gelrite media

Micro grafting after successful STG

- Micro-grafts are initially placed under shade cloth in the glasshouse to allow for adaption from the growth room.



Successful STG's ready to be micro grafted



STG micro grafts on Kinkoji rootstocks covered in plastic bags for 7 days

Biological indexing:

- Inoculate with one bud and one bark strip.
- Indicator plants are planted one per pot.
- Experimentation underway with additional lighting for better symptom expression.



Indexing room with additional ultraviolet lighting

Gene source:

- Facility will be moved to La Crosse
- Plants are kept in aerated bags for optimal root development



La Crosse: Bureau of Citrus Budwood Registration

We met Kristen Helseth, the interim Bio Administrator and James Coleman, Biological Scientist of the Citrus Germplasm Introduction Program. A tour of the facilities was conducted and a presentation of their programme was given.



New STG and glasshouse facilities at La Crosse (access to all glasshouses was restricted)

We were invited to sit in on a meeting with Florida role players to discuss their new protocols and procedures. This was an informative meeting in that they face similar challenges as to those faced by the South African citrus industry.

Concerns regarding the Winter Haven procedures:

- Excessive time spent on STG, need to cut time from 36 to 16 months.
- Out of date laboratory testing methods.
- Heavy reliance on biological indexing, which only includes testing for viroids, Psorosis and Concave Gum
- Limited qPCR personnel.
- Potential for undetected pathogens.
- STG indexing was determined by pre STG-biological indexing results; i.e. pre-STG indexing results were used and STG plants were not re-indexed. Some cultivars were found positive after release because of this practice.
- Cultivar mix-ups also occurred in Florida

Proposed changes to be introduced at La Crosse.

- Streamline biological indexing:
- Madam Vinous and Etrog will be the primary indicators for both Winter Haven and La Crosse
 - Madam Vinous – cool temperature indicator, Etrog – warm temperature indicator
- Both STG and biological indicators will be PCR screened
- Update laboratory testing and expand qPCR target panel
- Eliminate less sensitive tests: dsRNA, s-PAGE and ELISA
- Introduce new technology: Digital PCR and Ion Torrent Next Generation Sequencing (NGS)
- Do NGS at introduction, if “unknowns” found, you have 18 months to describe.

California

Citrus Clonal Protection Programme (CCPP) and Rubidoux Quarantine Facility, Riverside

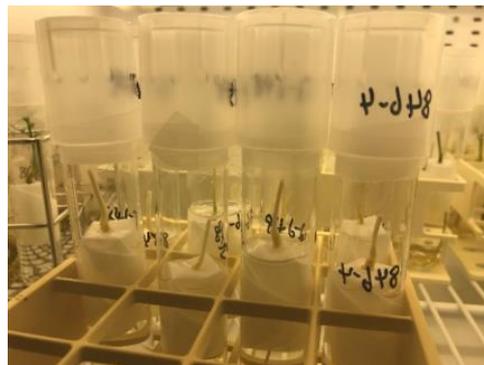
We were received by Prof. Georgios Vidalakis of the Citrus Clonal Protection Programme (CCPP) at the University of California, Riverside. He showed us the original experimental station, established in 1907. An overview of their programme was given and the online STG introduction system was demonstrated. Greg Greer, manager of the variety introduction and biological indexing programme, showed us the facilities and bio-indexing procedures. Ning Chen, the STG technician, showed us the STG facilities and the alternative triangle cut for STG. We were provided with their citrus disease detection protocols and STG procedural guides. The STG procedure observed was implemented in the ARC procedures and shows increased STG success rate.



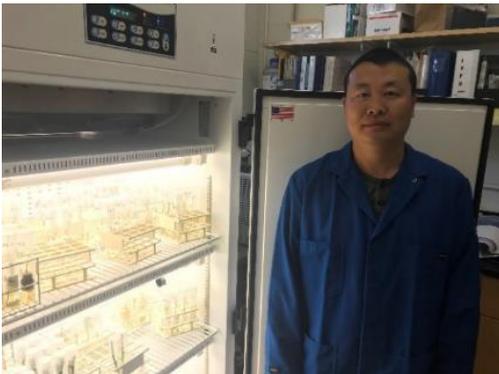
Prof. Georgios Vidalakis (middle)



STG laboratory



STG plants in liquid media with filter paper support



Ning Chen demonstrated the triangle cut for STG



Successful micro grafts after STG



Biological indexing: 3 plants per pot



Use only bark for inoculation



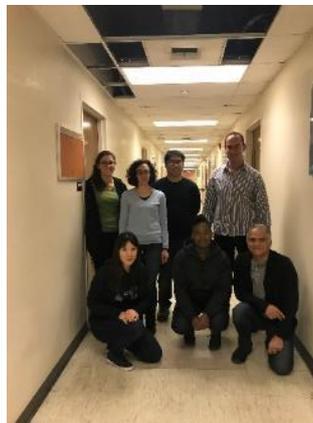
Gene source



Biological indicator seedlings

Diagnostic and Research Laboratory (CDRL), University of California, Riverside

We were hosted by Dr Irene Lavagi, quality manager, and Dr Sohrab Bodaghi, lead diagnostician.



Molecular detection:

A confidentiality agreement was signed with the ARC to obtain molecular procedures used at the CDRL laboratory. A follow up will be done to obtain the procedures.

National Clonal Germplasm Repository for Citrus and Dates (USDA), Riverside

Dr MaryLou Polek and her team, including Robert Krueger, the horticulturist, hosted us at the Agricultural Research Service. Their procedural guide was discussed and protocols were shared.



All new accessions go through thermotherapy for 12 weeks at 40°C for 18 hours and 30°C for 6 hours. Cultivars are established on Carrizo rootstocks, which is more heat resistant. By doing this they suppress most viruses, but not viroids.

In their laboratories, STG takes a minimum of 2 years.



STG laboratory



Micro grafting after successful STG



Thermotherapy Conviron



Biological indexing: indicator plants are planted 3 per pot and kept at different temperatures.

Positive controls and symptom expression:



Citrus tatterleaf virus



Citrus psorosis virus



Citrus Vein Enation Virus



Satsuma Dwarf Virus



Citrus Gene source at USDA Riverside

Lindcove Foundation Facility, Exeter

We were hosted by Dr Rock Christiano, Manager of budwood sources and budwood distribution.



Dr Rock Christiano



Mother tree facility



Root development of seedlings in aerated seed trays



Mother trees are either planted in open ground or maintained in pots



Multiplication trees on raised beds at Lindcove and multiplication block numbering

Wonderful Citrus Nursery in Visalia

Jose Lima, Director of Nursery Operations took us through the various stages of tree production from planting of the seed to tree delivery.



1. Chemical seed coat removal before plant 2. Seed tray preparation 3. Healthy root system in coir



Nursery totally enclosed due to HLB risk

Trees ready to be sent at 12 months



Trees are packed in sealed containers before dispatching

Acknowledgement

Zama Theledi (ARC) and Kobus Breytenbach (CRI) wish to thank CRI for this opportunity. The gained knowledge and technical experiences will improve functions of the CIS in RSA.

6.2 Third FAO/IAEA International conference on Area-wide Management of insect pests: Integrating the sterile insect and related nuclear and other techniques, 22-26 May 2017, Vienna Austria

Aruna Manrakhan, Citrus Research International, PO Box 28, Nelspruit, South Africa

Background

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) have over the last 50 years supported their Member States in the development and application of environmentally friendly methods to combat insect pests affecting agriculture and human health. The area-

wide approach for management of insect pests is control of the total population of an insect pest in a delimited area. This contrasts with the conventional pest control approach which consists of uncoordinated field by field actions. Area wide-pest management programmes integrating the sterile insect technique with other control technologies have been successfully implemented in a number of regions around the world to combat key insect pests affecting agriculture and human health.

The FAO/IAEA organized the first international conference on area-wide management of insect pests in 1998 in Penang, Malaysia. The second conference in this series took place in 2005 in Vienna. The third FAO/IAEA international conference on area-wide management of insect pests took place in Vienna, Austria between 22 and 26 May 2017.

The objective of the conference was to “familiarize participants with new developments, trends and challenges related to insect pest management, both in the fields of agriculture and public health, and to foster a broad exchange of information between sanitary and phytosanitary regulatory authorities, operational AW-IPM programme managers, scientists, human, animal and plant protection specialists, pest management experts, public health practitioners, medical personnel and epidemiologists, as well as the private sector” (Source: <http://www-pub.iaea.org/iaeameetings/50813/Third-FAO-IAEA-International-Conference-on-Area-wide-Management-of-Insect-Pests-Integrating-the-Sterile-Insect-and-Related-Nuclear-and-Other-Techniques>). The programme of the conference is available at: <http://www-pub.iaea.org/iaeameetings/50813/Third-FAO-IAEA-International-Conference-on-Area-wide-Management-of-Insect-Pests-Integrating-the-Sterile-Insect-and-Related-Nuclear-and-Other-Techniques>.

My participation at the conference was under the ERAfrica Fruit fly Project (2014-2017) funded by the Department of Science and Technology. A poster on the outcomes of the ERAfrica fruit fly project was presented (Annex A). The conference served as the last meeting point for partners in the ERAfrica fruit fly project. Two project partners: Royal Museum for Central Africa (RMCA) and CIRAD also attended the conference and the last ERAfrica meeting. The conference also served as a meeting point for discussion of 3 other fruit fly projects where CRI is involved: (1) Horizon 2020 FLYIN proposal on invasive fruit flies, (2) LEAP AGRI LOCATE proposal on surveillance and monitoring of fruit flies and (3) STDF/PPG/567: Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in Southern Africa.

In this report, key points from key oral presentations are provided. Key points from interactions with some participants are highlighted. The collaborative fruit fly projects where CRI is involved are summarized.

Key Points from Key Oral papers

Kenneth Bloem: Past, Present and future: a road map to integrated, area wide, systems and enterprise risk management approaches to pest control

In an area-wide insect pest management (AW-IPM) programme, a lot of focus should be placed on insect pest monitoring. Successful AW-IPM programme requires a collective approach whereby growers and stakeholders work together to manage key pests. IPM involves multiple tactics to optimize pest control. Area-wide systematic pest control is control applied to the total population of a key pest in a clearly defined area. In enterprise risk management, industry develops a business plan that specifically considers and addresses pest risk mitigation options. In enterprise led risk management the industry risk appetite is stated for example: reduce consignment rejections to zero. South African citrus growers and FCM control was given as an example of enterprise risk management.

Clemente de Jesus Garcia Avila: Holistic area-wide approach for successfully managing citrus greening in Mexico

Asian Citrus Psyllid was detected in Mexico in 2002. A containment programme was implemented in 2008. In 2009, CLAs was detected in Yucatan. Area wide management programme of citrus greening involves monitoring of psyllids, elimination of infected trees, chemical control, biological control using *Tamarixia radiata* and the entomopathogenic fungi: *Isaria fumosorosea*, *Metarhizium anisopliae* and *Isaria javanica*. In citrus growing areas in Mexico, monitoring traps for psyllids are checked every fortnight. With chemical and biological control integrated with removal of infected trees, 85% of citrus growing areas in Mexico are still free of the pest.

Cara Nelson: Putting SIT into the Modern IPM toolbox: over 20 years of successful area wide IPM in Canadian pome fruit

SIT of codling moth is funded mainly by municipal taxation. This amounts to approximately 11 dollars a year per property. Growers fund 40% of the SIT programme. The production capacity of sterile codling moth at the facility is 780 million sterile CM per year with 2000 sterile moths released per ha per week.

Joop Van Lenteren: Will the Nagoya protocol on access and benefit sharing put an end to biological control?

There have been 6000 introductions of natural enemies all over the world. Ten percent of these introductions resulted in satisfactory control. In the first 100 years of biological control, regulations were non-existent. ISPM No. 3 providing guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms was developed in 1995 and updated in 2005. In Africa, there are either none or complicated regulations for biocontrol agents and as such harmonization is required. Between 2000 and 2009, there was a slowdown in import of exotic biological control. There was a dramatic shift in use from exotic to native biological control agents. The NAGOYA protocol was the second phase of regulation of biological control agents under the Convention of Biological diversity. According to the Nagoya protocol, the state owns its biological control agents. Benefits of the state-owned biological control agents should be shared (Article 15 in Nagoya Protocol). Since October 2014, an agreement is required between parties that wish to export/import biological control agents. Recently, an article was published by Mason et al. 2017 in Biocontrol on "Best practices for the use and exchange of invertebrate biological control genetic resources relevant for food and agriculture". These best practices have been endorsed by the International Organisation for Biological Control.

Kaare Magne Nielsen: Regulatory and societal considerations of new genetic techniques

There are still unclear regulations on releases of genetically modified insects, in particular in organisms with site specific changes in their DNA (recombinant DNA, CRISPR-CAS, SNT-3). The regulation currently in Europe is a case by case approach. EFSA guidelines on environmental risk assessment of GM animals, including insects were published by Mumford et al. (2016) in IOBC-WPRS Bulletin Vol. 114, 2016 pp. 39-46.

Trang Vo: Methods to quantify invasive pest risk and socioeconomic impact to promote objective regulatory decision-making case studies on sterile insect techniques related programmes

APHIS, USDA quantifies invasive insect pest risks by (1) carrying out a prioritization of pests (done by entomologists) and (2) correlating entomologist grading and observed impacts (done by statisticians). FCM was listed as having a 67.3% probability of causing high impacts in the USA.

Manoukis et al. climate and medfly invasion persistence and insights from agent based simulations (ABS)

There are recurrent finds of medfly in pest free areas in California. The quarantine length following these Medfly incursions is set at 3 generations based on degree-days development. Agent Based simulation (ABS) is an alternative model which can be used to predict quarantine length. ABS is demographically explicit, realistic, stochastic and flexible. It simulates each fly. The model inputs the initial numbers of individuals trapped. Development parameters of the organism are required. Mortality data by control methods can also be added to the model. ABS is freely available from the web (MED-FOES). There are videos also available on the use of MED-FOES.

David Benavente: The role of drones in AW-IPM programmes: a drone for sterile tsetse releases in Ethiopia (Embenton)

Drones are currently being used in Ethiopia for releases of sterile tsetse flies. The drones are set on an autopilot split mission in different phases such as cruise phase and insect release phase. Medium size areas can be covered (e.g. 850 km² under drone releases of tsetse flies in the Rift Valley). Large number of insects being released would pose a challenge. The load that can be taken is currently at 2.5 kg. There are still technical limitations with the drone technology. There are also concerns about safety. For drones carrying heavier weights e.g. fruit fly bait, someone would have to be on the ground to direct drones.

Key points from interactions with conference participants

Kaare Magne Nielsen, Head of Department of Life Sciences and Health, Oslo and Akershus University College of Applied Sciences (Prof. Nielsen is an expert member of the GMO panel of EFSA)

I spoke to Prof. Nielsen about the new development on using engineered virus to combat Huanglongbing in the USA as published by Ledford (2017) in Nature, Vol. 245: 277-278. I asked whether the fruit would then be considered as a genetically modified organism. He said that this would depend on how much of the virus is found in the fruit. There is a threshold of GM that can be tolerated in food produce and beyond the threshold (0.9%) the food would be considered as GM.

Roger Vargas, United States Department of Agriculture

I spoke to Roger Vargas about levels of fruit fly catches tolerated in a systems approach to mitigate fruit fly risk in fruit commodities. He said there are fruit fly trap levels that have been established by USDA and gave me an example of the systems approach to mitigate the risk of Oriental fruit fly in Sharwil Avocados where 6 *B. dorsalis* flies per trap per week is the actionable level established by USDA APHIS on this crop.

Collaborative fruit fly projects with external institutions

Horizon 2020 FLYIN proposal on invasive fruit flies threatening Europe

The project: FLYIN under Horizon 2020 framework deals with invasive fruit flies that are of concern to Europe. The project is led by Prof. Papadopoulos, University of Thessaly, Greece, and involves 14 institutions from Europe (including UK), South Africa, Australia and China. On Wednesday 24 May 2017 and Friday 26 May 2017, project partners who attended the FAO/IAEA conference met to discuss activities proposed in the FLYIN project. The feedback received from Prof. Papadopoulos is that the FLYIN proposal successfully went through the first round of evaluation and an invitation was sent to the project leader to submit a full proposal. In the second round, the project has a 30% chance of succeeding. In the FLYIN project, activities will be centered on five fruit fly species: *C. capitata* (currently expanding to Northern Europe with Austria as its northern limits), *C. quilicii*, *C. rosa*, *B. zonata* (present in Israel) and *B. dorsalis* (recently detected in Reunion Island, France). The main research foci are to (1) obtain biological knowledge on the pests (environmental stresses, life history traits, establishment and dispersal linked to propagule density), (2) determine the sensitivity of detection methods (field detection using e-traps, detection of infested fruit using the e-nose technology, identification methods (LAMP and other molecular techniques), (3) development of simulation tools for decision making on pathway risk attenuation, incursion response and pest management (including eradication) and (4) testing of management tools and strategies to deal with fruit fly invasions. CRI's involvement will be mainly in testing of e-traps and management tools and strategies.

LEAP AGRI LOCATE proposal on surveillance and monitoring of fruit flies

The LEAP AGRI LOCATE project proposal is focused on surveillance and monitoring of pest fruit flies in Africa. The project proposal is led by Dr Christopher Weldon, University of Pretoria. Other project partners are RMCA, CIRAD, CRI, Stellenbosch University (SU) and ISRA (Senegal). The main research foci are (1) development of trapping strategies through optimal placement of traps and (2) development of the LAMP method and other molecular techniques for fruit fly identification. The pre-proposal was submitted. If approved, the project will be funded by the National Research Foundation in South Africa.

STDF/PPG/567: Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in Southern Africa

A grant of 40 000 USD was approved for the project preparation on "Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in Southern Africa" by the STDF. The project will bring together five institutions: RMCA, DAFF, Eduardo Mondlane University (Mozambique), SU and CRI, who will assess the development of Pest Free Areas (PFA) and Areas of Low Pest Prevalence (ALPP) for fruit flies in Southern Africa. The project will be administered by RMCA who will then subcontract tasks to other partners. The project will involve (1) obtaining baseline data to determine the feasibility of PFAs and ALPPs for fruit flies in South Africa and Mozambique, (2) cost-benefit analyses for establishment and maintenance of PFAs and ALPPs in South Africa and Mozambique and (3) formulation of a project proposal on establishment of PFAs and ALPP in South Africa and Mozambique on the basis of the findings of the project preparation.



DEVELOPING EFFECTIVE DETECTION TOOLS FOR AFROTROPICAL FRUIT FLY PESTS- THE ERAFRICA 'FRUIT FLY' PROJECT

A. Manrakhan¹, J.-H. Daneel¹, M. De Meyer², M. Virgilio², P.F. Duyck³, H. Delatte³ and F. N. Hala⁴

¹Citrus Research International, P.O. Box 28, Nelspruit 1200, South Africa
²Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium
³CIRAD, UMR PVBMT, 7, Chemin de l'IRAT, 97410 Saint-Pierre, La Reunion, France
⁴Centre National de Recherche Agronomique, 13 BP 989 Abidjan, Côte D'Ivoire
 Corresponding author: E-mail: aruna@cri.co.za



Introduction

- Fruit flies heavily impact production and trade of fruit and vegetables in Africa and the Indian Ocean region.
- With low tolerance of insecticide residues in fruit and vegetables, an integrated management approach which combines effective detection and control should be followed for fruit fly pests.
- The ERAFRICA 'Fruit Fly' project is a joint Africa and Europe partnership project which was initiated in 2014 with an aim to develop effective and accurate detection methods.
- The project assembled a team of fruit fly experts from two African countries and two European countries (Fig. 1): South Africa (Citrus Research International), Côte D'Ivoire (Centre National de Recherche Agronomique), Belgium (Royal Museum for Central Africa) and France (CIRAD Reunion) to address aspects of detection systems for Afrotropical fruit fly pests.

Activities

Fruit fly detection trapping:

- Attractant and trap combinations were tested in natural areas and commercial fruit orchards in South Africa (Fig. 2) and Ivory Coast.
- The sensitivity of the novel EGO lure based trapping system for detection of three *Ceratitids* fruit fly pests was evaluated in South Africa using a mark-release-recapture method.
- The effects of lure dispensers and trap types on monitoring efficacy of two male lures: trimedlure and methyl eugenol were tested under South African field conditions.
- The potential of fruit volatiles for monitoring females of the melon fly- *Zeugodacus cucurbitae* (Coquillett) and the peach fruit fly - *Bactrocera zonata* (Saunders) was investigated in Reunion island (Fig. 3).

Fruit fly identification:

- The online multi-entry key for identification of African frugivorous tephritids was tested and optimized.
- Field collected fruit fly specimens were identified using DNA barcoding.

Major findings

- Two relatively new male lures were found to be promising for monitoring of important Afrotropical fruit fly pest species
 - ❖ EGO lure (Fig. 4) was found to be a promising attractant for a number of *Ceratitids* pest species.
 - ❖ Zingerone was a promising attractant for some cucurbit infesting *Dacus* species.
- Three fruit fly pests: *Ceratitids capitata* (Wiedemann), *Ceratitids rosa* Karsch and *Ceratitids cosyra* (Walker) were found to respond equally well to EGO lure.
- Types of lure dispensers and traps influenced efficacy of trimedlure and methyl eugenol for monitoring *C. capitata* and *Bactrocera dorsalis* (Hendel) respectively.
- Potential fruit volatiles were found which were similarly attractive to both *Z. cucurbitae* and *B. zonata*.
- New DNA barcodes were generated for some fruit fly taxa and were deposited in the Barcoding of Life Data Systems.
- DNA barcoding was found to be a suitable tool for the identification of *C. cosyra* and some of its closely related *Ceratitids* (*Ceratalaspis*) species.

Information transfer and publications



Figure 5. Factsheets were compiled within the framework of two network projects: The "ERAFRICA_NL 027 Fruit Fly" project and the networking project "BL/37/FWI08 FRUITFLY" funded by the Belgian Science Policy.

- Project outcomes were presented at the 3rd International Symposium of Tephritid Workers of Europe, Africa and Middle East, 11-14 April 2016, Stellenbosch, South Africa

- Peer-reviewed publications from this project include:

- ✓ Manrakhan A. *et al.* 2017. Efficacy of trapping systems for monitoring of Afrotropical fruit flies. *Journal of Applied Entomology*: n/a-n/a. doi:10.1111/jen.12373.
- ✓ Manrakhan A *et al.* 2017. Sensitivity of an enriched ginger oil based trapping system for *Ceratitids* fruit fly pests (Diptera: Tephritidae). *Crop Protection* 99: 26-32.
- ✓ Virgilio M. *et al.* 2017. The complex case of *Ceratitids cosyra* (Diptera, Tephritidae) and relatives. A DNA barcoding perspective. *Journal of Applied Entomology*: n/a-n/a. doi:10.1111/jen.12379
- ✓ Virgilio M. *et al.* 2015. Population structure and cryptic genetic variation in the mango fruit fly, *Ceratitids cosyra* (Diptera, Tephritidae). *ZooKeys* 540: 525-538.

- Factsheets covering taxonomy, molecular diagnosis, biology and detection methods for 30 fruit fly species of economic importance in Africa and the Indian Ocean region were made available through the website of the Royal Museum for central Africa. (<https://fruitflykeys.africamuseum.be/en/index.html>) (Fig. 5)

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Figure 1. Participating countries in the ERAFRICA fruit fly project (shaded in brown)



Figure 2. Trapping in natural areas in Mpumalanga Province, South Africa



Figure 3. No choice fruit odour and oviposition tests

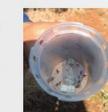


Figure 4. EGO Pherolure (Insect Science, Tzaneen, South Africa) in a Sensus Bucket trap (River Bioscience, Port Elizabeth, South Africa)

6.3 Report on attendance of the 11th Congress of the International Society for Citrus Nurserymen (July 2017)

Paul Fourie (CRI)

The congress was held in Mildura, Australia, and was attended by approximately 130 delegates from the major citrus growing countries in the world. The congress was organised by Gary Eyles (Past President of the ISCN, and owner of Eyles Citrus, a citrus nursery specialising in producing citrus trees for the retail sector), Wayne Parr (Variety Access, an Australian private cultivar management company) and Simon Jurisich (owner of Kwan Nursery, a citrus tree nursery in New Zealand), with assistance from Tim Herrmann (Auscitrus manager) and

Dr Nerida Donovan (Department of Primary Industries, New South Wales), work colleagues and family members.

This meeting is a very good forum to get best-practice advice on practical nursery matters, but also for world updates on cultivars and biosecurity matters. Since schemes and nurseries provide the first line of defence against pest and disease incursions, it is also important to get exposure to biosecurity systems and practices implemented by other industries.

Highlights from the symposium talks are summarised below:

FRED GMITTER – “The Eternal Tale of the Dragon and the Phoenix”

Dr Gmitter presented a keynote address on the current chapter on Huanglongbing (HLB; or Asian Citrus Greening) in Florida, USA. He presented a time line of disastrous pest and disease incursions into Florida. This was firstly citrus leafminer in 1994, which was followed by Citrus Canker in 1995, and Asian Citrus Psyllid (ACP) in 1998.

From 2000, the USA attempted to eradicate Citrus Canker through a very aggressive programme: all trees within 1900 ft (579 m) from an infected tree had to be removed. More than 5 million nursery trees and >11 million residential and commercial citrus trees were destroyed; this included the Florida Citrus industry’s budwood foundation block, which at that stage was planted outdoors. This aggressive eradication programme was necessitated by prevailing weather conditions in Florida, specifically warm conditions and regular wind-blown rain events that are highly favourable for canker and its spread. The presence of leafminer exacerbated the problem, as the leafminer wounds were ideal infection portals for the canker bacterium. In 2006, the mandatory canker eradication programme was ended; it essentially failed after several hurricanes, finally Hurricane Katrina, spread the bacterium across the state and nullified the eradication efforts.

ACP was first detected in 1998 and was limited to backyard citrus trees; ACP was not controlled and by 2000 the pest has spread to 31 Florida counties. ACP is not an economically important pest of citrus when occurring in the absence of HLB. However, ACP is a highly efficient vector of HLB. HLB was first detected in Florida in 2005, but the widespread presence of ACP and slow response after first detection resulted in a large proportion of “hot” psyllids (i.e. psyllids carrying the HLB pathogen); this presented an overwhelming hurdle to effective eradication. It was considered that the “horse has bolted” and with the failed Canker eradication fresh in mind, HLB eradication was not attempted. The three-pronged approach, as employed effectively in Brazil [1 – plant disease-free trees; 2 – effective control of the vector; 3 – inoculum control through eradication of all infected trees], did not work due to poor grower participation. This was unfortunate, as the HLB destruction surpassed initial predictions and today Florida citrus yields are down by a staggering 70%! “The dragon has arrived”, as Fred Gmitter presented as a metaphor.

“But out of the ashes rise the Phoenix”, Dr Gmitter continued optimistically. Signs of a potential saviour, the metaphoric “Phoenix”, are escape trees in rootstock and cultivar evaluation trials, which indicate potential tolerance to HLB. These include the following:

- ‘Sugar Belle’ is HLB tolerant mandarin hybrid that showed growth and production despite HLB
- ‘Bingo’ is another mandarin hybrid of which trees have grown to 10 yrs before declining to HLB [*getting young trees to a productive age of 10 yrs before HLB decline is regarded as an economic benchmark*]
- Various rootstock cultivars (UFR1 to UFR17) have been “fast-track” released based merely on the fact that they were escape trees in rootstock trials in which all the other trees succumbed to HLB (resistance or tolerance has not been empirically proven).

Asked what Florida should have done differently, Dr Gmitter answered (1) that they should have controlled ACP upon first detection, (2) increased efforts for early HLB detection and (3) eradication early before the horse has bolted.

TIM HERRMANN – “Citrus Nursery Scene in Australia”

Tim Herrmann is the manager of AusCitrus, which is the Australian equivalent of the South African Citrus Improvement Scheme. AusCitrus was started in 1928. Presently it has some tunnels but most budwood is

supplied from a 3-ha open ground budwood orchard. The mother material is kept inside an insect-secure greenhouse structure, and it is planned that all budwood sources will move 'indoors'; however, funding has yet to be approved. AusCitrus also has 2 ha of rootstock seed trees.

AusCitrus supplies budwood and rootstock seed to 100 nurseries, which include many retail nurseries. However, seed supply (on a per tree basis) outstrips budwood supply, which is indicative of the common practice of using field material as budwood source by nurseries. They acknowledge that this presents a significant biosecurity risk. Most nurseries grow citrus trees in pots (using bark as medium; coir is increasingly being used) in shade house structures; there are still some open ground nurseries.

The biggest limitations for Australian nurseries are:

- Cost of labour - AU\$21-26 per hour for unskilled labour (25% extra for seasonal workers)
- Disease Management - only mild CTV, except for severe CTV in Queensland; some exocortis viroid is found in field-cut material; HLB and ACP are present in neighbouring islands and present a serious threat
- Illegal imports –post-entry quarantine (PEQ) via the use of shoot-tip-grafting (STG) is mandatory, but is very expensive (AU \$12,000 per cultivar)
- Tree production is not in insect-secure structures and present a biosecurity risk
- No nursery certification scheme
- Funding for AusCitrus is limited and exacerbated by the use of field-cut material (reduced income from budwood sales)

AusCitrus has started an initiative "Citrus Secure Australia", which is a voluntary budwood certification scheme, without any government endorsement yet. It was acknowledged, however, that a compulsory, government endorsed scheme is required to face the imminent biosecurity threats.

NERIDA DONAVAN – "Supply of Healthy Propagation Material to Australian Citrus Nurseries"

Dr Donovan, leading Citrus Pathologist providing diagnostic and technical services to AusCitrus and the Australian citrus industry, highlighted the major exotic threats: HLB and ACP, stubborn disease and Citrus Variegated Chlorosis. As part of an early warnings programme, they have active surveillance and awareness programmes on the Australian coast as well as the islands in Torres Strait. Dr Donovan's team also conducts diagnostic tests for AusCitrus, testing sources for viroids and viruses, seed source testing against *Citrus Leaf Blotch virus* (which is seed transmissible), CTV cross protection (she noted that they do not have a cross protection strain against the severe orange stem pitting CTV that occurs in Queensland), STG introductions of local selections, and development of awareness material and diagnostic tools.

GRAEME SANDERSON – "Variety Evaluation in Australia"

Graeme Sanderson has many years of experience in evaluation of citrus cultivars. He presented a talk on his programme, and the field trip also visited some of his trial sites. It is recommended that CRI's cultivar evaluators contact him to exchange information, particularly on rootstock cultivars.

NATHAN HANCOCK – "Citrus Australia National Overview"

Citrus production in Australia spans 26,000 ha, mostly for domestic market but with export increasing after concerted market access efforts. Exports are mostly to Japan, Hong Kong China and Asia. At present demand for citrus outstrips supply. Records indicate increased mandarin, lemon and early navel plantings, and declines in Valencia and mid-navel plantings. Private cultivars are increasingly being planted.

VERONICA HERRERA – "New Cultivars and the Challenge on the Obtention, Distribution and Commercialisation"

The talk presented an overview of models of private cultivar management. These models are all being implemented in South Africa and it appears that the extent of our experience with the private cultivar 'business' in SA surpasses those of other citrus producing countries. One aspect that was not addressed in the talk, was the implications of private cultivar management on citrus nurseries, particularly where nurseries are authorised / licenced to grow certain cultivars.

WU HOUJIU – “Citrus nursery industry in China”

Chinese citrus production is below 32° latitude, and is adversely affected by the presence of HLB. Following improved production practices China has recorded big increases in yield based on moderate increases in plantings. Plantings are 67% mandarin, 19% orange, and 12% pummelo. China has twenty large-scale nurseries spanning more than 300 hectares with 180 hectares of greenhouses; 30 million citrus trees are produced annually.

GEORGIOS VIDALAKIS – “Californian Citrus Protection Program and Californian Citrus Nursery Diagnostics”

Dr Vidalakis gave an excellent overview of the Californian Clonal Protection Programme (CCPP; their citrus improvement scheme) and its diagnostic support to nurseries amidst their fight against ACP and HLB incursion. As an exclusion control measure the cost of running the CCPP (\$ 0.5 million/yr) was relatively cheap compared with the cost of the surveillance and eradication programme in California (\$ 20 million/yr), and pales in comparison to Florida’s losses incurred following the widespread outbreak of HLB (\$ 5 billion/yr). As part of its biosecurity strategy, the CCPP supplies budwood to 1322 CCPP clients, of whom only 20 are citrus nurseries. Through improved methods, STG time was reduced to an average of 2 years, and improved and high throughput diagnostics reduced infection in multiplication stock at nurseries from 5% to <0.5%.

KLAUS BEDERSKI – “Soil Substrates for Certified Citrus Nursery Trees”

Mr Bederski’s nursery in Peru is in an area with minimal rainfall, and they face few pest and disease threats. It was therefore possible for them to convert to an organic nursery, with the biggest challenge being the availability of an organic substrate and fertiliser. Their recipe is composted and washed (to leach Na and Cl salts) dry manure, shredded green refuse, chicken feathers (it has a mineral composition of 11 N, 9 P, 2 K), river sand and river loam sand, and a fermented, germinated corn seed and molasses mixture. Before use, they test their substrate by germinating grass, cucurbit and legume seeds. Mr Bederski also mentioned the use of *Citrus depressa* (Taiwan tangerine) as excellent rootstock for grapefruit in Peru.

GRAHAM BARRY – “Global Trends in Citrus Varieties”

Growth in citrus production was observed in the East (7%) and in South Africa (3%), while a decline was recorded in the Americas, most probably as a result of HLB. China, USA and Brazil produce 50% of world citrus, but only 13% of this volume is exported; 25% of world production is in the southern hemisphere. Fresh exports are mostly (82%) from the northern hemisphere, 25% from Spain. Big (>50%) increases in export volumes were recorded from Turkey, Egypt, Peru and Chile. Mandarin and lemon plantings generally increased in recent years.

FRED GMITTER – “University of Florida-CREC Breeding program: New Scion and Rootstock Cultivars”

The Florida industry needs are mostly cultivars with better juice quality in oranges, seedless easy-to-peel mandarins, grapefruit without the detrimental “Grapefruit Juice Effect” (in combination with high blood-pressure medicine) and disease resistant cultivars, specifically against HLB. He presented an overview of the HLB tolerant UF950, SugarBelle, and also mentioned the fast-track release of 17 putative HLB tolerant rootstocks, which are available for evaluation elsewhere. These cultivars were not bred specifically for HLB tolerance, but were evaluation trees that exhibited tolerance amidst orchards where other trees were succumbing to HLB.

KIM BOWMAN – “Treatments to Improve shoot growth of newly budded nursery plants”

Slow growth from buds budded onto Swingle and US802 rootstocks were leading to slower field readiness. Treatments evaluated (during winter) were bud paint onto unwrapped bud, bud orientation and extra light (increasing daylight to 16 hours by artificial light from 01h30-0830). Benzyl adenine (BA) in paint increased budbreak and more on sun facing buds, but the orientation effect was not significant after 10 weeks. Longer shoots were measured in the BA paint treatment, while additional light increased rootstock sprouts. He did warn that previous experience with BA resulted in multiple sprouting from buds.

NATE JAMESON – “Rootstock effects on Substrate pH”

Brite Leaf Citrus Nursery grows all their citrus trees in 100% coir with drip fertigation. They find that uniformity in some rootstocks was poor, whilst others did not exhibit these problems. In order to investigate, they

“measured to manage”. Leaf sample mineral content data showed big differences between rootstocks and they started to measure input (fertigation) and output (leachate) and looked at trends in EC and pH for each irrigation. The pH and EC varied based on scion-rootstock combination. EC was better controlled when controlled throughflow was kept at 20%. They postulate that rootstocks affect OH⁻ and H⁺ release into substrate (perhaps related to rootstock pH tolerance) and will study this further.

TAHIR KHURSHID – “Citrus Nursery Management and Production Practices in Pakistan”

Dr Khurshid gave an overview of the industry in Pakistan where 90% of production is Kinnow mandarin and some blood orange production in the cooler north. Additional cultivars were needed to expand the supply window, and Australian researchers supported this new cultivar project with the additional aim to improve nursery hygiene. They helped by establishing a mother block and developed and provided information packages, which included a nursery manual, and rootstock and scion topworking manual. A copy of the citrus nursery manual was donated to the Citrus Improvement Scheme.

IAN TOLLEY – “Seed and Cultivar selection”

Mr Tolley stressed the importance of seedling grading and selection and noted that poor seedling selection results in poor orchard uniformity and performance, often accentuated by compatibility issues with off-type rootstocks. Mr Tolley is one of the founding members of the ISCN and wrote a book summarising his citrus nursery experience, titled “Commonsense Citrus: a hands-on guide to propagation & planting”. The book is available to view at <http://commonsensecitrus.com/>

JOHN McDONALD – “Nursery production farm management system in Australia”

The Nursery Production Farm Management System is an industry developed on-farm system for Production Nurseries that encompasses Best Management Practice, Biosecurity, Environmental and Natural Resource Management. The system is applicable to all plant nurseries and includes three programmes: the Nursery Industry Accreditation Scheme, Australia (NIASA) Best Management Practice, the environmental and natural resource management system EcoHort and the on-farm biosecurity programme BioSecure HACCP. Whilst not mandatory, the system enjoys government recognition and streamlines other auditing requirements and improves market access, particularly interstate movement of plant material. Feedback from nurseries that have implemented the system, was that improved production was experienced due to the improved management.

JOSE LIMA – “Nursery Techniques: Results of small trials run in the nursery situation”

Jose Lima manages Wonderful Citrus Nursery, which is a new state-of-the-art nursery in California and presented some practical nursery observations based on their experience. They perform seed coat removal prior to sowing, and sow one seed per Ellepot (<http://www.ellepot.com/>). Seed orientation is important and seeds should be placed flat in the pot. They transplant 45-180 days after sowing and bud 60 days after transplant. They force the buds by cutting, girdling or half-girdling above the stuck bud. For interstocks, they bud into the bark shield of the interstock, instead of waiting for the interstock shoot to grow out. They grow trees in containers and ship trees inside a biodegradable sock, and keep the container at the nursery.

ERICK VALLE – “Production of citrus trees in greenhouses”

California citrus tree production has mostly moved into greenhouses and feedback is that they generally experience at least a 30% faster productivity (6-8 vs. 12-14 month growing period). Erick Valle from Tree Source Citrus Nursery presented a talk on the management thresholds for environmental parameters. These are listed below:

- Temperature: manage daytime temperatures between 13 and 35°C, with optimal at 29-32°C
- Light: a quantity of 800-1000 micromoles/m²/second is required (a summer day is 2000 outside). A minimum of 12 h light duration is required for citrus growth and daylight should be extended in winter by additional light; alternatively, night time can be interrupted for 1 hour (around midnight) with low-level light (only 2-3 mM needed)
- CO₂: the minimum CO₂ requirement for citrus is 800 ppm, and seeing that trees are grown in closed systems they must supplement CO₂ levels in winter by using propane burners. Fresh air also works, but might reduce temperatures during cold periods.

- Relative Humidity: 70-80% RH is ideal

CESAR GRAF – “Citrus Nursery Tree Certification System in Brazil”

Cesar Graf is one of the largest citrus nurserymen in Brazil. He presented the citrus nursery tree certification system in Brazil. Vivecitrus is a society of citrus nurserymen with 11 members. It was established in 1986 and developed an ISO 9001-2008 certification system for citrus nurseries. The Vivecitrus board develops the certification criteria, but they use independent auditors to audit nurseries two times per year. One interesting criterion is a Systems Performance Indicator, where nurseries have to show year-on-year improvement. Through the requisite worker training (at least 8 hours training per employee per year) and participation, nurseries have experienced improved productivity.

JOHN CHAVARRIA – “Citrus variety trends in the world”

Based on experience in Spain and Australia, Mr Chavarria presented an overview of citrus variety trends. Modern variety trends are predominantly market driven: more pull from markets than push from a nursery level. This is somewhat different to the advisory role of traditional citrus nurseries. For varieties, shelf life was most important, apart from quality. Market opportunities exist for oranges in Asia with strong growth opportunities in China. Pigmented oranges offer new opportunity, but requirements are to extend the Cara Cara window and for a Cara Cara fruit with rind blush, and also the need for blood oranges with more consistent colour and better size. Strong soft citrus growth was observed in western markets, particularly for seedless varieties. Clementine declined due to mandarin growth, largely due to poorer shelf life. Large lemon growth was observed worldwide, and Mr Chavarria speculated that it was perhaps overplanted. Seedless lemons will become more important in future. Early navels before Navelina were in demand, as well as late navels, of which the Australian cultivars were generally not as good yielding as the South African varieties.

PAUL FOURIE – “Dynamic growth in South African citrus industry - coping with the demand for propagation material”

I presented an overview talk on the dynamic growth in the SA citrus industry, which has seen the number of citrus trees in nurseries double from 3.2 million to 6.7 million within a period of 4 years. The SA Citrus Improvement Scheme is unique in world terms as it is a central budwood supply scheme, and the initiatives employed by the Citrus Foundation Block to maintain at least a 70% primary budwood supply record were discussed. These include rapid multiplication in heated tunnels, expansion in greenhouse capacity, improved climate control and monitoring, irrigation water management and expanded trueness to type systems.

An initiative under investigation is *in vitro* rapid multiplication and was discussed with various experts. Dr Kim Bowman from USDA-Florida has a lot of experience in this field and noted that the risk of inducing mutations when using *in vitro* multiplication of developed nodes is very low (similar to that for conventional multiplication). This is supported by experience in other crops. However, the risk of multiplying an off-type bud in unstable lines to the large numbers possible should be carefully considered.

In vitro multiplication (or tissue culture) of rootstock cultivars is employed in Florida where there is a big demand for new rootstock varieties (without rootstock seed orchards) or where rootstock seed orchards were killed by HLB. Nate Jameson received a large quantity of tissue culture rootstocks, but experienced some problems with these: poor root development, bushy development as opposed to liner development of seedlings, slow bud push, and generally a lot more work in nursery with 20 to 30% loss and slower tree development. Kim Bowman is doing research comparing rootstock seedling vs cuttings vs tissue culture development in nurseries as well as orchards.

DAZHI LI – “Identification Technology of Citrus Seedlings”

Radio frequency technology using electronic chips implanted into citrus trees was developed in China. Various small (1.2 × 4 mm) chips from PVC or steel are stapled to trees with a staple gun and read with a scanner. Wounds healed and did not influence growth and chips lasted >5 years. Cost is estimated at about 10 US cents per chip, but will depend on quantity.

KIM BOWMAN - Successful Propagation of Rootstock Stem Cuttings

Dr Bowman presented an efficient method for propagation of rootstock plants from stem cuttings. Bowman and his co-worker Ute Albrecht recently published this method in the *Scientia Horticulturae* journal in 2017

(volume 225, pages 681-688) in an article titled “Efficient propagation of citrus rootstocks by stem cuttings” and is briefly summarised here. Single-node cutting (with leaf attached to each node) is cut from 2-5 month old rootstock plants in the nursery. The cuttings are prepared by reducing the leaf area to 20-30% of its original size; thereafter the basal end is dipped in a commercial rooting powder containing 0.3% IBA (indole-3-butyric acid), and immediately inserted into pre-moistened soilless potting mix, with the leaf base and node above the potting media. Their mist-bed was operated using an automated system controlled by both a wet leaf sensor and timer (Mist-a-matic controller, www.growerssolution.com); under their conditions in Florida, the cutting were misted for 6 s every 5-10 min during daylight hours, and rarely at night. During the fifth week, the plants received a liquid fertilizer application of water-soluble fertilizer (20N-10P-20 K) at a rate of 400 mg per litre N. The misting was stopped after 6 weeks and the plants received another liquid fertilizer application that included chelated iron (Sequestrene 138 Fe). Greenhouse conditions were 27-35°C, and a shade cloth in the house was closed from 09h00 to 18h00 during the first 6 weeks. At the beginning of the seventh week, the shade cloth was left open continuously. Subsequently, plant care was the same as applied to normal citrus greenhouse plants, with alternating water irrigation and liquid fertilizer application, and periodic insecticide applications, as needed.

In their study, Bowman & Albrecht compared the growth of various rootstock cultivars, and there was a significant difference between different cultivars. Rootstock plants (12 week old) grown from seedlings had a similar plant weight as cutting-grown rootstock plants (12-16 week old), and a similar 80:20 shoot to root ratio, but pronounced differences were observed in the structure of root development (more adventitious root development and multiple primary roots in cuttings compared with seedlings, which generally developed one primary root only). Their research is continuing and will also compare field performance.

Bowman proposed that single node cuttings be made from tops of rootstock plants in the nursery, but it should be kept in mind that citrus tree production in Florida is exclusively inside insect-secure structures. It is important to consider the risk of spread of graft transmissible diseases that might have infected the young rootstock seedlings in the nursery. Whilst very few citrus pathogens are seed transmissible, young seedlings can be infected by means of mechanical or insect transmission of pathogens. Rootstock cuttings should therefore be sourced from inside an insect-secure structure and strict cutting tool sterilisation should be employed.

ROGER SMITH / ERICK VALLE – “IPM and Biological control in Citrus Nurseries”

Tree Source Citrus Nursery in California used to be an open-ground nursery, but moved to container-grown citrus nursery trees inside insect-protected structures following the mandatory California biosecurity rules. One of their biggest pests inside the greenhouses is two-spotted spider mites, and they soon exhausted the miticide options. They were forced to investigate alternative options, and since 2014 tested and developed a biological control programme. This programme was successful and was expanded to all their greenhouses. The benefits from this biological control programme include sustainable pest control, a safer work environment, better treatment coverage for high density crops, no re-entry restrictions into greenhouses, relatively little training required to apply biocontrol agents. They highlighted certain challenges of a biocontrol programme: successful implementation can take up to 3 years; successful biological control programmes require preventing insect and mite problems rather than reacting to them; predicting pest pressures and planning releases according to those pressures to prevent pest explosions; chemicals that are safer for beneficial insects are often more expensive; California government regulations require treatment of all shipments out of the ACP quarantine with both systemic and foliar pesticides.

Against two-spotted spider mites and citrus red mite they employed *Amblyseius californicus* and *Phytoseiulus persimilis*. Thrips can be controlled with *Orius* minute pirate bug, but will require a pepper purple flash as banker plant system as food source and breeding ground to maintain the *Orius* population. *Amblyseius swirskii* and *A. cucumeris* are both generalist predators and will feed on some other insect and mite species besides thrips. Both mites feed only on the first larval stage of thrips, making both of these predators suitable for prevention.

Aphids can be controlled using the parasitoid *Aphidius colemani*, which has flying and searching capabilities. Oils and insecticidal soaps are used to knock down aphid populations in hot spots. The aphid banker plant system uses grain plants (such as barley, wheat, or oats) and a cereal aphid (bird cherry oat aphid) to sustain the parasitoid population. The banker plants provide a constant source of aphids readily available to parasitize.

This allows for *A. colemani* to continuously increase their population leaving enough parasitoids to control the pest in the surrounding area.

Bacillus thuringiensis can be used as alternative to insecticides against leaf roller, whilst entomopathogenic nematodes can also be used as general insecticide treatment.

A successful programme requires effective scouting. They require at least 10 hours each week to complete 2.5 ha greenhouse space, and use a colour coding system in their scouting reports to indicate pest freedom, low, moderate and high levels of infestation.

Suppliers of their biocontrol products are Biobest (www.biobestgroup.com), Bioline Agrosiences (www.biolineagrosiences.com), and Beneficial Insectary (www.insectary.com).

FIELD TRIPS

DARETON RESEARCH STATION

The research station spans 250 ha and is used for research and cultivar trials as well as for commercial purposes. Graeme Sanderson demonstrated some Citrus Viroid dwarfing trials. It is allowed in the AusCitrus scheme where CVd-infected material is made available to nurseries to inoculate trees. However, it is not popular as these CVd-infected trees were more susceptible to drought situations. CVd-dwarfed plantings were also generally irregular, since trees on which the inoculation was not successful will not be dwarfed. They also found that CVd dwarfing accentuated in growth limiting rootstocks, such as C35. An interesting trial was demonstrated where Macrophylla rootstock was used in a CTV cross protection trial. The CTV sensitive rootstock declined when preimmunised with CTV, while the CTV-free trees were clearly more vigorous (Fig. 1).



Fig. 1. Poor growth of CTV pre-immunised trees on the CTV sensitive Macrophylla rootstock

AUSCITRUS

The AusCitrus farm and facilities outside Dareton was visited. AusCitrus is staffed by a manager, Tim Herrmann, an admin assistant and three field staff. They maintain, multiply and supply 215 cultivars; 108 of these are open cultivars. The open cultivars are funded by grower levy, while the private cultivars are funded by the owner/agent.

Introduction of all cultivars are subject to mandatory shoot-tip-grafting (STG) and diagnosis to ensure pathogen freedom. Following STG, AusCitrus receives one tree, which is then multiplied and tested for trueness-to-type (TtT). AusCitrus will not sell material prior to TtT designation. If private cultivar owner/agent insist on early release, they will have to acknowledge responsibility.

Budwood production is still in field orchards and foundation (mother) trees are grown in an insect-proof greenhouse (Fig. 2). Mother trees are grown in 50-L pots of pine bark or coir in greenhouses with permanent shading and misting for cooling. Mother trees are tested every 3 years to ensure pathogen freedom and testing is funded by budwood sales. For TtT identification, nucleus block and mother trees are fruited, and cultivars are also submitted to evaluation trials at the nearby Dareton Research Station by Graeme Sanderson.



Fig. 2. Insect-proof greenhouse at AusCitrus

They also have some multiplication in shade-houses (not in insect-secure structures); these trees are used for 4 years only to minimise the disease threat. There are plans to move into insect secure structures, but funding is a constraint.



Fig. 3. Shade-house for multiplication trees at AusCitrus

Irrigation water is drawn from the Murray River, via a sand filter and chlorine dioxide is used to sanitise the water. Fertigation is completely computerised with EC set at 1.2, and an alarm set at EC of 3. They monitor pH and EC of leachate, or test bottoms of pots. For pest control, they use a fogger (Fig. 4), which is fully automatic on a timer. However, they struggle with miticide options and two-spotted spider mite is a problem that requires more regular treatments.



Fig. 4. Fogger used to apply pesticides in the greenhouses at AusCitrus

About 2000 L of seed is harvested annually. Seed extraction is done using a similar, but more automated system than South Africa's Citrus Foundation Block (Fig. 5). Extracted seed is triple-washed in water, then sodium bicarbonate is added to lift last debris before final rinse. Seed is heat treated (52°C for 10 min) and dried in an ambient air drier. Dried seed is treated with Thiram Flo in a fungicide slurry treater then quick-dried in closed system with a dehumidifier before it is packed and coldstored (Fig. 6).



Fig. 5. Seed extraction machine at AusCitrus



Fig. 6. Fungicide slurry treater and treated seed (blue) at AusCitrus

Labour cost is very expensive and many tasks are supported by mechanisation; for example, back-mounted forklift on tractor, forklift, seed slurry treater, fogger, etc. Another interesting innovation was the shoe sanitation sponge mats, which are incorporated with a sanitiser. Since these mats are easy to move, it is also used to sanitise shoes of visitors when exiting cars or buses (Fig. 7).



Fig. 7. Shoe sanitation sponge mats used in Australian nurseries

6.4 A report on the Norman E. Borlaug international agricultural science and technology fellowship program at the department of Botany and Plant Sciences at the University of California Riverside (UCR) in California, USA from 17/08/2017 to 13/11/2017

Ockert (Jakkie) PJ Stander, Citrus Research International (Pty) Ltd, Department of Horticultural Sciences, University of Stellenbosch, South Africa.

PI: Dr. Martha Orozco-Cardenas, Academic coordinator & director Plant Transformation Research Center, department of Botany and Plant Sciences, UCR.

Mentor: Dr. Carol J. Lovatt, Professor of Plant Physiology, Emeritus & Plant Physiologist, Professor in the graduate division, Department of Botany and Plant Sciences, UCR.

Co-operator: Dr. Songjin Pan, Academic coordinator & Director Proteomics Core Facility, Department of Botany and Plant Sciences, UCR.

Introduction

The primary objective of the fellowship was to undergo training under the guidance of an experienced mentor, to conduct three complimentary lines of research at the early stage of my scientific career:

- (i) Quantification of fruit-induced changes in plant phyto-hormone profiles;
- (ii) Identification of the mechanisms underlying these changes through assessment of the activity of genes regulating hormone titers (i.e., genes regulating hormone synthesis, catabolism, reversible and irreversible inactivation, export, and import) in fruit, floral buds, or other tissues; and;
- (iii) Quantification of expression of genes regulating *Citrus* spp. floral development.

This training would develop and enable me to conduct complimentary research to my current research project investigating alternate bearing in *C. reticulata*. The fellowship would assist me in establishing these protocols at my home institution in South Africa, and to share the knowledge with fellow researchers and students to the benefit of the broader Southern African community.

Other important objectives were to develop relationships with the host research institutions and establish possible opportunities for research collaboration; to receive specific lectures from my mentor on topics related to my field of research interest; to visit citrus production areas and important industry role players; and for the mentor at the USA host institution to visit my home institution in South Africa.

This is a report on my fellowship, which consists of a summary of activities for the period of stay in Riverside, California in the USA, protocols and results for analysis of gene expression and phyto-hormone quantification, and a report on the visit to citrus producers in the Central Valley of California.

Summary of activities

Date	Day	Trainer	Activity
8/21	Mon.	CL ^z /MOC ^y	Introduction to gene expression. Gel electrophoresis training.
8/22	Tues.	MOC	Cowpea RNA isolation training using Trizol reagent.
8/23	Wed.	MOC	Cowpea DNase treatment training.
8/24	Thur.	MOC	Preparation of report and reagents for cowpea cDNA synthesis.
8/25	Fri.	CL/MOC	Feedback of progress and cowpea cDNA synthesis training.
8/26	Sat.	CL	Citrus tissue sampling, preparation and storage protocols training.
8/27	Sun.	CL	Citrus tissue sampling, preparation and storage protocols training.
8/28	Mon.	MOC	On-boarding at UCR and cowpea normal PCR training.
8/29	Tues.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
8/30	Wed.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
8/31	Thur.	MOC	Cowpea q-PCR training and citrus samples to bioanalyzer.
9/1	Fri.	MOC	Preparation of feedback on results.
9/2	Sat.	-	-
9/3	Sun.	-	-
9/4	Mon.	-	-
9/5	Tues.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
9/6	Wed.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
9/7	Thur.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
9/8	Fri.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
9/9	Sat.	-	-
9/10	Sun.	-	-
9/11	Mon.	MOC	Isolation of citrus RNA using Trizol reagent (experiment).
9/12	Tues.	MOC	Citrus DNase treatment and citrus cDNA synthesis (experiment).
9/13	Wed.	MOC	Primer set design training using NCBI and IDT websites.
9/14	Thur.	MOC	Normal PCR of citrus samples (experiment).
9/15	Fri.	MOC	Results of normal PCR of citrus samples (experiment).
9/16	Sat.	-	-
9/17	Sun.	-	-
9/18	Mon.	MOC	q-PCR of citrus samples (experiment).
9/19	Tues.	MOC	Feedback preparation.
9/20	Wed.	MOC/CL/SQ P ^x	Meeting with Jim Remcheck.

9/21	Thur.	MOC	Normal PCR for ref. gene and LFY in citrus samples (using a new primer).
9/22	Fri.	CL	Ordering of new hormone internal standards (ISs) and lecture on hormones (CL).
9/23	Sat.		-
9/24	Sun.		-
9/25	Mon.	SQP	Introduction to UPLC and mass spectrophotometry.
9/26	Tues.	SQP	Testing of old ISs in UPLC and mass spec.
9/27	Wed.	SQP	Testing of old ISs in UPLC and mass spec.
9/28	Thur.	MOC	Testing of old ISs in UPLC and mass spec.
9/29	Fri.	MOC	Normal PCR of citrus samples (exp.).
9/30	Sat.		-
10/1	Sun.		-
10/2	Mon.	Wonderful citrus	Central valley production area visit.
10/3	Tues.	Wonderful citrus	Central valley visit production area visit.
10/4	Wed.	Wonderful citrus	Central valley visit production area visit.
10/5	Thur.		Research day Visalia.
10/6	Fri.	Wonderful citrus	Ventura production area visit.
10/7	Sat.		-
10/8	Sun.		-
10/9	Mon.	SQP	Preparation 1 for hormones extraction and freeze-dry of samples.
10/10	Tues.	SQP	Extraction attempt 1 of hormones from citrus tissue (overnight).
10/11	Wed.	SQP	Extraction attempt 1 of hormones from citrus tissue.
10/12	Thur.	SQP	Extraction attempt 1 of hormones from citrus tissue.
10/13	Fri.	SQP	Sample batch 1 on UPLC/Mass Spec.
10/14	Sat.		-
10/15	Sun.		-
10/16	Mon.	SQP	Samples batch 1 on UPLC/Mass Spec.
10/17	Tues.	SQP	Preparation 2 for hormones extraction attempt 2, and freeze-dry of samples.
10/18	Wed.	SQP	Extraction attempt 2 of hormones from citrus tissue (overnight).
10/19	Thur.	SQP	Extraction attempt 2 of hormones from citrus tissue.
10/20	Fri.	SQP	Sample batch 2 on UPLC/Mass Spec.
10/21	Sat.		-
10/22	Sun.		-

10/23	Mon.	SQP	Preparation 3 for hormones extraction attempt 3.
10/24	Tues.	SQP	Extraction attempt 3 of hormones from citrus tissue (overnight).
10/25	Wed.	SQP	Extraction attempt 3 of hormones from citrus tissue.
10/26	Thur.	SQP	Sample batch 3 on UPLC/Mass Spec.
10/27	Fri.	SQP	Sample batch 3 on UPLC/Mass Spec.
10/28	Sat.		-
10/29	Sun.		-
10/30	Mon.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
10/31	Tues.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/1	Wed.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/2	Thur.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/3	Fri.	MOC	Primer set design and order.
11/4	Sat.		-
11/5	Sun.		-
11/6	Mon.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/7	Tues.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/8	Wed.	MOC	cDNA synthesis and q-PCR of citrus samples (exp.).
11/9	Thur.	SQP	Method development and analysis of UPLC/Mass Spec. results.
11/10	Fri.	SQP	Laboratory clean-up and pack-up.
11/11	Sat.	MOC/CL/SQ P	Farewell dinner.
11/12	Sun.		-
11/13	Mon.	CL	Return to South Africa

^z Prof. Carol Lovatt

^y Dr. Martha Orozco Cardenas

^x Dr. Sonqin Pan

Protocols: Quantification of expression of genes regulating citrus floral development

1. RNA extraction

(See addendums A1 and A2 for detailed protocols)

Always be cleanly shaven and wear a lab coat, closed shoes, clean gloves and safety goggles. Cover the bench space with sanitised protective covering, and clean your bench space and hands beforehand (and throughout) with 70% ethanol. Organise your equipment and chemicals in a synchronised pattern. Prepare for the extraction process before starting, by dating and writing the method and work plan in a size A4 notebook. Use calibrated pipettes, and sterilized pipette tips and Eppendorf tubes. Water should always be RNase-free. Do not process too many samples at once – less samples provide shorter working time and greater chance for uniform results; therefore ideally use 9 [3 biological (tree) replicates × 3 technical (tubes) replicates] to 12 [4 biological (tree) replicates × 3 technical (tubes) replicates] samples per batch of analysis. Handle all samples uniformly and always work in a consistent and ordered manner. First sterilize equipment by wrapping scalpels, tongs, and pestle and mortar with aluminium foil (Fig. 1A), and placing the wrapped equipment in an autoclave (Fig. 1B) for 1 h in a *gravity* cycle, or, if the equipment are sterilised in the same cycle as that of liquids, in a *liquid* cycle. Sterilise enough equipment so that each sample can be extracted cleanly. While waiting for the autoclave cycle to finish, clearly label 12 × 1.5 mL Eppendorf tubes with a permanent marker (e.g. 1.1, 1.2, 1.3...), and pipette 1 mL of Trizol reagent (Fig. 2) to each labelled tube on ice. Subsequent to use, the Trizol reagent should always be kept cool at 2 °C. During the latter part of the fellowship a Qiagen® Isolation Plant RNA Mini was used. The protocol is provided in the manufacturer handbook. Always make sure that the caps of the tubes are fully closed when kept on ice. If they are not, water droplets from melted ice can enter and contaminate the reaction. Remove the equipment from the autoclave and remove the foil from the equipment. Before using the equipment in the grinding and extraction processes, make sure it is dry and thoroughly cooled down with liquid nitrogen (N). It is very important to ground the fresh, frozen tissue to an as fine as possible texture. To disintegrate frozen, fibrous or woody tissues such as roots, shoots or buds to a finer structure, a sterilized and cool automated aluminium coffee grinder can be used in a quick, initial grinding step, but ALWAYS keep the sample in a frozen state. When cleaning the coffee grinder in-between samples, avoid using alcohol on its plastic surfaces. Transfer the frozen tissue from the grinder to the mortar in liquid N, and continue grinding with the pestle until the tissue is very fine. Before transferring the ground and frozen tissue to an Eppendorf tube, cool down the scalpel and tong with liquid N. Zero the tubes on a scale and quickly add ≈100 mg fresh and frozen sample tissue. It is extremely important to have a ratio of sample to Trizol reagent in the Eppendorf tube as close as possible to 100 mg sample: 1 mL Trizol reagent, but do not waste too much time to get the ratio perfect, since the frozen tissue will defrost before it can lyse with the reagent. Lyse the sample by vortexing the Eppendorf tube vigorously for ≈1 min, until the sample tissue and Trizol reagent is in a uniform solution. Subsequent to use, the Trizol reagent should always be kept cool at 2 °C. The goal is that every structure and each surface of the frozen sample tissue should make similar contact with the reagent. Incubate the tubes at room temperature (15 to 30 °C) for 5 min. In a fume hood, add 200 µL chloroform (Fig. 3A) to each tube. Close the caps of the tubes and vortex vigorously for ≈15 to 20 s. Incubate the tubes at room temperature for 3 min. Centrifuge the tubes at 10 500 rpm and 4 °C for 15 min. While waiting for the centrifugation step to finish, label a new set of sterilised 1.5 mL Eppendorf tubes. Very carefully remove the tubes from the centrifuge. In each tube, a separation of different phases will be visible – at the bottom of the tube a pellet containing proteins, pigments and structural carbohydrates, and a clear aqueous phase containing the extracted RNA at the top of the tube (Fig. 3B). Do not disturb or mix the layers. If the layers mix by accident, centrifuge again until the layers are separated. Carefully transfer the top, ≈380 µL aqueous phase to the new labelled tube on ice. In the fume hood, add 500 µL isopropanol alcohol to each tube. Close the caps and incubate the tubes at room temperature for 10 min. Do not mix or vortex the tubes after adding the isopropanol alcohol. Centrifuge the tubes at 10 000 rpm and 4 °C for 10 min. During centrifugation, the isopropanol alcohol will precipitate the RNA contained in the chloroform to a small, nearly invisible pellet at the bottom of the tube. Remove the samples from the centrifuge and carefully discard the supernatant. To wash the RNA in the pellet, add 1 mL cold 75% ethanol to each tube. It is important to prepare the 75% ethanol solution using RNase-free and not regular or distilled water. Close the caps of the tubes and vortex for ≈10 s. Centrifuge the tubes at 8000 rpm and 4 °C for 5 min. Very carefully discard the supernatant. Add 1 mL cold 75% ethanol to each tube. Close

the caps of the tubes and vortex for ≈ 10 s. Centrifuge the tubes at 8000 rpm and 4 °C for 5 min. Very carefully discard the supernatant. Open the caps of the tubes and leave the tubes to air-dry on paper towels for at least 15 min (Fig. 4). The tubes must be completely dry. To solubilize the clean and extracted RNA from the pellet, add 50 μ L RNase-free water (Fig. 5) and mix the gel-like precipitate containing the RNA, by using the pipette tip to dissolve in water, and keep on ice. The RNA is now isolated from the fresh sample tissue and ready for total RNA quantification and testing of the RNA quality using NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The RNA is not yet ready for use in a polymerase chain reaction (PCR), but only isolated from the tissue. It still has to be cleaned and a cDNA template has to be created. The NanoDrop Spectrophotometry gives an indication of the success of the extraction, or quality of the sample tissue (Fig. 6). Addendums B1 and B2 are protocols for determinations without NanoDrop, using manual spectrophotometry. Open the software and select the nucleic acid function. Type in RNA and first add 2 μ L RNase-free water to zero/blank/calibrate the machine for subsequent readings. Clean the reader with clean paper towel after reading the blank, and continue the reading of the samples.



Fig. 1. A) Sterilize equipment by wrapping scalpels, tongs, and pestle and mortar with aluminium foil. B) Place the wrapped equipment in an autoclave for 1 h in a *gravity* cycle.

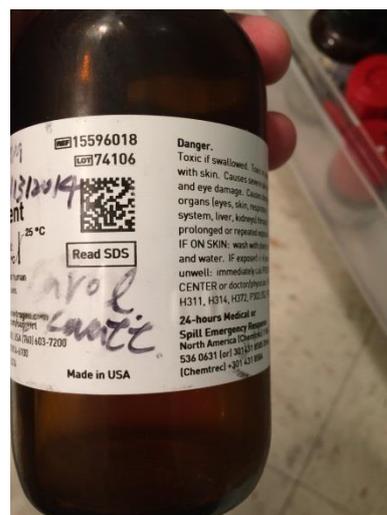
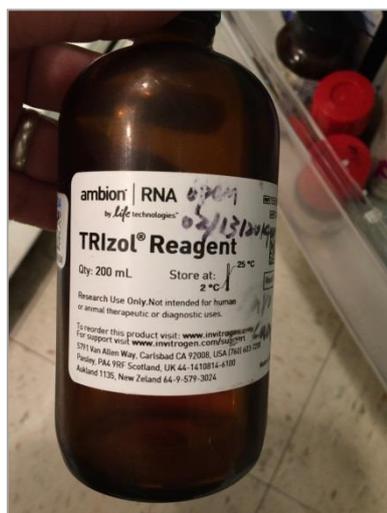


Fig. 2. The Trizol reagent should always be kept cool at 2 °C.

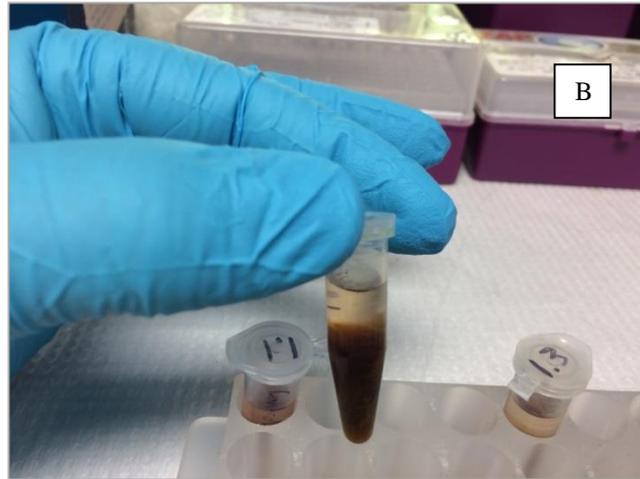


Fig. 3. A) Subsequent to adding 0.2 mL chloroform to the sample, B) a separation of different phases will be visible – at the bottom of the tube a pellet containing proteins, pigments and structural carbohydrates, and a clear aqueous phase containing the extracted RNA at the top of the tube.



Fig. 4. After washing the precipitate with 75% ethanol, discard all of the supernatant and be sure to allow the pellet to air-dry thoroughly, for at least 15 min.



Fig. 5. Be sure to always use certified RNase-free water. Do not contaminate and store at room temperature.



Fig. 6. Total RNA quantification and testing of the RNA quality are done using NanoDrop Spectrophotometry.

2. Testing the quality of the extracted RNA or DNA using gel electrophoresis

Subsequent to extraction of RNA from sample tissue, the total and quality RNA extracted can be determined using NanoDrop Spectrophotometry. The NanoDrop reading will give a concentration value (concentration, ng per μL), as well as a 260/280 value - the ratio of the absorbance measured at wavelengths 260 and 280 nm, which is an indication of the RNA quality. A ratio value close to 2.0 is good quality RNA. The quality of RNA can also be confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Carla, CA, USA). It is of critical importance to extract good quality RNA from the fresh and frozen sample tissue. Gel electrophoresis is an additional method to evaluate and confirm the quality of the extracted RNA. From these three methods of checking RNA quality, the best of the three technical replicates should be selected to represent the biological replicate (e.g. a tree replicate) in the subsequent normal and q-PCR assays. To check RNA quality using gel electrophoresis, first prepare an agarose gel. Prepare ≈ 50 mL agarose for every 6 samples – 1% agarose for DNA or 2% for RNA testing (see addendums C1 and C2 for agarose recipes). For testing of RNA quality (2% agarose) of 12 samples, prepare ≈ 150 mL of the following agarose solution (using 15 x 25 cm loading trays), stepwise, in a 200 mL Erlenmeyer flask:

Add 3 mL 50x TAE^Z solution (already prepared), 147 mL de-ionized water and 1.5 g molecular biology grade agarose (Fig. 1). Heat the solution in the microwave to boiling point for ≈60 s and let stand. Add 4 uL ETBr (10 mg/mL) and stir gently. Be careful when handling ETBr. Always wear gloves and discard of the pipette tip in a separate container, since the chemical is highly carcinogenic. Also be careful in handling the gel, always wear gloves and clean the surfaces that made contact with the gel properly. Before loading the agarose solution to the loading trays (Fig. 2), seal the 15×25 cm loading tray thoroughly with electrical masking tape on the open 15 cm ends, and insert the white combs. The 15 cm ends are open so that the electrical current in the TAE solution can move through the gel. The tape is added before loading the agarose solution in the loading tray. Do not let the agarose solution in the Erlenmeyer flask stand for longer than 10 min, because it will dry and solidify in the flask. Add the solution in the Erlenmeyer flask to the loading tray, and let stand for ≈20 min to form a gel. For preparation of the reaction to load in the agarose gel, carefully label 12×200 uL Eppendorf tubes (Fig. 3), close the caps and keep on ice. Calculate the correct ratio of RNA: water: loading buffer^Y to add in each 200 uL Eppendorf tube. The volume loading buffer (see addendum D for loading buffer recipes) added to each sample should always be one sixth the volume of the total eventual sample volume to be loaded to the agarose tray (20 to 30 uL). The volume RNA to add to each tube should be 600 to 1500 ng calculated from the NanoDrop readings. The volume RNA required always amounts to between 2 to 20 uL, depending on the quantity of RNA extracted from the fresh sample tissue. Store the rest of the extracted RNA at –80 °C for later use. Make up to final volume with RNase-free water and centrifuge at 14000 rpm and 4°C for 30 s (quick) to mix all the reagents. Remove the white combs from the tray, make sure the TAE solution covers the gel, and carefully add 15 uL Hind III marker (molecular weight) in the first opening on the left side of the agarose gel and continue loading the respective samples in the next openings to the right. Try and leave spaces between samples of different treatments. Add the cover of the loading tray and provide electrical current of 90 to 120 Volts for 40 to 60 min (Fig. 2). Turn off the electrical current, remove the cover of the loading tray and very carefully remove the agarose gel to take a picture for quality check (Fig. 4). Factors affecting the quality of the RNA include ineffective grounding of samples that causes insufficient exposure of cells to the Trizol extraction solvent; incorrect ratio of sample tissue to Trizol reagent, or DNA contamination, i.e. the equipment was not sterile. Figure 5 is an example of good and bad RNA quality. From here on forward the best of the three technical replicates will be selected to represent the biological replicate in the subsequent normal and q-PCR assays.

^Z 100 mL 0.5 M edta (pH 8.0)
1 L/242 g TRISBASE;
57 mL Glacial acetic acid glacial;
10 mL water
Add: 1) Edta 2) trisbase 3) acetic acid 4) water

^Y Recipe for purple 6x or 10x gel loading buffer (New England Biolabs, #70245):
30% (V/V) glycerol;
0.25% (w/v) bromophenol blue;
0.25% (w/v) xylene cyanol FF,
store at 4°C

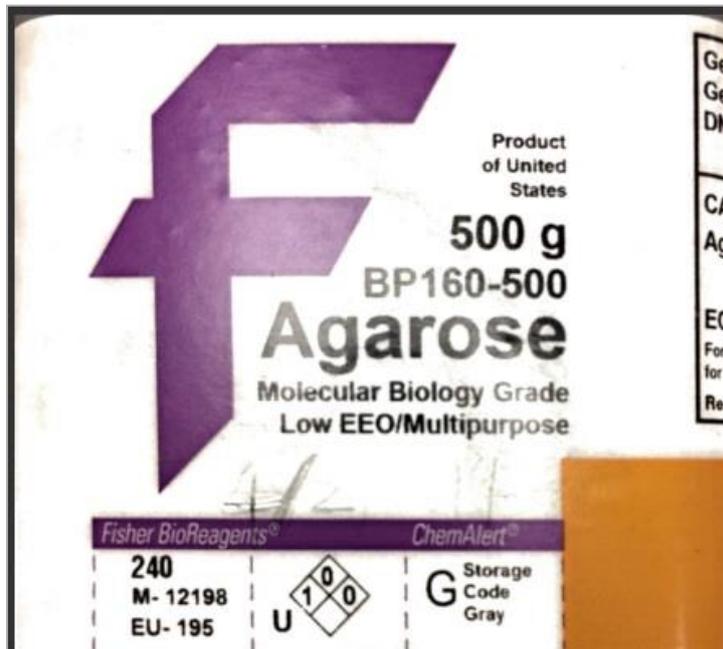


Fig. 1. It is important to use molecular biology grade agarose with a low melting point.

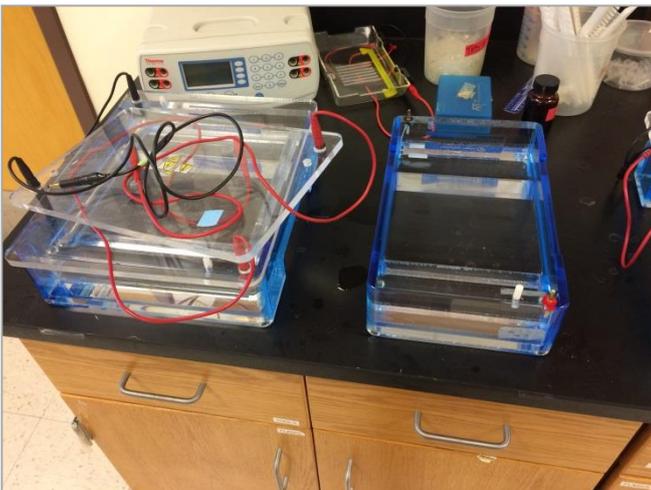


Fig. 2. Gel electrophoresis system used to test the quality of RNA or DNA.

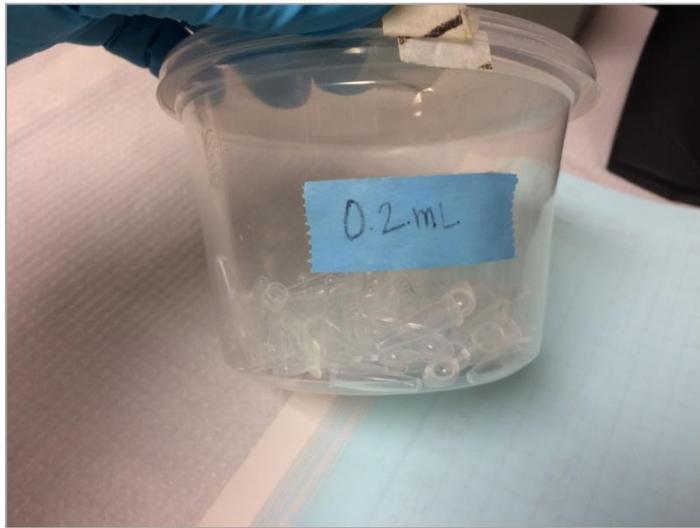


Fig. 3. Use smaller tubes when testing RNA quality.

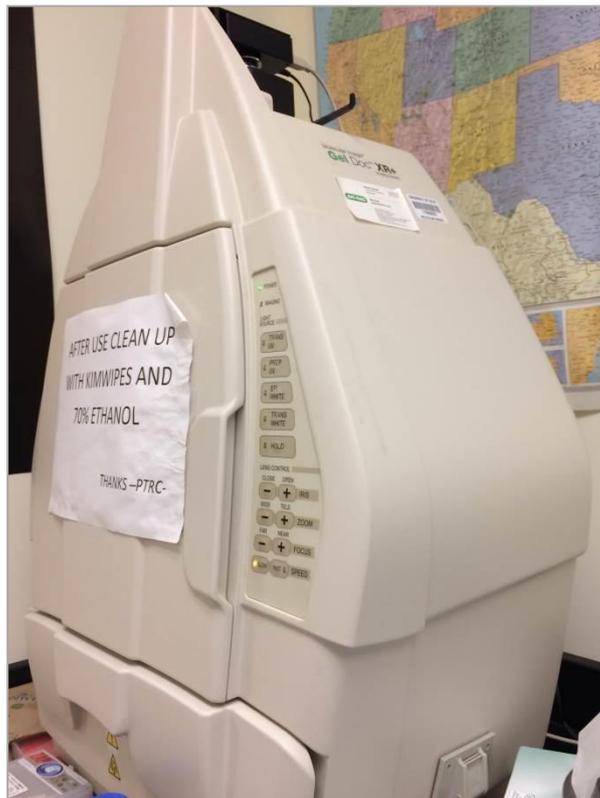


Fig. 4. The gel doc station used to photograph the agarose gel and evaluate the RNA quality subsequent to gel electrophoresis.

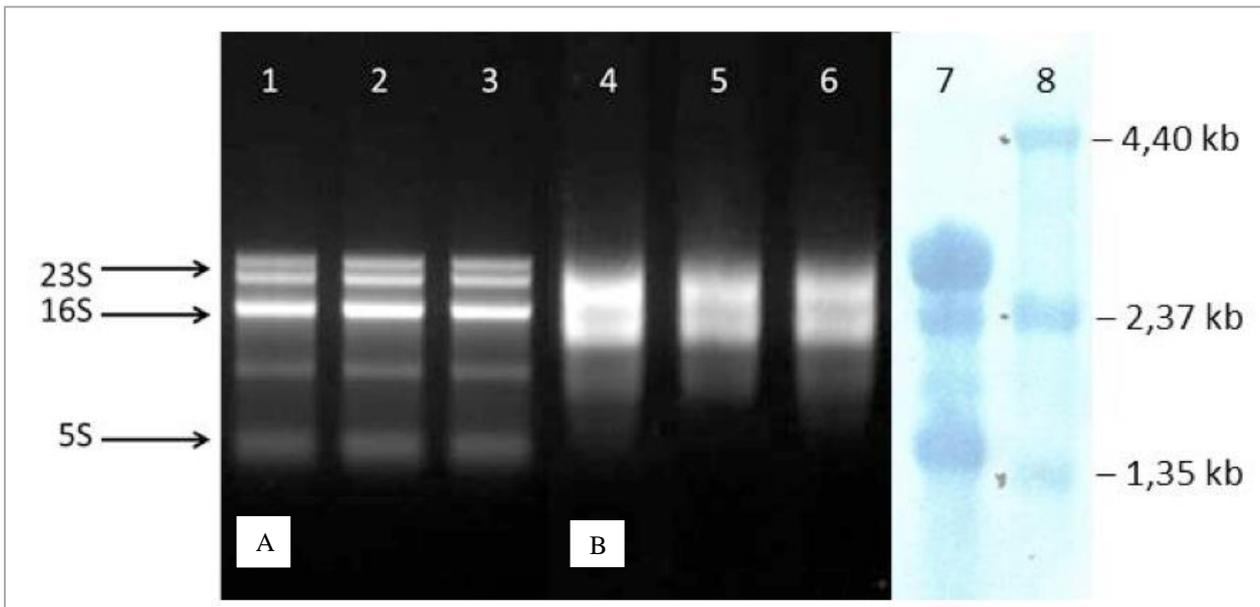


Fig. 5. An example of A) good and B) bad quality RNA. In A the bands can be clearly distinguished, in B the bands are blurred and indicate signs of tissue contamination.

2.1. Citrus results: RNA quality check

Table 1. An example of citrus RNA quality tested with a NanoDrop Spectrophotometer and a Bioanalyser.

Biological replicate (tree fruiting status)	Technical replicate	[RNA] using NanoDrop Spectrophotometer ng/uL	260/280 ratio (a ratio of 2 is good)	[RNA] measured with Bioanalyser ng/uL
OFF 1	1.1	388	1.89	368
	1.2	642	2.06	302
	1.3	590	2.08	418
OFF 2	2.1	345	2.05	452
	2.2	552	2.00	390
	2.3	279	2.07	984
OFF 3	3.1	267	2.07	1051
	3.2	424	2.09	525
	3.3	887	2.05	91
OFF 4	4.1	555	2.03	758
	4.2	333	1.98	364

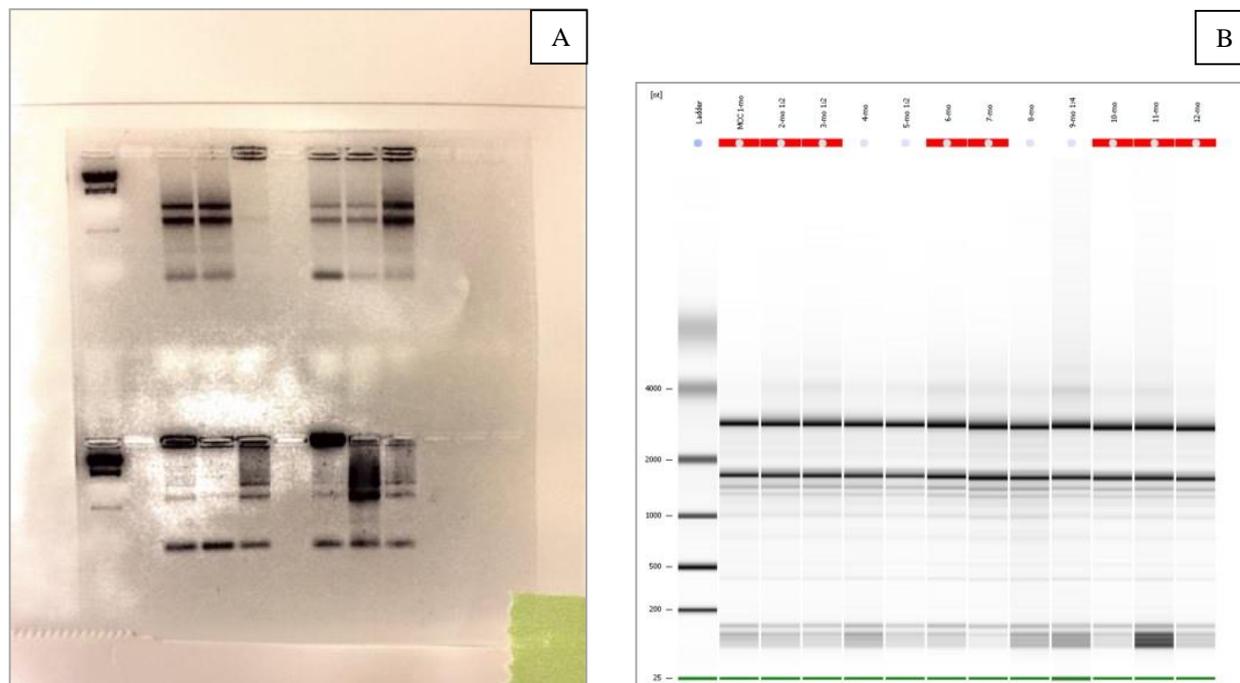


Fig. 1. Tests of citrus RNA quality using A) gel electrophoresis, and B) a Bioanalyser.

3. DNase treatment of extracted RNA

(See addendum E for full protocol)

At the end of the RNA extraction, the RNA precipitate at the bottom of the tube was made up to a volume of 50 μ L with RNase-free water, from which 2 to 20 μ L was used to check the RNA quality using gel electrophoresis. From the remaining volume, 1000 ng RNA should be used in subsequent steps to clean the RNA, create a cDNA template and for use in normal and q-PCR assays. In this step, any contaminant DNA will be removed from the extracted RNA. It is very important to do all steps on ice, since you are now working with extracted RNA that is sensitive to high temperature. Make sure that the caps of the tubes are always fully closed when kept on ice. If they are not, water droplets from melted ice can enter and contaminate the reaction. You are also working with very small volumes, and even a small droplet of contamination can completely wreck the results. Record the calculations for every reaction of each sample in your notebook. It is very important that the order of pipetting (in 200 μ L tubes) should always be the following:

- 1) RNase-free water
- 2) 1 μ g RNA (each of 1 μ g RNA should be treated with the same volume DNase enzyme)
- 3) DNase buffer (comes with kit)
- 4) RQ1 RNase-Free DNase (Promega, Madison, WI, USA)

Incubate the tubes at 37°C for 15 min in the thermal cycler.

Add 1 μ L EDTA (25 mM) to stop the DNase activity.

Incubate the tubes at 65°C for 15 min in the thermal cycler.

Place on ice for 1 min.

Spin the tubes quickly at full speed (14 000 RPM) and keep on ice.

Freeze the samples at -20 °C.

4. Firststrand cDNA synthesis using Superscript II RT using random primers

You now have 1000 ng of clean RNA, from which you have to create 500 ng (20 μ L) cDNA template (see addendum F for full protocol) to use in PCR assays.

If the RNA was frozen, remove from freezer and defrost on ice. Centrifuge the sample quickly at full speed to collect the contents before using it in subsequent reactions. Clearly label new tubes to which you will pipette the following to a final volume of 12 μ L:

10 μ L RNA (500 ng)

1 μ L random primers (comes with kit)

1 μ L dNTPs (comes with kit)

Once again, it is very important to close the caps of the tubes thoroughly – if the caps of the small tubes are not tightly closed they tend to flip open in the thermal cycler, which will cause evaporation of the solution and destruction of the sample.

Quickly (30 s) centrifuge the tubes at full speed (14 000 rpm) to mix the reagents.

Incubate in the thermal cycler at 65 °C for 5 min and quickly chill on ice.

Quickly (30 s) centrifuge the tubes at full speed (14 000 rpm) to collect the contents of the tube.

Add 4 μ L of 5x first strand buffer (comes with kit).

Add 2 μ L DTT (0.1 M) (comes with kit) and mix the reagents gently by slowly pipetting the contents of the tube up and down for 5 s.

Incubate in the thermal cycler at 25 °C for 2 min.

Add 1 μ L (200 units) of superscript II RT (comes with kit) and mix the reagents gently by slowly pipetting the contents of the tube up and down for 5 s.

Add 1 μ L RNase-free water.

Incubate in the thermal cycler at 25 °C for 10 min.

Inactivate the reaction in the thermal cycler at 70 °C for 15 min.

You now have 20 uL or 500 ng cDNA of each sample. Make sure each cap are fully closed and store the samples at -80°C .

Normal PCR to determine gene expression

Normal PCR can be used as a qualitative mean to determine if certain genes are expressed in the sample tissue at a certain time-point, or in plants in reaction to two contrasting treatments, for example. The method uses an agarose gel electrophoresis test and cannot be used to determine fold-expression of a gene of interest relative to another. Use equipment, i.e. pipettes, pipette tips etc. that are allocated for PCR assays only. The equipment should be clean and sterile, and be kept in a clean prep station that reduces the risk of sample contamination while performing PCR assay experiments (Fig. 1). Use the 20 uL (500 ng) cDNA template. Calculate volumes for reagents to prepare a master mix for each gene of interest that contains the forward and reverse primers (primer sets) of each gene, Tag polymerase (comes with kit, see addednum G for description), and certified RNase-free water (this water should only be used in PCR assays, and be lkept in the clean prep station). A volume of 23 uL of the master mix will be added to 2 uL (50 ng) of the cDNA template in each clearly labelled 200 uL tube. There will be enough cDNA to do 10 PCR reactions, i.e. 10 different genes of interest for each sample (10 reactions \times 2 uL = 20 uL cDNA template).

The following is an example of the reagent details of PCR assays used for analysis of expression of the reference (ref.) gene *ELONGATION FACTOR1-alpha (EF1- α)*, *FLOWERING LOCUS T (FT)*, *APETALLA 1 (AP1)*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* and *LEAFY (LFY)* in citrus samples used in the experiment in citrus:

- A) *EF1- α*
- B) *FT*
- C) *AP1*
- D) *SOC1*
- E) *LFY*

Prepare separate master mixes for each of the genes A, B, C, D and E, and add 23 uL of each master mix in each 200 uL tube:

- 1) Tag polymerase – 12.5 uL \times 15 samples = 187.5 uL
- 2) 10 uM Forward (F') primer – 1 uL \times 15 samples = 15 uL (different for A, B, C, D and E) (diluted from 100 uM stock)
- 3) 10 uM Reverse (R') primer – 1 uL \times 15 samples = 15 uL (different for A, B, C, D and E) (diluted from 100 uM stock)
- 4) RNase-free water – 8.5 uL \times 15 samples = 127.5 uL (pipetted 1st)

Always include a non-template control (NTC) which is a tube that contains a reaction with all the reagents, bar the cDNA template. The NTC is used to indicate whether or not contamination occurred in any of the reactions, i.e. the equipment dirty or was there carry-over of plant sample tissue from a pipette to another tube. The gel channel that contains the NTC should not show anything subsequent to gel electrophoresis. The ref. gene is a gene that is always expressed in the organism of interest. It should show expression in all the treatments and is of specific importance in q-PCR, to “normalize” the intensity of expression of other genes or of different treatments relative to one another. If the ref. gene is expressed in one treatment, but not the other, there is probably something wrong with the treatment’s sample tissue or with the RNA that was extracted and cDNA that was subsequently created. After adding all the reagents, make sure the caps are tightly closed. Mix the reagents by brief centrifugation before loading in the Thermal Cycler (Fig. 2). The reaction detail for the normal PCR reaction in the experiment in citrus are shown in Figure 3.



Fig. 1. To reduce the risk of sample contamination all equipment for PCR assays should never leave the clean prep station.

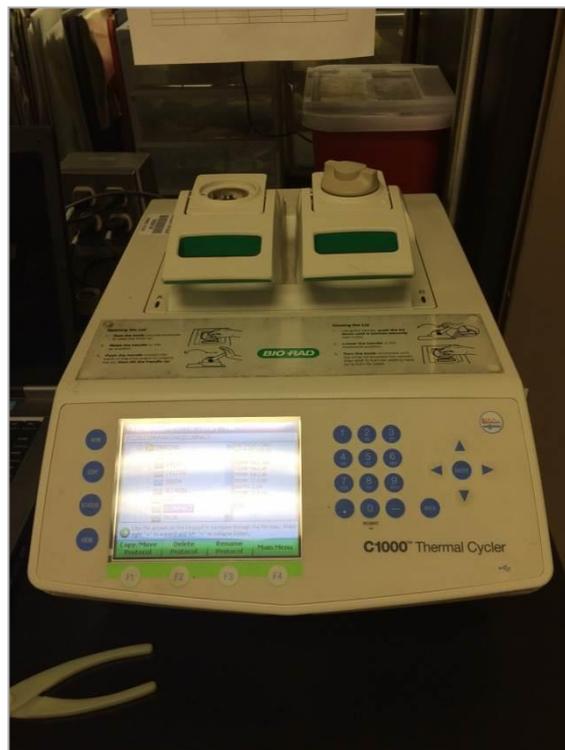


Fig. 2. The BioRad C1000 Thermal Cycler used for PCR reactions.

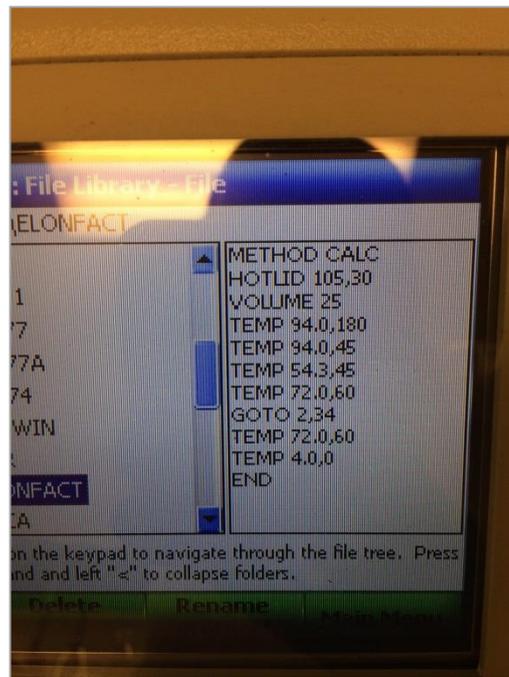


Fig. 3. The conditions for the normal-PCR assays in the Thermal Cycler.

4.1. Citrus results: Normal-PCR

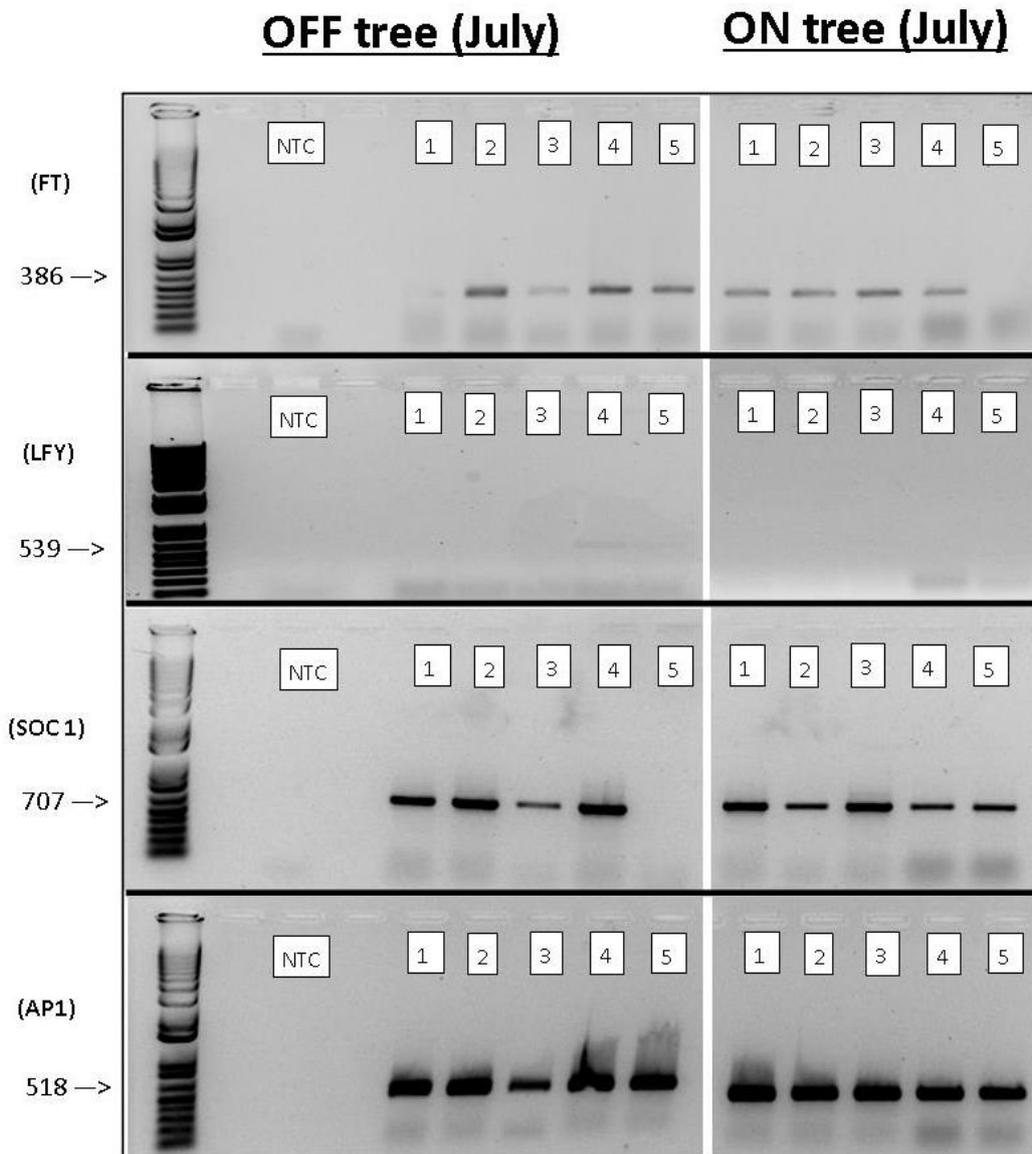


Fig. 1. An example of normal-PCR results from bud samples in the citrus experiment. Normal-PCR results are not quantitative, but indeed qualitative. The *LFY* gene, for example, is not expressed in any of the replicates (1 to 5) in either of the two (OFF or ON trees) treatments; whereas the *FT* gene is weakly expressed in both treatments, and the *AP1* gene is highly expressed in all the replicates of both treatments. The non-template control shows that no contamination occurred, i.e. no DNA was present in the specific tubes.

5. Quantitative real-time PCR (q-PCR) to quantify gene expression

Similar to normal PCR, q-PCR can be used to determine if genes are expressed or not, but as opposed to normal PCR, q-PCR is used as a mean to quantify the expression of gene of interest in the sample tissue and compare expression across treatments or different samples relative to each other. Similar to normal-PCR, the reaction of the q-PCR assay will consist of the forward and reverse primers (primer set) of each gene, Tag polymerase (comes with kit), and certified RNase-free water. Calculate volumes for reagents to prepare a master mix for each gene of interest that contains the primer sets² of each gene, SensiMix™ SYBR & Fluorescein (2X) (Bioline USA Inc., Taunton, MA) (comes with kit, see addednum G for description) and

certified RNase-free water. Be very careful and concentrate when pipetting all the reagents to the q-PCR plate. A total volume of 20 μ L, containing 2 μ L [50 ng, according to SensiMix™ SYBR & Fluorescein (2X) manufacturer] of the cDNA template will be added to each of the tubes in the q-PCR plate. Use equipment, i.e. pipettes, pipette tips etc. that are allocated for PCR assays only. The equipment should be clean and sterile and be kept in a clean prep station that reduces the risk of sample contamination while performing PCR assay experiments. Always include a NTC, which is a tube that contains all the reagents except the cDNA template. The NTC is used to indicate whether or not contamination occurred in any of the reactions. It should have no expression, i.e. a Ct value reading of N/A. The ref. gene is a gene that is always expressed in the tissue of the organism of interest. It should show expression in all the treatments, and its Ct value is of specific importance in q-PCR to “normalize” the intensity of expression of other genes and compare the strength of the expression of the genes relative to each other and relative to different treatments. Make sure loading plate is tightly sealed, and mix the reagents by brief centrifugation before adding to the loading plate to the Thermal Cycler. The q-PCR assays in the citrus experiment were carried out using the CFX96 Touch™ real-time PCR detection system with C1000 Touch™ thermal cycler (Bio-rad Laboratories, Hercules, CA, USA) in a 20- μ L reaction system containing 2 μ L (50 ng) cDNA, 0.5 μ L gene-specific forward and reverse primer mix (10nM), 10 μ L SensiMix™ SYBR & Fluorescein (2X) (Bioline USA Inc., Taunton, MA, USA), and 7 μ L PCR-grade water. Each reaction was run at 95 °C for 10 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. Using Ct values less than 35 obtained from q-PCR, relative levels of expression (fold change) of the genes of interest were calculated using the Pfaffl method for the experiment in citrus (see section 6.1). The expression or Ct values of the OFF trees in July were used as the control, to which the rest of the treatments and treatments in other months were compared.

^z The sequences of the genes of interest can be obtained from GenBank and Reference Sequence databases (National Center for Biotechnology Information [NCBI] <http://www.ncbi.nlm.nih.gov>) and the gene-specific primers designed using the web-based Integrated DNA Technology PrimerQuest program (<http://www.idtdna.com/primerquest/Home/Index>).

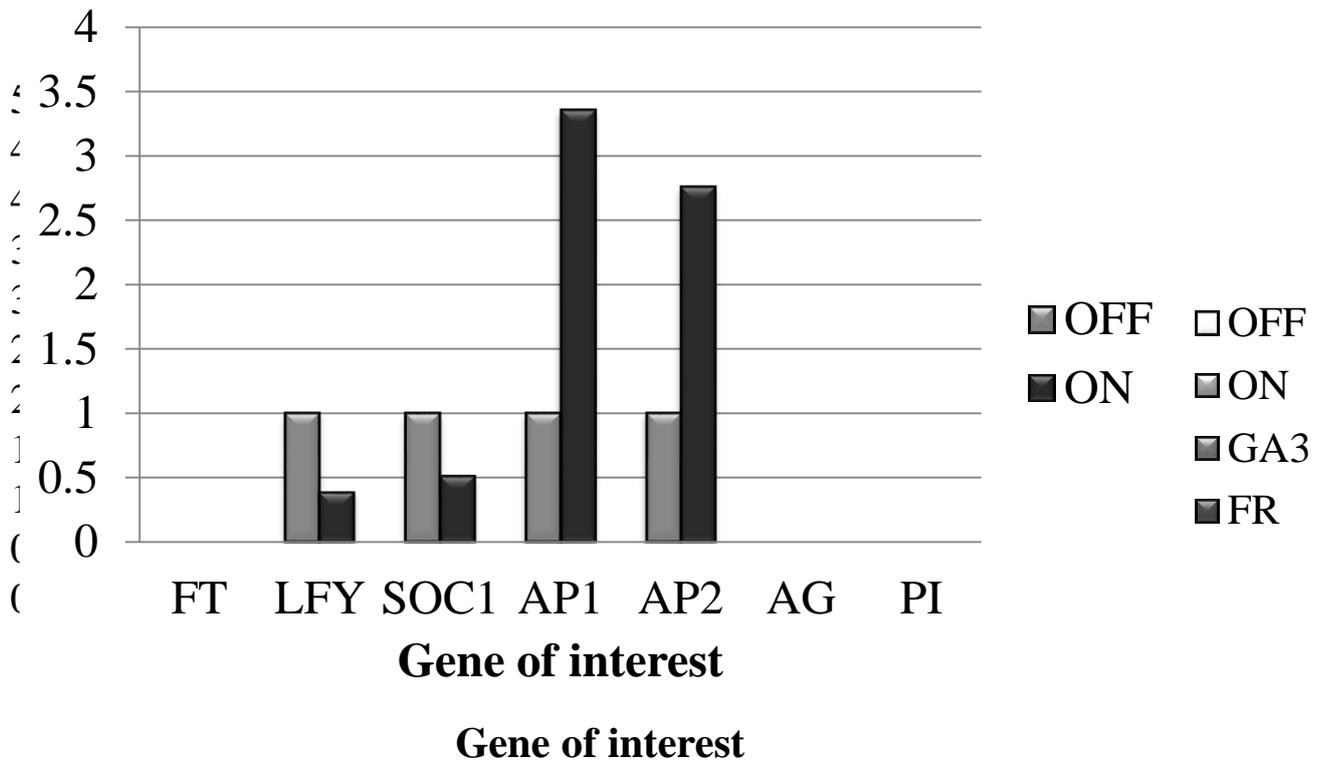
The sequences and the product sizes of the primer pairs used in the experiment to analyse genes of interest in citrus were obtained from Lisa Tang's research papers forming part of her PhD study (Table 1).

Table 1. Forward and reverse primers for the citrus target and reference genes used in the q-PCR assays in the citrus experiment.

Annotation	Accession number (<i>Citrus spp.</i>)	Forward primer (5' to 3') Reverse primer (5' to 3')	Product size (bp)	PCR	product
				sequence against target sequence E-value	blast target gene Identity
<i>FT</i>	AB027456.1 (<i>C. unshiu</i>)	CCGCGTTGTTGGTGATGTTCTTGA ATTTAGCCCTAGGCTGGTTTCAGA	132	6E-37	95%
<i>SOC1</i>	EU032532.1 (<i>C. sinensis</i>)	TCGACCCAACGGAAAGAAGCTGTA TGCCTAGAAGATTGCAGGAAGCCA	139	5E-46	98%
<i>LFY</i>	AY338976.1 (<i>C. sinensis</i>)	TCTTGGGACAAAGCATCAACAGCG TCAAAGCTGCTGTTAGGGCTGAGA	112	3E-25	92%
<i>AP1</i>	AY338974.1 (<i>C. sinensis</i>)	ACCGCTCTCAAACACATCAG GCAGCCTTCTCTCTCTCC	137	7E-38	96%
<i>AP2</i>	EU883665.1 (<i>C. trifoliata</i>)	AAATGAAGCTGACTGGCACAACCG AGCGATGATGAAGCTGGTGACTGA	138	9E-18	95%
<i>SEP1</i>	AB329715.1 (<i>C. unshiu</i>)	TGCTGAGGTGGCTCTCATCATCTT TCTCGAGCTCCTTTGCTGGCTTAT	146	1E-32	90%
<i>PI</i>	XM_ 006472790.1 (<i>C. sinensis</i>)	ATGGCCTTAGAGGATGCCCTTGAA AGCTATCTCCTGTTGCCAGAACA	144	2E-36	92%
<i>AG</i>	HM246523.1 (<i>C. sinensis</i>)	GGGAAGTTGACTTGCACAACAGCA TAGCTCCGGGAATCAAATGGCTGA	142	1E-30	97%
<i>ACT</i>	GU911361.1 (<i>C. sinensis</i>)	TCACAGCACTTGCTCCAAGCAG TGCTGGAAGGTGCTGAGGGA	130	7E-34	98%

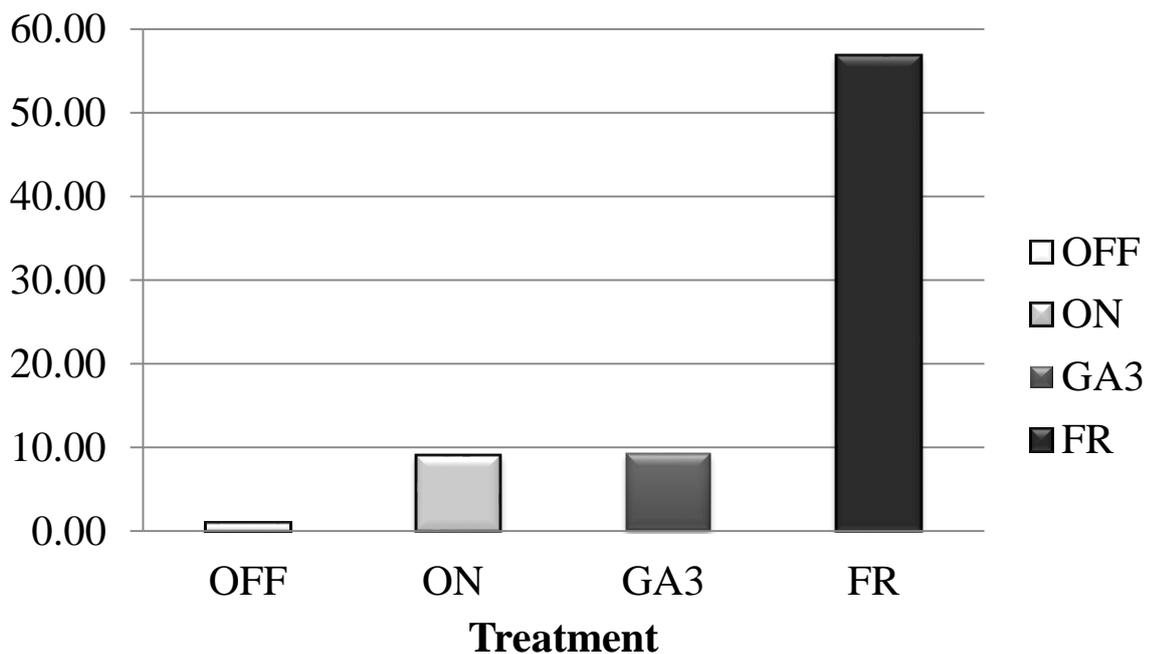
The database sources for the accession numbers: NCBI GenBank and Reference Sequence databases (<http://www.ncbi.nlm.nih.gov>)

Fold change expression: July



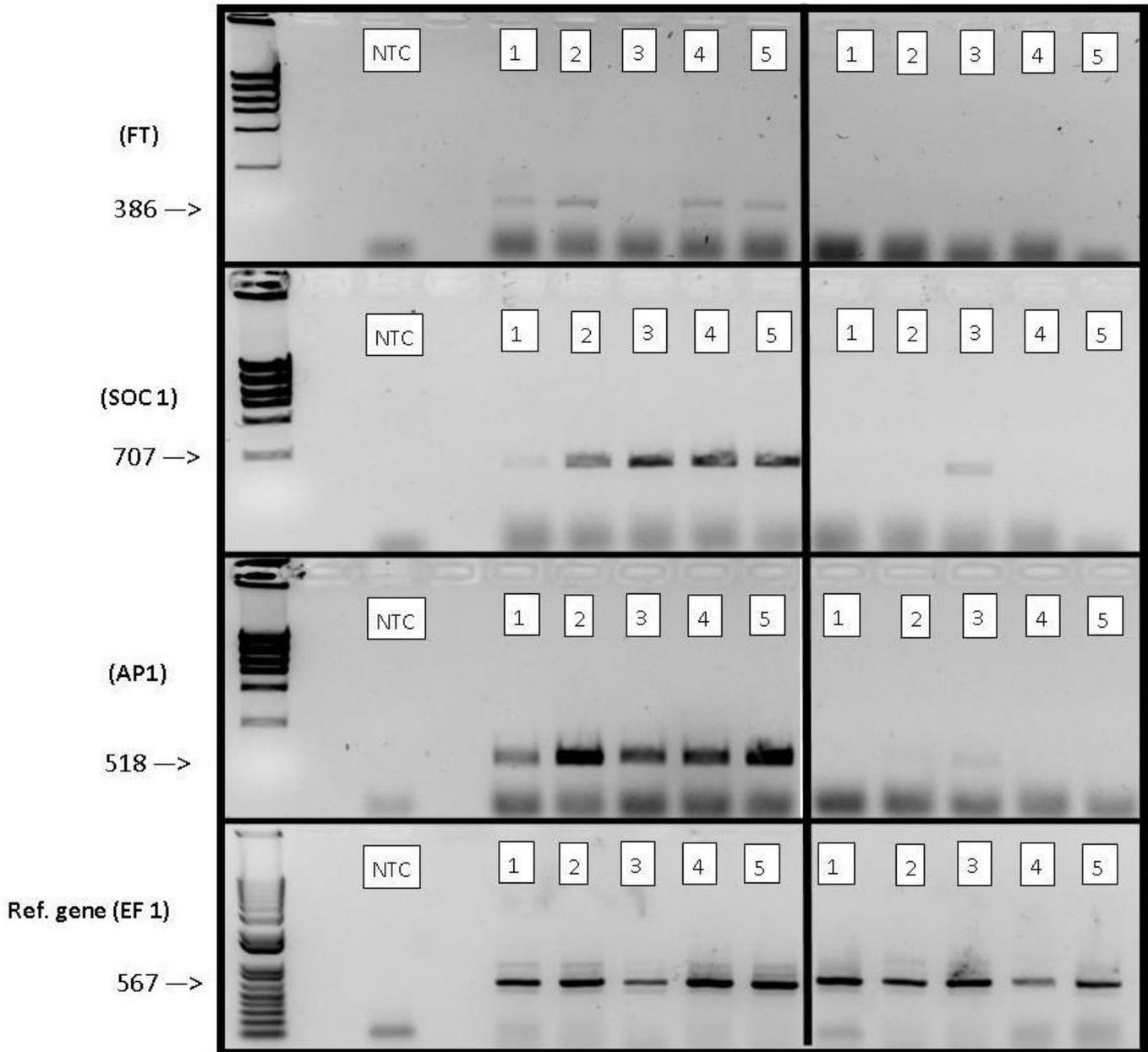
Fold change expression: July

Fold change expression: SEP1 August



OFF tree

OFF + GA₃



Protocols: Attempts to extract and quantify phyto-hormones in citrus in a single Ultra-high Performance Liquid Chromatography and Mass Spectroscopy run

1. Optimisation of conditions for Ultra-high Performance Liquid Chromatography and Mass Spectroscopy (UPLC-MS)

There is currently no protocol at UCR or the University of Stellenbosch for the quantification of multiple phyto-hormones from plant tissue using a single UPLC-MS run. The fellowship provided an opportunity to initiate a collaborative attempt to develop such a protocol using citrus as a model plant. Most of the method development on extraction and quantification of phyto-hormones from citrus tissue was based on previous methods of extraction of phyto-hormones from citrus leaves developed in the laboratory of Prof. Carol Lovatt, and by Kojima et al. (2009, 2012) for extraction and quantification of phyto-hormones from leaves of tobacco (*Nicotiana tabacum* L.) using UPLC-MS. In the first step in the attempt we had to determine the optimal conditions of the mobile phase, i.e. the correct solute [e.g. the % methanol, formic acid (FAC) or acetic acid (HAC)] and pH in which to dissolve all the phyto-hormone substances in order for them to remain stable and be detected using Mass Spec. We also had to determine the concentration limit of detection for each of the phyto-hormones, and, depending on the molecular charge of the derivitized phyto-hormone substances subsequent to dissolving, the mode in which the hormone is detected i.e. a positive or negative ion mode. The publication of Kojima et al. (2012) provided the pattern of derivitization of the various phyto-hormone catabolites which could be used to positively identify the respective phyto-hormones in the Mass Spec. analysis. We prepared a master mix of synthetic phyto-hormones, viz. abscisic acid (ABA), the cytokinin 6-benzyl adenine (6-BA), the gibberellin gibberellic acid (GA₃) and the auxin indole-3-acetic acid (IAA) and its derivatives, indole-3-pyruvic acid and indole-3-acetamide in a range of stock solution concentrations (1mM to 10 nM) in 100% methanol, to a 50% methanol working solution with either 0.1% FAC or 0.1% HAC.

The results of detection in the Mass Spec. analysis are presented in Table 1.

The phyto-hormones ABA, GA₃, and the auxin-like compounds indole-3-pyruvic acid and indole-3-acetamide could all be detected in the negative ion mode, whereas 6-BA could only be detected in the positive ion mode and IAA in both the positive and negative ion modes. In concurrence with findings of Kojima et al. (2009), the positive ion mode was most sensitive for detection of phyto-hormones at low concentrations. Gibberellic acid and IAA, for example, were poorly detected by Mass Spec. at a concentration as high as 1 uM, whereas 6-BA and indole-3-acetamide could be detected at concentrations as low as 10 nM. For all the hormones to be quantified by a single UPLC-MS run, a single mode has to be decided on, which would mean that some of the hormones such as GA₃ and IAA (in this case) would not be detected if the run was made in the negative mode and if the concentrations of the endogenous hormones in the plant tissue were low.

Table 1. Method development for phyto-hormone using Internal standards (9/25 to 9/28).

Compound	Mono-isotopic-mass	1 mM	0.1 mM	0.01 mM	1 uM		100 nM	10 nM
		FAC ^z	FAC	FAC	FAC	HAC ^y	FAC	FAC
ABA	264.136	(-) ^x	(-)	(-)	(-)	(-)	(-)	X
GA₃	346.141	(-)	(-)	(-)	(-)*LOW	(-)*LOW	(-)*LOW	X
6-BA	225.101	(+) ^w	(+)	(+)	(+)	(+)	(+)*LOW W	X
Indole-3-acetic acid (IAA)	174.055	(+)/(-)	(+)/(-)	(+)/(-)	(-)*LOW	(-)*LOW	X	X
Indole-3-pyruvic acid	203.058	(-)	(-)	(-)	(-)*LOW	(-)*LOW	X	X
Indole-3-acetamide	174.199	(-)	(-)	(-)	X	X	X	(+)*LOW W

^z 50% methanol (100%) and 50% formic acid (0.1%)

^y 50% methanol (100%) and 50% Acetic acid (0.1%)

^x Detected in negative ion mode

^w Detected in positive ion mode

*Detection was low

2. 'MS-probe' modification

To enhance the sensitivity of negatively charged compounds such as GA₃ and IAA, we introduced a chemical modification with bromocholine developed by Kojima et al. (2009), called 'MS-probe'. Bromocholine possesses a positively charged quaternary amine moiety (Honda et al. 2007) and the modification transforms a natural compound containing a carboxyl group into a positively charged compound by conjugation. The method therefore enables detection of phyto-hormones such as GA₃ and IAA using the positive ion mode in Mass Spec. The method is described in detail by Kojima et al. (2009) and further modified by Kojima et al. (2012). Briefly, all the phyto-hormones were reconstituted in 75 µl of 1-propanol. A 20 µl aliquot of water, 4 µl of 500 mM bromocholine in 70% acetonitrile and 0.8 µl trimethylamine were added to the solution. The mixed solution was incubated in a water bath at 80°C for 130 min, and then moved to ice. The solution was evaporated and reconstituted with 50 µl of 0.05 % formic acid, and subjected to UPLC-MS analysis using the positive ion mode. We applied this MS-probe method to the analysis of plant samples. After fractionation by Oasis MCX 96-well plate, the Elution 1 fraction that contains auxins, ABA and gibberellins is partially purified by anion-exchange spin column chromatography. After recovery, the products are reacted with MS-probe and then subjected to UPLC-ESI-qMS/MS analysis using the positive ion mode. The modification with MS-probe permits these hormone derivatives to be measured in a single UPLC run. The analysis of the pure synthetic phyto-hormone standards revealed that the quantification limits of the modified compounds were greatly enhanced. For example, GA₃ and IAA could be detected at a concentration of 1 nM in the positive ion mode. The implication of the 'MS-probe' modification is that the different phyto-hormones would still have to be extracted from plant tissue in different elutes, e.g. cytokinins and acidic phytohormones such as ABA, gibberellins (GAs) and IAA in separate elutes. The elute containing the acidic hormones would have to be modified with bromocholine and then analysed in the positive ion mode together with the cytokinins.

3. Extraction and detection of phyto-hormones from citrus buds using UPLC-MS

We had to undergo several attempts to optimize the extraction process of phyto-hormones from citrus buds. We managed to successfully extract and detect some individual phyto-hormones from citrus buds, such as GA₁, GA₇ and GA₉ (which we apparently managed to successfully modify by the 'MS-probe'), and the cytokinins 6-BA (surprisingly), trans-Zeatin, and cis- and trans-Zeatin-O-Glucoside from a few replicates in one treatment. Unfortunately, there was not enough time to repeat the analysis in the same tissue or to use more samples and/or different tissue types (e.g. leaves, fruit and roots). We could also not determine what the cause(s) was of the variation in results, e.g. why only some of the phyto-hormones could be detected and others not, and why some of the phyto-hormones were only detected in some replicates of the same treatment. It could be that some phyto-hormones were not present in the plant tissue at the moment of sampling, but there are a few factors in the extraction process that need to be optimized in future collaborative endeavors. For example, avoiding exposure of sample tissue to direct light throughout the extraction process, and regulating and lowering the temperature and drying time of the extract solutions in the various extraction steps. Some of the samples dried down substantially faster than others, which might have contributed to the non-uniformity in results. We also had problems to regulate the temperature of some of the speed-vac runs – in some of the runs the temperature inside the speed-vac rotary evaporator rose to very high levels, which might have contributed to the destruction of some compounds and explains the variation in results! Nevertheless, the development of a protocol to quantify multiple phyto-hormones from plant tissue using a single UPLC-MS run is complex and very difficult and we managed to get some positive results in a very short period of time. We are therefore excited to continue our collaborative attempts to develop a protocol to successfully extract and quantify phyto-hormones.

Citrus industry visit: Citrus production in the Central Valley of California

1. History

Citrus was introduced to California in 1769 by missionaries, or so-called 'padres'. Back then, citrus trees were mainly grown in and around San Diego in the South, towards San Jose in the North, and in gardens of Los Angeles and Riverside. The first small citrus orchard was planted in 1804 at the San Gabriel mission east of

Los Angeles. The first commercial citrus orchard, however, was established by William Wolfskill in 1841, near the current center of downtown Los Angeles. The industry was initially concentrated in three Southern Californian counties, Los Angeles, San Bernardino and San Diego (Fig. 1). In 1870, the first 'Washington' navel sweet orange trees were introduced to California from the United States Department of Agriculture, which sent two trees to Eliza Tibbetts in Riverside. Completion of the transcontinental railway enabled fruit distribution to eastern states. Together with the discovery of gold, these events led to a massive boom in citrus production and the subsequent establishment of the famous Californian citrus industry.



Fig. 1. The state of California and its counties. Close to 90% of California’s citrus fruit is now produced in five southern and central counties, viz. Fresno, Kern, Tulare, Ventura and Riverside.

One of the first 'Washington' navel trees is still growing in Riverside today (Fig. 2), amidst threats from the dreaded Huanglongbing (HLB) or Asian Greening Disease that was recently reported in this Southern Californian county. In 1875, the total commercial citrus plantings in California were made up of ±90000 'Washington' navel trees. This number increased rapidly to approximately 2 million trees by 1885 after introduction of lemon and grapefruit, and to 4.5 million trees by the early 1900s when the first commercial 'Valencia' orchards were planted.



Fig. 2. One of the first 'Washington' navel sweet orange trees is still growing in Riverside in southern California on the corner of Arlington and Magnolia Avenue.

2. The Californian citrus industry today

Urbanization has ringed the necks of southern citrus businesses in and around the major cities of Los Angeles, San Diego and Riverside. Close to 90% of California's citrus fruit is now produced in five southern and central counties, viz. Fresno, Kern, Tulare, Ventura and Riverside, but most of the fresh citrus fruit is produced towards the regions north of the Tehachapi mountains in the central counties of the San Joaquin Valley, viz. Fresno, Kern and Tulare. The total hectares of bearing citrus orchards in California amounts to $\pm 108\,000$, from which a total of 4 million tons of fruit was produced in the 2016/17 season, which is more than half of the total USA production. Sweet orange hectares make up 70% of the total Californian citrus acreage, mandarin plantings 15%, lemons 10% and grapefruit 5%. Marketing of high-quality easy-peelers under brands such as *halo* and *cutie*, has established a popular demand for a premium seedless soft citrus fruit in a massive, but highly-competitive local market. Twenty years ago, the total mandarin plantings in California amounted to approximately 2000 hectares. This figure has increased ten-fold since then, and by 10% in the last three years alone. More than half of this area consists of 'Tango' mandarin trees, with the rest made up mainly of 'W. Murcott' and 'Clementine' orchards (Fig. 3).



Fig. 3. A new 'Tango' mandarin planting in the Maricopa region in the Central Valley.

3. The Central Valley

The Central Valley is one of the most productive agricultural regions in the world. The Valley is located at the heart of California and is bound by the Sierra Nevada Mountains towards the east and the Pacific Ocean towards the west. The Valley is 60 to 100 km wide and stretches approximately 750 km from north to south. Of the total 47000 km² more than half is under irrigation with intensively cultivated vegetables, citrus, grape, pome and stone fruit orchards, as well as pistachio, walnuts and almonds. The Valley has a semi-desert Mediterranean climate and receives an average annual rainfall of ± 100 mm from October to March. Fruit production is highly dependent on water-supply from melting snow falls on the Sierra Nevada Mountains that are intricately canaled towards production regions across the Valley floor. Other water sources are mostly that of groundwater and banked or district water. The irrigation water has a high pH that has to be adapted with sulfuric acid. The soils are of deep sandy loam character and have a pH of between 7 and 8. In the Central Valley, citrus fruits are mainly produced for fresh consumption and are harvested almost throughout the entire season. For most parts of the year, the major citrus production regions experience low relative humidity, which makes pathogen disease pressure on fruit in the orchards almost negligible. The snow-capped peaks of the Sierra Nevada's, and cool South-Westerly winds continuously breezing through the orchards further enable fruit to hang on the trees to very late in the season. In some late navel and 'Valencia' orchards, fruit are held on the tree as late as June, which, in South Africa, relates to mid-summer or the month of December.

Quarantine insect pests include the Glassy-Winged Sharpshooter, the Fullers Rose Beetle, and Bean thrips, with the latter being especially problematic in navels as the cavity at the stylar-end provides refuge for these insects when they move over to citrus orchards from neighboring summer crops. The major insect pests causing damage to citrus orchards include Red mites, Citrus thrips and Californian Red scale. Due to an abnormally warm season this year, a 5th generation of red scale was detected in citrus orchards in Kern County in October (April in South Africa), which according to local entomologists is a very rare occurrence and extremely problematic. Control options are extremely narrow towards harvest due to strict MRLs.

4. Production challenges and technology

Major frosts that used to happen only every ten years now occur more frequently. Catastrophic frosts hit the Valley in 2012 and 2014. To protect fruit and young plantings, almost every orchard has fans installed at a cost of six to eight thousand dollars per hectare (Fig. 4). When cloudless, cold nights are anticipated, orchards are irrigated with micro-jet sprinklers to increase humidity, and fans are activated to break and mix inversion layers of warm and cold air. Early frosts before the major winter rainfalls are the most dreaded, as orchards are dry and air humidity low. The autumn flush has not yet fully hardened by then, and fruit at this stage have a low sugar content which makes them highly susceptible to freeze damage.



Fig. 4. Almost every orchard has gas-driven fans installed to break and mix inversion layers of warm and cold air and protect fruit and young trees from freeze damage. The foothills of the Sierra Nevada's are seen in the background.

Californian citrus growers have mastered the art of producing seedless 'Clementine' and 'W. Murcott' fruit on a massive scale. To prevent pollination and fertilization, growers cover their trees each spring with $\pm 12 \times 400$ m temporary drape-nets that are mechanically erected over individual rows and cover the complete tree canopy, dirt scooped over the edge of the net holds them in place. After 60 to 90 days, the drape-nets are individually removed, rolled up, covered with a plastic sheet and left at the edge of each row until the next spring (Fig. 5). According to growers, the specially-woven drape-nets have no effects on penetration of foliar sprays, and successfully avoid cross-pollination and unwanted seed development without significantly reducing fruit set, or negatively affecting fruit quality.



Fig. 5. To avoid cross-pollination and unwanted seed development, temporary drape-nets are mechanically-erected over individual rows and left covering the complete tree canopy. After 60 to 90 days, the drape-nets are individually removed, rolled up, and covered with a plastic sheet and left at the edge of each row until the next spring.

5. Cultural practices

The predominant rootstocks used are 'Carrizo' and 'C-35', especially in 'Tango' and 'W. Murcott' plantings. Trees on 'C-35' appear less vigorous in growth habit and are planted at narrower spacing. A 'W. Murcott' \times 'C-35' combination appears to be a good option. General tree spacing is 5.5×2.5 m, or 6×3 m. Drip irrigation is applied to young trees and a micro-jet-sprinkler system in older orchards. Almost all the trees are planted on ridges and orchards are free of weeds, which is critical to effectively manage spells of frost. Major pruning is done mechanically and mainly focused on increasing the efficiency of the drape-net process, optimizing penetration of foliar sprays, and increasing maneuverability and vehicle access in and around the orchards (Fig. 6). The majority of mineral nutrients are applied via fertigation and spread throughout the season until as late as September. The total nitrogen (N) application averages between 150 to 180 kg N per hectare per annum (at 555 trees per ha) and rate of potassium fertilization is approximately two thirds that of N, slightly higher for mandarins. Micro-nutrients such as Zinc, Copper and Manganese are applied by foliar sprays, with up to 6 sprays per season.



Fig. 6. A 'W. Murcott' orchard in the Central Valley that received an early-summer mechanical pruning.

Only a few plant growth regulators, which must be registered for use in California, are available for citrus production. The more important are: (i) 2,4-D to prevent preharvest fruit drop used with GA₃ to prevent rind senescence of late-harvested navels; (ii) GA₃ to increase fruit set or delay fruit senescence in mandarins; and (ii) use of 2,4-D on most cultivars to increase fruit size. Late navels such as 'Barnfield' are kept on the tree until mid-summer, but a problem arising from this appears to be a negative effect of the previous crop on flowering and fruit set of the next. The practice is nevertheless rewarding, considering the financial returns from navels in the summer market.

6. The Maricopa area

The Maricopa area is located at the foot of the Central Valley in a basin south of Bakersfield and north of the Tehachapi Mountains (Fig. 7). The area is the earliest, in which mostly 'Clementines', early navels and lemons are grown. Trees are planted in a North-to-South row-direction and general spacing for 'Clementine' orchards are 5.5 × 2.5 m, or 6 × 3 m. Flowering period for 'Clementines' is spread for 4 to 5 weeks, and fruit set strategies consist of two to three GA₃ foliar sprays combined with girdling of the main trunk. Girdling is effective on young trees, but less frequently applied to adult trees. 'Clementine' orchards visited included 'Nules', 'Orogrande', 'Clemenpons' and 'Fina' selections. The selection most suited to the region appeared to be 'Nules' with trees producing generous fruit loads, with a good fruit size distribution, and smooth rinds.



Fig. 7. The Maricopa area is located at the foot of the Central Valley in a basin south of Bakersfield and north of the Tehachapi Mountains. The area is the earliest, in which mostly 'Clementines', early navels and lemons are grown. Early navel orchards visited in the Maricopa area included young orchards of the ultra-early Australian 'M7' navel, adult 'Beck' navel orchards, 'Fukumoto', and 'Newhall' navels. The 'M7' trees bear its fruit mostly inside the tree canopy, underneath the foliage (Fig. 8). Unlike the other early navels, 'M7' fruit are round, the fruit have a smooth rind, and variable size of navel-end openings. The 'Beck' navel fruit were more elongated than the M7, had thick rinds, but fruit had a very good taste.



Fig. 8. The 'M7' navel trees bear its fruit mostly inside the tree canopy, underneath the foliage.

Acknowledgements and general conclusions

I am grateful to the United States Department of Agriculture for providing me the opportunity to undergo The Norman E. Borlaug International agricultural science and technology fellowship program at the department of Botany and Plant Sciences at the University of California Riverside (UCR) in California in the USA, and to Prof. Carol Lovatt for making this dream a reality. A special thankyou to Dr. Martha Orozco-Cardenas and Dr. Sonqin Pan, for opening your laboratories, and for making the time to train me and share your secrets and experience in a friendly and helpful manner. My personal opinion is that I was successfully trained to do analysis of floral gene expression in citrus tissue. Together with Dr. Orozco-Cardenas and Prof. Lovatt, we managed to successfully measure expression of various floral genes in buds of different treatments related to altered tree fruit load in 'Washington navel' sweet orange, and obtained novel results. We are therefore continuing the research in subsequent experiments in a collaborative research attempt, to better understand the roles of these floral genes in the citrus flowering model. My next endeavor is to successfully establish a similar protocol for analysis of gene expression at my home institution, at the department of Horticultural Sciences at the University of Stellenbosch so that I can explore the expression of genes related to regulating hormone synthesis, catabolism, reversible and irreversible inactivation, export, and import in fruit, floral buds or other tissues. I was fortunate to successfully obtain funding for most of the equipment required for analysis of gene expression, and would hopefully start implementing the equipment in 2018. In the attempts to optimize the extraction process of phyto-hormones from citrus buds, we managed to successfully extract and detect some individual phyto-hormones from citrus buds, such as GA₁, GA₇ and GA₉, and the cytokinins trans-Zeatin, and cis- and trans-Zeatin-O-Glucoside. Unfortunately, there was not enough time to repeat the analysis in the same tissue or to use more samples and/or different tissue types to determine what the cause(s) of the variation in results was. The development of a protocol to quantify multiple phyto-hormones from plant tissue using a single UPLC-MS run is complex and very difficult; however, we nevertheless managed to get some positive results in a very short period of time. We are therefore excited to continue our collaborative attempts to develop a protocol to successfully extract and quantify phyto-hormones.

7 EXTENSION / VOORLIGTING April 2017 - Maart 2018

By/Deur Hennie le Roux, Hannes Bester, M.C. Pretorius, Keith Lesar, Dawid Groenewald, Andrew Mbedzi en Melton Mulaudzi (CRI)

7.1 VOORLIGTINGOORSIG

7.1.1 Die 2017 Seisoen

Verpakking op pomelo's het aan die begin van die seisoen goed afgeskop, met pryse wat belowend gelyk het. Vroeë aanduidings was dat pryse op lemoene en sagtesitrus ook goed sou wees. Pryse op suurlemoene het reeds op 'n vroeë stadium heelwat geval, veral a.g.v Argentinië wat swak gehalte vrugte in veral Rusland geland het. Die wisselkoers het ook 'n negatiewe invloed op inkomste op DIP-vlak gehad.

Die Oos-Kaap, en tot 'n mindere mate die Wes-Kaap, het wesenlike probleme met vrugbars en gevolglike vrugval op nawels en selfs Valencias ervaar, veral Midknights. Hierdie tendens was die gevolg van buitensporige hoë temperature, gepaardgaande met lae humiditeit, gedurende die blom en vrugset periode.

Heelwat probleme met suurvrot is ervaar. Dit kan toegeskryf word aan die feit dat guazatine nie meer gebruik mag word nie, terwyl propiconazole op sy beste slegs 80% so effektief as guazatine is.

Dis duidelik dat die implementering van 'n Valskodlingmot Bestuurstelsel, om in die toekoms marktoegang tot die EU te verseker, 'n geweldige groot sprong van die hele sitrusbedryf gaan vereis en groot uitdagings aan verskeie rolspelers gaan stel. Hierdie komplekse stelsel bied die Suid-Afrikaanse sitrusbedryf egter die geleentheid om alles moontlik te doen om te verseker dat koue-sterilisering na die EU nie 'n vereiste word nie.

Hoewel die oes oor die algemeen ligter was as die 2016 seisoen, was daar uitsonderings in sekere areas met sekere nawel en Valencia kultivars waar 'n baie goeie oes afgehaal is. Die hoër insidensie van witluis, rooidopluis, valskodlingmot, vrugtevlug, karobmot en sitrusbladspringer was duidelik sigbaar in meeste boorde, asook vrugte wat by die pakhuis gelewer is. Die uiterlike voorkoms van vrugte was goed, maar windskaede in sekere areas was meer prominent. Boordsanitasie en plukpraktyke, asook algemene oesbestuur by meeste van die produsente was van 'n hoër standaard as in die verlede. Pakhuispraktyke t.o.v 'pre-sorting' was ook noemenswaardig beter en het die afkeuring van boorde, palette met VKM en swartvlek probleme tot die minimum beperk. 'n Hoër insidensie van *Alternaria* kernvrot is hoofsaaklik in nawels opgemerk, maar sekere Mandaryn tipes en midseisoen Valencias was ook besmet. Die grootste na-oes uitdagings was die *Alternaria* bruinvrot voorkoms, asook suurvrot wat in al die areas op vrugte voorgekom het.

Daar was groot onsekerheid en kommer oor waterbesikbaarheid teen die einde van 2016 en sitrusprodusente moes al hulle fokus op boordseleksie en vrugset plaas. Met die goeie reën wat ervaar was in Januarie kon almal damme volmaak, vrugset en die kwaliteit van die vrugte was goed. Nuwe marksegmente het oopgegaan en produsente kon 2de klas pomelo's uitvoer teen uitstekende pryse, asook klas 3 Valencias. Kommentaar van produsente is dat dit 'n besondere jaar was vir sitrus-uitvoere as alles in ag geneem word.

Terugvoer uit die markte was dat gehalte oor die algemeen goed was, met die normale sporadiese bederf en skildefekte wat uitgedop het. Pryse was oor die algemeen goed, hoewel beduidend laer op suurlemoene. Uit 'n fitosanitêre oogpunt het dit goed gegaan totdat die laat Valencias probleme met onderskeppings vir swartvlek in die EU opgetel het. Hopelik sal die suider Afrikaanse bedryf uiteindelik leer om nie Valencias laat in die seisoen na die EU te stuur nie, aangesien dit elke jaar die stert van die seisoen is wat die probleme veroorsaak.

Die 2017 seisoen is afgesluit met uitvoervolumes wat 'n rekord 123 miljoen kartonne bereik het. Met die uitsondering van nawels was die volumes op al die variëteite hoër in vergelyking met 2016. Pomelo's het geëindig op 15,7mil, sagtesitrus op 13,4mil, suurlemoene op 19mil, nawels op 21,1mil en Valencias op 53,8mil kartonne. Indien dit nie vir groot verliese op nawels was a.g.v vrugsplit nie, asook hael in sekere streke, sou

die totale volume baie naby aan 130mil kartonne gekom het. Dit beteken dat 130mil kartonne maklik haalbaar behoort te wees in 2018, veral as die groeiende volumes in suurlemoene en sagtesitrus in ag geneem word.

Baie tyd en energie is veral tydens die tweede helfte van die seisoen spandeer om die valskodlingmot-bestuurstelsel (FMS) betyds in plek te sit vir uitvoere na die EU in 2018. Hierdie stelsel is een van die grootste uitdagings tot nog toe wat die Suid-Afrikaanse sitrusbedryf in die gesig staar.

7.1.2 Die 2018 Seisoen

Die vooruitskouing vir die seisoen lyk belowend, met die verwagte skattings t.o.v. uitvoerkartonne as volg: pomelo's 14,5mil, suurlemoene 20,5mil, nawels 25,6mil, Valencias 54,6mil en sagtesitrus 17,7mil kartonne, met 'n totaal van 131,7mil kartonne. Die verwagting is dat die vruggroote effens aan die klein kant gaan wees, maar dat die kwaliteit goed sal wees.

Terugvoer uit die markte dui daarop dat meeste ander produserende lande vroeg uit die markte sal wees en dat suider Afrikaanse vrugte dus in 'n redelike sterk mark behoort in te kom, teen goeie pryse, op voorwaarde dat die volumes na elke mark sinvol bestuur word. Marktoegang na veral Europa gaan egter hierdie komende seisoen 'n groot uitdaging aan feitlik alle rolspelers stel om die valskodlingmot bestuurstelsel effektief te implementeer.

7.1.3 CRI-PTF

Verskeie pakhuse het vroeg in die seisoen navraag gedoen oor die nuutste situasie rondom plastiese palette (nuwe sowel as herwinde plastiek). CRI-PTF is op 'n voortdurende basis besig met ondersoeke, maar ongelukkig is plastiese palette nie naastenby koste-effektief nie. Al sou plastiese palette meer bekostigbaar wees, sal die vervaardigers nie hoë volumes kan vervaardig nie. Die oorgrote meerderheid van die paletvervaardigers is tog van mening dat houtpalette in die toekoms beskikbaar sal bly, maar die verwagting is dat pryse aansienlik verhoog sal moet word. Wat die huidige palet se spesifikasie/konstruksie betref het, was daar ook verskeie navrae oor die rigting van die bo-dek planke. Die bo-dek planke loop in die 1010mm rigting en palette word in die 1210mm rigting in rakstelsels geplaas. Die vraag is gevra of palette met bo-dek planke in die 1210mm rigting nie stewiger in rakstelsels sal wees nie. 'n Volledige ondersoek is gedoen en terselfdertyd is ook gekyk na die vertikale lugvloei met bo-dek planke in wydtes van 75- en 100mm. Die ondersoek het baie duidelik gewys dat die huidige palet, soos vervat in die "CRI Packaging Material Specifications and Palletisation Protocols", die beste is. Die hoofrede hiervoor is die drie 25mm dik "Top deck stringers" wat in die 1210mm rigting loop.

"Hemingway Plastics" het vroeg in 2017 laat weet dat hulle vër gevorder het met die ontwikkeling van 'n bekostigbare plastiese palet vir uitvoer-sitrus. Tien palette is na Schoeman Boerdery gestuur en 'n proef is met telling 105 Valencias in A15C kartonne (80 per palet) gepak. 'n Rak is in die pakhuis gesimuleer en die deurbuiging in die gesimuleerde rak op die standaard houtpalet is vergelyk met die eksperimentele plastiese palet. Die deurbuiging op Hemingway plastiese palette was onaanvaarbaar hoog en daar is besluit dat die risiko om die palette verder te vat net eenvoudig te hoog is.

'n Pakhuis in die Ohrigstad omgewing maak gebruik van 'n "robot" palettiseerder. Probleme was ondervind met die palettisering van A15C kartonne, hoofsaaklik met tellings 48 en 56 nawels wat langwerpige is. Op versoek van die eienaar is die probleem saam met hulle kartonvervaardiger ondersoek. Die ondersoek het weer gewys dat akkurate groottegradering van langwerpige vrugte moeilik is. Dit lei tot uitermatige hoë uitbuiging (bulging) wat palettisering dan moeilik maak. Let daarop dat langwerpige sitrusvrugte ekwatoriaal en polêr gemeet moet word. Na etlike pogings met verskillende verstellings aan die groottegradeerders kon daarin geslaag word om die uitbuiging te verminder en die palettisering met die "robot" baie te verbeter.

Aanvanklike navorsingswerk is gedoen op 'n nuwe ontwerp vrugtevlieg lokval. Plaaslik vervaardigde "bleached pulp sheets" is gebruik. Op 'n stadium was die spesifieke materiaal nie meer plaaslik vervaardig nie en CRI was toe verplig om 'n ingevoerde produk te gebruik. As gevolg van kostes is Sappi genader en daar is toe

besluit om proewe te doen met 'n ander soortgelyke plaaslik vervaardigde "pulp sheet". Voorlopige proewe het baie goeie resultate gelewer en groter-skaalse proewe word gedoen. Indien die proewe suksesvol is, sal Sappi die produk aan die sitrusbedryf beskikbaar stel.

Gedurende die eerste kwartaal is 'n proef met ligter A15C kartonne met suurlemoene vanaf 'n pakhuis in Tshipise gedoen. Hi-cube palette is per pad na Durban vervoer en na die M/O en Europa uitgevoer. Die kontrole kartonne was met 165g/m² Superflute en die eksperimentele kartonne met 150g/m² Ultraflute vervaardig. Die proef was baie suksesvol en, gebaseer daarop, word die 150g/m² Superflute nou op 'n semi-kommersiële basis gebruik.

Ongelukkigheid bestaan oor die sogenaamde hoë kostes van pakmateriaal. Die baie groot reeks/verskeidenheid van verskillende ontwerpe en groottes is een van die bydraende faktore vir die sogenaamde duur kartonne. Daar word op 'n voortdurende basis 'n beroep op al die rolspelers gedoen om meer te standardiseer en die aantal ontwerpe en groottes te verminder.

Vrug-toedraaipapier (fruit wrapping paper) word nie meer plaaslik vervaardig nie. Die ingevoerde papier is ongelukkig duur en nie vrylik beskikbaar nie. Op versoek van plaaslike verskaffers van toedraaipapier word op 'n voortdurende basis na ander oorsese verskaffers gesoek. 'n Nederlandse maatskappy (NZZ), verskaffers van pakmateriaal, kan die toedraaipapier verskaf, maar ongelukkig is hulle prys hoegenaamd nie kompetend nie. Onderhandelinge met Sappi het begin en daar is baie vordering gemaak met die verkryging van 'n geskikte papier by Sappi.

Die gebruik van NZZ se rekbare net om paletvragte te stabiliseer word oorsee gebruik. Die eerste kleinskaalse proef met hierdie net is met A15C kartonne by Schoeman Boerdery gedoen. Twintig palette is met dié net toegedraai en per pad na Durban vervoer. Ongelukkig het die net op sekere palette, wat styf teen mekaar gelaai was, deurgeskuur. Die palette het nogtans in 'n aanvaarbare toestand aangekom en is gedurende die eerste week in Julie in 'n hi-cube skeepshouer na Europa verskeep. Voorlopige berekeninge het getoon dat die gebruik van die rekbare net 'n 50% besparing per palet kan meebring. Opvolgproewe is gedurende die jaar by SRCC, Schoeman Boerdery en die Komati Groep se Vergenoeg en Riverside pakhuis gedoen. NNZ het te kenne gegee dat die gebruik van hulle net 'n besparing van R15.00 per palet kan meebring. Ongelukkig was die proewe nie 100% suksesvol nie. Die net het op 'n groot aantal palette tydens padvervoer van die pakhuis na die hawe geskeur en deurgeskuur en dit het veroorsaak dat die palette in 'n ontstabelle toestand in Durban aangekom het. Van die palette moes eers weer met hoekstukke en plastiese bande vasgemaak/gestabiliseer word voordat dit verskeep kon word. Om die probleem te voorkom moes meer lae net gebruik word en dit verhoog dan weer die kostes, soveel so dat daar feitlik geen besparing met die gebruik van net is nie. Meer lae maak ook die skandering van die "Bar codes" op die palette moeilik, asook die trek van monsters vir inspeksie doeleindes. Die eerste terugvoering van oorsee af was ook nie positief nie. Kopers het gekla dat die net om die aste van vurkhysers draai en dat wanneer die net van palette verwyder word, om net enkele lae van die palette af te haal, die palette dan onstabiel en onhanteerbaar is.

Vroeër is gerapporteer dat 'n "mulching" proef met 'n ligte ingevoerde papier gedoen is. Dit was nie suksesvol nie. Die papier het binne drie weke geskeur en begin verweer. Proewe is herhaal, maar 'n baie swaarder 300g/m² (Virgin Linerboard) is gebruik. Die proef word weer op die plaas in die Brits omgewing gedoen, bloot om te bepaal hoe lank die papier gaan hou.

Carmoc is 'n kartonvervaardiger in Mosambiek. Hulle het CRI-PTF genader met 'n versoek dat hulle geakkrediteer wil word. Die proses is begin deur die eerste A15C kartonne wat by die SANAS geakkrediteerde laboratorium getoets is. Die eerste eksperimentele kartonne het aan die spesifikasies voldoen. Die proses sal gedurende die derde kwartaal van 2018 voortgesit word.

Gedurende die periode Maart tot Augustus was kartonne op 'n geskeduleerde basis deur die geakkrediteerde kartonvervaardigers, pakhuis en CRI personeel na Sappi se SANAS geakkrediteerde laboratorium gestuur vir akkreditasie toetse, ten einde te verseker dat die kartonne aan die spesifikasies voldoen. Bo en behalwe bogenoemde is eksperimentele kartonne waarmee daar gedurende 2017 proewe gedoen is, ook getoets. Die

toetsresultate was oor die algemeen baie goed. Daar was gedurende 2017 slegs twee gevalle waar die kartonne nie aan die BCT vereistes/standaarde voldoen het nie. In beide gevalle was dit a.g.v. papierkombinasies. Dit is met die betrokke kartonvervaardigers opgeneem en in altwee gevalle het die produsente die kartonne te laat bestel en hulle was verplig om ligter papier te gebruik. Produsente is versoek om bestellings vir kartonne betyds te plaas. Die toets van die kartonne, asook die volledige verslae wat na elke stel toetse uitgereik word, is 'n groot en belangrike taak en Sappi se laboratorium-personeel verdien 'n groot kompliment vir 'n taak welgedaan. Sappi het gedurende 'n vergadering met hulle senior bestuur aangekondig dat hulle gedurende 2018 sal voortgaan om kartonne op hulle kostes te toets. Daar was gedurende die 2017 seisoen ook slegs twee gevalle waar kartonne op die oorsese markte probleme veroorsaak het en in altwee gevalle was rowwe hantering beslis die oorsaak van die probleme.

Na die 2017 se akkreditasie toetse en verskeie ander CRI waarnemings is die volgende kartonvervaardigers goedgekeur as geakkrediteerde verskaffers vir 2018. In alfabetiese volgorde:

- APL Cartons
- Corruseal
- Houers Koöperatief Beperk
- Mpact Corrugated
- Neopak
- New Era Packaging
- Sunnypacks

Nuwe besigheidsgesleenthede in samewerking met Sappi is ondersoek. In Oktober is begin met samesprekings oor proewe met lignosulphonate. Lignosulphonate word met water gemeng en dan op grondpaaie gespruit om stof te verhoed. Proewe in sitrusboorde is beplan vir 2018. CRI het met Ambrosia Citrus in Hoedspruit gereël dat proewe in boorde en rondom hulle pakhuis in April gedoen gaan word.

Al die Wes-Kaap se sitrus wat na die VSA uitgevoer word, word op die sogenaamde "11 Slat" dennehout palet gepak. Ander streke mag moontlik later in 2018 ook na die VSA uitvoer. Om verskeie redes gaan dit geweldig moeilik wees om die "11 Slat" palet in die res van SA te vervaardig. Alles moet gedoen word om goedkeuring by DAFF te kry sodat sitrus buite die Wes-Kaap op die standaard sitrus palet gepak mag word. 'n Skrywe in dié verband is opgestel om aan DAFF te stuur. Tesame met bogenoemde is vergelykende vertikale lugvloei toetse gedoen. Die toetse het gewys dat vertikale lugvloei met die standaard sitruspalet op die oop vertoonkartonne aansienlik beter as die "11 Slat" palet is. Wat die A15C Supervent karton betref is die verskil in vertikale lugvloei nie groot nie.

Sekere uitvoer organisasies het dit onder CRI-PTF se aandag gebring dat hulle gedurende 2017 klagtes ontvang het van oop vertoonkartonne wat inmekaar gesak het. Hulle het versoek dat die stapelsterkte (BCT) vereistes van die oop vertoonkartonne met 10% verhoog word en ook weer die papier en basiese massa van die papier te spesifiseer. 2017 se toetsresultate van die kartonne is krities bestudeer en na deeglike oorweging is daar besluit om dit nie te doen nie. Daar is wel besluit om weg te doen met die 5% toleransie op alle oop vertoonkartonne se BCT vereistes. 'n Skrywe in hierdie verband is aan die geakkrediteerde kartonvervaardigers gestuur. Pakhuise sal ook weer versoek word om asseblief streng te bly by die aanbevole palettiserings protokolle.

7.1.4 **Produksie areas**

Die somerreëns wat eers middel van die somer in Mpumalanga en Limpopo begin het, het redelik verspreid oor die gebied voorgekom. Die oes was oor die algemeen ligter as die vorige seisoen. 'n Hoër insidensie van witluis, rooidopluis, valskodlingmot, vrugtevlieg, karobmot en sitrusbladspringer het meer algemeen in die sentrale sitrusareas voorgekom. Die algemene voorkoms van vrugte was goed met windskaide in sekere areas wat meer prominent was.

CRI het goeie sigbaarheid getoon in die Noorde met gereelde voorligtingsbesoeke en studiegroepbywoning. Op aanvraag van die produsente was 'n snoeiwerkswinkel gehou in Letsitele met goeie bywoning van meer

as 40 persone. In Tshipise en Weipe is die studiegroepe weer aktief, en toon goeie bywoning en interaksie van so ver as Pontdrift. CRI Voorligting het ook besoeke aan die Suidelike Zimbabwe produsente en CGA verteenwoordiger, Paul Bristow, gebring. Dit is duidelik dat CRI Voorligting in die toekoms 'n groot rol gaan speel in die sukses van Zimbabwe se sitrusbedryf.

Valskodlingmot, karobmot en witluis was 'n groot bron tot kommer by alle produsente in die noordelike sitrus areas. Dopluis-druk was sporadies hoog en het tot 'n mindere mate probleme veroorsaak. Probleme wat produsente ervaar het was vrugte wat nie goed opkleur nie en uit die markte was daar bietjie terugvoer van suurvrot en ook ander tipes bederf. FCM infestasië in die Letsitele boorde het begin toeneem in Julie en meeste besmette vrugte kon in die pakhuis verwyder word. Maar vir sommige produsente het afkeurings op pomelo's begin deurmaak, wat getoon het dat dit 'n erge FCM jaar was. Verder het CBS infestasië in Letsitele ook toegeneem. Tydens 'n nabetraging in Letsitele is uitgelig dat buiteseisoen vrugte moontlik die oorsaak was, gepaardgaande met die warm winter. Letsitele produsente het Bennie Valencias later laat hang as in vorige jare, wat ook kon bydra tot die ontwikkeling van CBS simptome op die vrugte. Oor die algemeen het alle produsente die uitdagings goed bestuur en pakhuis het hulle beste pogings aangewend om by te dra tot die goeie uitpakte.

Die suide, veral die Oos-Kaap, het groot uitdagings in die gesig gestaar a.g.v. bogemiddelde vrugval op nawels en sekere Valencia seleksies. Dit kon toegeskryf word aan die uitermate hoë temperatuur en lae humiditeit wat gedurende blom en vrugset voorgekom het. Groot verliese is as gevolg van hierdie probleem gelei. Die erge droogte in die suide het ook sy tol ge-eis. As gevolg van die erge vrugsplit was valskodlingmot baie aktief en addisionele beheermaatreëls moes in plek gesit word. Suurvrot het wydverspreid voorgekom, ook voor-oes en selfs tot bo in die bome. Die afwesigheid van guazatine vir na-oes beheer van suurvrot het veroorsaak dat heelwat terugvoer van suurvrot uit verskeie markte ontvang is.

Navorsingsprioriteite is gedurende Junie en Julie in al die produksie areas bespreek. Daar was nie veel addisionele versoeke wat tot die huidige projekte toegevoeg is nie. Prioriteite is ook tydens die onderskeie CRI werksinkels in die verskillende streke gelys, asook by die Exporters Technical Panel en Cooling Working Group se onderskeie vergaderings.

Na aanleiding van 'n versoek deur die sitrusprodusente vanuit die noordelike Zimbabwe produksie streek dat regerings-amptenare voorgelig word t.o.v. biosekuriteits gevare wat die Suidelike Afrikaanse streek in gevaar mag stel, is 'n besoek beplan en gefinaliseer, wat in Augustus plaasgevind het. Die besoek is uitgebrei om sitrusprodusente, konsultante, chemiese verteenwoordigers, asook beleggers voor te lig oor sitrusproduksie wat die volgende dissiplines ingesluit het: voor-oes vrug- en blaarsiektes, grondgedraagde siektes, na-oes pakhuispraktyke, asook entomologiese plae. Twee areas is besoek waar boordbesoeke plaasgevind het, nl. noord van Harare – Mvurwi en Mazoe sitrus en suid-wes van Harare – Chegutu area. Daar word beplan om weer 'n gesertifiseerde CIS kwekery hier te begin. Die kwekery is besoek en aanbevelings is gemaak volgens CIS riglyne. Die besoek is afgesluit met 'n werksinkel wat deur John Perrot gereël is en wat bygewoon is deur regerings amptenare van Zimbabwe Plant Quarantyn, produsente, konsultante en chemiese verteenwoordigers. Aspekte wat hanteer is, het ingesluit; Biosekuriteits siektes en plae wat 'n bedreiging inhou vir die suider-Afrikaanse sitrusprodusente, relevante kultivar en onderstam aanbevelings, entomologiese aspekte, marktoegang spesifiek vir Zimbabwe, uitvoere na China, die sitrusverbeteringskema en na-oes pakhuispraktyke. Opvolgbesoeke deur die area voorligter vir Limpopo en Zimbabwe, asook CRI se ondersteuning, was bevestig.

In die Noorde was 2017 'n jaar met 'n gemiddelde opbrengs, maar pryse en selektiewe markte het opgemaak vir enige tekortkominge. Sommige produksie-eenhede het nogtans rekord-getalle kartonne gepak. Produsente in die Noorde het wel hulle kant gebring om op hoogte te kom met die addisionele beheermaatreëls vir FCM. CRI het 'n groot dryf in al die produksiestreke, asook Zimbabwe, om die FMS en gepaardgaande FCM bestuur suksesvol te implementeer. Zimbabwe gaan inval met die FMS en sal ook gebruik maak van "PhytClean". Witluis in die Tshipise en Weipe gebied het toegeneem in Oktober en teen die einde van Desember het dit gelyk asof die populasies wel onder beheer gebring is. In ander gebiede waar die blaaspootjie-beheer meer aggressief is, kan dit verwag word dat witluis reperkussies hier en daar gaan uitbreek. As gevolg van 'n paar

CBS onderskeppings in Europa is daar 'n hele paar PUC's vanuit die Noorde wat op die "Blacklist" opgeëindig het, en gevolglik is ondersoek ingestel om die redes vir die verhoogde voorkoms van CBS te bepaal. Ondersteuning van CRI gaan krities wees in die 2018 seisoen wat voorlê.

In die suidelike produksiestreke was die klimaat uitstekend vir goeie vrugset vir 2018. Volumes vir al die sitrustipes sal na verwagting hoër wees as die 2017 seisoen, veral op nawels wat verlede jaar ernstige vrugsplit gehad het a.g.v. uiterste klimaatstoestand. Produsente is positief en optimisties dat dit 'n goeie seisoen sal wees, selfs in die droogtegeteisterde gebiede van die Wes-Kaap en Gamtoosriviervallei. Nuwe tegnologie word toenemend ingespan om produsente te help om water te bestuur, soos bv. FruitLook in die Wes-Kaap wat kosteloos aangebied word. Die Gamtoos het van tyd tot tyd bietjie reën gekry om die oes deur te trek. Produsente in die Wes-Kaap voel dat hulle voldoende water behoort te hê om die huidige oes groot te maak, en verder dui die langtermyn weervoorspelling dat dit 'n normale reënvalseisoen gedurende April tot Junie in hierdie streek behoort te wees.

Die vooruitsigte in die sentrale produksiestreke vir 2018 lyk op hierdie vroeë stadium belowend vir 'n baie goeie oes die komende seisoen. Die uitgerekte blom van gemiddeld drie sette per boord mag dalk 'n uitdaging later in die seisoen raak. Die blaaspootjie-druk was steeds oor die algemeen baie laag, alhoewel daar beweging laat in Maart in sekere areas voorgekom het. Dit wil voorkom of windskade in meeste van die areas effens hoër is as wat verwag is. Wydverspreide laat somerreëns het in meeste van die streke voorgekom, wat moontlik 'n positiewe effek op vrugsgroottes op die later kultivars kan hê. Hoë dagtemperature het einde Februarie en Maart 2018 voorgekom, die nag temperature het eers aan die einde van Maart begin afneem wat voordelig sal wees vir kleurontwikkeling op die vroeër kultivars. Vrugval op sekere mandaryne en vroeë nawels het in van die streke voorgekom. Die rede vir die vrugval nadat alle bekende oorsake ondersoek is, is steeds onbekend. Hierdie boorde het wel 'n bogemiddelde oes aan en vrugval is nie ongekend in sulke gevalle nie.

Beheerstrategieë vir Valskodingmot is hoog op elke streek se prioriteitslyste en waar nodig word behandelings betyds gedoen om sodoende die FMS riglyne noudeset te volg. Geen abnormale probleme in die verband is aangemeld nie. Al die areas fokus sterk op die VKM beheerstrategie en implementering van die FMS. Ten spyte van die uitdagings wat die FMS vereis, is die gesindheid van die produsente om dit te maak werk ongekend positief.

Produsente in die Noordelike produksiestreke is opgewonde oor die 2018 seisoen wat voorlê. Die skatting op volumes is hoër as verlede seisoen en die verwagting is dat die kwaliteit ook goed sal wees. Vrugval was opmerklik laag gewees tot in die eerste kwartaal van 2018. Die eerste pomelo's uit Tshipise het op telling 60 gepiek en die kommer vanuit die res van die noorde is ook oor die vrugsgroottes van die pomelo's. Daar is heelwat navraag by kartonvervaardigers oor verskillende kartonne, wat wys daar word gekyk na alternatiewe bemarkingsopsies van die kleiner vrugte. Met die reën wat geval het in Februarie en Maart is daar geen bekommernis oor vrugsgroottes op ander variëteite nie. Pryse op suurlemoene het gedaal in vergelyking met verlede jaar. Blaaspootjie-druk op sitrus was laag gewees hierdie seisoen en FCM-druk is laag oor die algemeen. Produsente doen regtig moeite met sanitasie en FCM beheer. Witluis bly ongelukkig een van die groter uitdagings tans op sitrusproduksie.

Produsente in Zimbabwe verwag baie goeie oeste vir 2018 in die suidelike gebied by Beitbrug. Vrugsgroottes op Valencias behoort goed te wees, maar pomelo's sal kleiner wees hierdie seisoen. Ongelukkig was daar net een houder Satsumas uitgevoer uit die Noorde van Zimbabwe. Die baie reën het gepla, gehalte van vrugte was nie op standaard nie en dopluis was ook 'n probleem. CRI het deeglik tyd spandeer met Zimbabwe produsente in hierdie seisoen, ondersteuning gegee op produksie, IPM & DM en na-oes aspekte asook tyd ingesit met die Zimbabwe Department Landbou om al die nodige reëlings te tref vir marktoegang na die EU, wat Phytclean insluit. Zimbabwe het 'n protokol vir koue-steri ontvang van die Chinese regering af, asook 'n aanbod om sitrusbome uit Asië te ontvang. Die nuwe verhouding met die Chinese word baie streng dopgehou deur die CGA verteenwoordigers in Zimbabwe.

7.1.5 Na-oes voorligting

'n Suksesvolle reeks sitruspakhuis besoeke/konsultasies, is gedurende die 2017 pakseisoen afgehandel. Die pakhuis is weer op 'n een tot een basis besoek en die houding en terugvoering was weereens baie positief, met goeie interaksie en samewerking met die pakhuis. Die pakhuis "check" lys is aangepas en elke lys is deeglik by die pakhuis op 'n een tot een basis voltooi. Pakhuisbestuur was meer tegemoetkomend en gewillig om hulle idees en vertroulike informasie t.o.v terugvoering oor bederf, residu-resultate ens. te bespreek, en was ook bereid om die nodige aanbevole veranderinge aan te bring, as gevolg van die toename in bederf.

Na drie tot vier lae-bederf jare het die situasie gedurende 2017 heeltemal verander. In beide die Noordelike en Suidelike produksiegebiede was al die vername patoogen-infeksies (groen/blouskimmel, suurvrot, latentepatogene, Phytophthora bruinvrot, selfs Trichoderma) waargeneem. Die meeste infeksies was hoofsaaklik op suurlemoene, nawels en sagtesitrus waargeneem. As gevolg van laaghangende vrugte en deurdrenkte boorde is meer gevalle van Phytophthora bruinvrot op sekere vrugte in die markte sowel as die boorde waargeneem. Daar was 'n noemenswaardige toename in suurvrot bederf, veral in die Noordelike streke op nawels en sagtesitrus kultivars. *Penicillium* infeksies is ook uitgewys in terugvoering vanaf die markte, met foto's van besmette vrugte wat "wit" en groen- en blouskimmel in kartonne wys. Dit is 'n bekommernis, omdat dit of 'n probleem met swamdoder-aanwending of moontlike bestandheid kan wees. Baie sporulerende *Penicillium* infeksies is ook op die laat en ouer Valencias op foto's van die verskeie markte waargeneem. Heelwat *Diplodia* stingelentverrotting op vroeë suurlemoene is ook waargeneem. *Alternaria* kern- en nawelent verrotting op nawels en sommige Valencias was ook 'n geweldige probleem hierdie seisoen.

'n Baie nuwe konsep, waar pakhuis nou gewillig is om hulle probleme en ondervindinge openlik te bespreek, en nog met ander pakhuis te deel, is die nuut gestigte "Pakhuis Forum Vergaderings". Hierdie vergaderinge het in die SRV (Sondags Rivier Vallei) begin en vandaar na die Gamtoos Vallei (Patensie) en ander areas uitgebrei. CRI het al drie vergaderings in die SRV en een van die twee in die Gamtoos Vallei bygewoon. Die terugvoering na bywoning en interaksie onder die pakhuis en die tegniese afdelings was uiters belowend. Daar word huidiglik deur Voorligting daaraan gewerk om hierdie Pakhuis Forum Vergaderings in die ander produksiegebiede te stig.

Dit is positief om te sien hoeveel pakhuis poog om hulle pakhuiskritiesebeheerstelsels reg te bestuur, veral die sanitasie van die pakhuis. Baie pakhuis het meer "klinies" geword, wat pakhuis-sanitasie betref, maar daar is nog steeds pakhuis wat nie genoeg aandag op pakhuis-sanitasie toespits nie. Die bestuur van pakhuis-sanitasie gaan ook hand aan hand met die bestuur van die pakhuisbehandelings en die toediening van die regte konsentrasies en residuladings van die belangrike swamdoders. Dit skep dan 'n hoë risiko vir die ontwikkeling van swamdoder bestandheid, veral waar daar nog behandelde vrugte en uitskot vrugte in die pakhuis is wat nog in plukkratte rondstaan en vrot word en die pakhuis met miljoene swamspore besoedel. Dit is uiters gevaarlik vir moontlike bestandheid.

Die bestuur van waksaanwending in sitruspakhuis is nogsteeds baie wisselvallig, a.g.v. nat vrugte voor aanwending, oor- en onder-aanwending, en dan ook swamdoder residu oorskrydings van tyd tot tyd.

Skilprobleme was 'n bekommernis a.g.v. die uitermatige wisselvallige omgewingstoestande wat 'n rol hier gespeel het. Terugvoering van die markte en markagente af het heelwat fotos gewys van gevalle van meestal "pitting" en skilafbraak op sagtesitrus kultivars, en "pitting" op sekere Valencia kultivars.

Die oorsaak van die groot verliese a.g.v. vrugsplit in die Oos- en tot 'n mindere mate die Wes-Kaap, was as gevolg van die abnormale en wisselvallige omgewingstoestande, d.w.s. uitermate hoë temperature en lae humiditeit. 'n Tekort aan water het ook hier 'n rol gespeel. Hierdie toestande het die ontydige vrugval van hierdie gebarste vrugte veroorsaak. Saam met die skade was die ontwikkeling van sekere na-oes patoogen infeksies soos *Penicillium* (groenskimmel) en *Alternaria* (nawel-ent en kernverrotting). Die meerderheid *Penicillium* infeksies het op die gevalle vrugte onder die bome ontwikkel. Die infeksies saam met die geweldige skade het veroorsaak dat sommige produsente hulle hele nawel-oes verloor het. In ander gevalle het die meerderheid *Alternaria* infeksies tydens verskeping ontwikkel, en die skade van hierdie infeksies is eers tydens aankoms in die markte waargeneem.

Die eerste tekens van bestandheid wat met die ontleding van die swamspoor pluismonsters van pakhuis waargeneem is, het bewys hoe belangrik sanitasie is. Pluismonsters sal op 'n gereelde basis by pakhuis tydens pakhuisbesoeke gedurende die 2018 pakseisoen geneem word en die pakhuis sal in die komende pakseisoene aangeraai word om hulle eie monsters ook op 'n gereelde basis na die CRI Diagnostiese laboratorium te stuur vir maandelike bestandheid-ontleding. Die meerderheid pakhuis poog om hulle kritiese beheerpunte/stelsels goed te bestuur, maar daar is nog steeds baie pakhuispraktyke wat gunstig is vir die ontwikkeling van hoë bederf. Al die “groen bomme” wat nog in die vrugwasstelsels gedompel word, word 'n resep vir bestandheid en 'n toekomstige ramp, veral tydens 'n hoë-bederf jaar.

Die gebruik van ongeregisteerde middels in die sitruspakhuis is kommerwekkend. Dit sluit hoofsaaklik die “saniteermiddels” en ook die benatters in wat deur die chemiese verskaffers versprei word, sonder om eers vir CRI te raadpleeg of die middels goedgekeur is of nie.

Daar sal gepoog word om gedurende die 2018 seisoen pakhuisforums in al die produksiestreke op die been te bring waar sodanige forums nog nie gestig is nie. Bydraend hiertoe sal verdere na-oes voorligting fokus op een-tot-een pakhuisbesoeke, soos die behoeftes ontstaan. Die 2018 oes-seisoen het reeds in die Noordelike en Suidelike produksiegebiede afgeskop. 'n Toename in oesvolume met kleiner vruggrootte word verwag. Wat pakkapasiteit betref is daar 'n toename in nuwe paklyne, asook in die aantal nuwe pakhuis in die produksiestreke.

Die eerste Satsuma besendings hierdie seisoen vanaf die Noordelike gebiede het goed verloop. Die interne gehalte was baie goed, maar ongelukkig het daar na ontgroening 'n bietjie fitotoksiteit op 'n klein persentasie vrugte voorgekom. Die eerste Star Ruby pomelo's en suurlemoene vanuit die Noorde se gehalte is ook baie goed. Alternaria kernverrotting en 'n paar gevalle van endokserose op suurlemoene uit die Noorde is egter waargeneem. Oleo het ook sporadies voorgekom. Die eerste latente patogeen-infeksies (Diplodia stingelentverrotting) op suurlemoene is ook in die Noordelike gebiede waargeneem, as gevolg van die hoë swamspoorlading op dooiehout in die bome.

7.1.6 CRI Produksiewerkswinkel

Die CRI Produksie-werkswinkels is vir die eerste keer in drie jaar weer in vyf van die ses groot produksiestreke aangebied. Beplanning van die produksiewerkswinkels is met produsente, sitrus konsultante, navorsers en voorligtings personeel se insette gedoen. Meer as 700 persone het bygewoon, wat 'n rekord getal is vir die produksie-werkswinkels. 'n Ongekende belangstelling in die werkswinkels het veroorsaak dat van die registrasies in die Oos-en Wes-Kaap gesluit moes word. Baie positiewe terugvoer is ontvang en die sukses van die werkswinkels kan toegeskryf word aan kort relevante aanbiedings deur die sprekers, boordbesoeke en 'n fokus op kultivars in die onderskeie areas, asook 'n kultivar-uitstalling.

7.1.7 CRI Geïntegreerde Plaagbeheer en Siektebestuur Werkswinkels

Die CRI IPM en DM is in Limpopo, Mpumalanga, Oos Kaap en Weskaap aangebied. Die formaat van die werkswinkel was dieselfde met kort en relevante aanbiedings en aan die einde van elke afdeling het 'n paneelbespreking goeie interaksie van die gehoor uitgelok. Die raamwerk van die FMS was aangebied as die hoof onderwerp, en die geleentheid vir vroeë en kommentaar was in 'n spesiale besprekingsessie afgehandel.

Daar was heelwat deelname en interaksie vanaf die produsente. Positiewe terugvoer vanaf produsente oor CRI se metode van aanbieding van hierdie werkswinkels waar daar gefokus word op toegepaste, wetenskaplike en relevante onderwerpe het bygedra tot die sukses van hierdie tipe forums om die nuutste inligting aan produsente deur te gee.

7.1.8 CRI Na-oes Werkswinkels 2018

Die CRI Na-oes werkswinkels vir 2018 het suksesvol afgegaan, met alle maandelike onderwerpe wat gedek is om in die voorligtingsbehoefte van die pakhuis te voorsien. Die formaat van die werkswinkels is suksesvol

aangepas om voorsiening te maak vir die groeiende bywoningsgetalle. Minder en korter aanbiedinge is aangebied om tyd vir paneelbesprekings toe te laat. 'n Wye reeks onderwerpe is aangespreek, wat strek van voor-oes praktyke, regdeur tot die logistieke ketting. 'n Totaal van meer as 1200 persone het bygewoon. Die werksinkels is in Tzaneen (30-31 Januarie), Loskopdam (1-2 Februarie), Nelspruit (12-13 Februarie), Durban (15-16 Februarie), Simondium (20-21 Februarie) en Jeffreysbaai (22-23 Februarie) aangebied. Wenkem/Citrosol en ICA was onderskeidelik weer die hoofborg en platinumborg gewees.

7.1.9 10de CRI Sitrusnavorsings simposium 2018

Die beplanning vir die simposium is goed op koers en die totale borgskappe tot op hede is feitlik reeds genoeg om die totale kostes, om die simposium aan te bied, te dek. Die "scientific committee" het gedurende Februarie vergader om die beplanning van die wetenskaplike program vir die simposium aan die gang te sit. Gesprekke met buitelandse navorsers om as "keynote speakers" genooi te word, vind op die oomblik plaas.

7.1.20 Opsomming van aktiwiteite

Opsomming van aktiwiteite deur Hannes Bester vir die jaar

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
4 Apr 2017	Dawid Groenewald	Beplanning	Hannes Bester
	Teunis Vahrmeijer	Navorsings projekte	Hannes Bester Teunis Vahrmeijer
1-3 Mei 2017	Nelspruit	Voorligting beplanning	Hannes Bester MC Pretorius Wayne Mommsen Keith Lesar Liezl vd Linde
4 Mei 2017	Hoopstad: Dr Schalk van Wyk	Bespreek nuwe produkte: Restore & Crop Candy	Hannes Bester
16 Mei 2017	Nampo	Borge vir simposium	Hannes Bester
17-18 Mei 2017	Teunis Vahrmeijer	Aanbiedinge vir werksinkels	Hannes Bester Teunis Vahrmeijer
19 Mei 2017	Brits	Boordbesoeke met Ryno Prins	Hannes Bester
22 Mei 2017	Letsitele	Groep 91 Houers Kooperatief Laeveld Sitrus: Snoei	Hannes Bester Wayne Mommsen
23 Mei 2017	Letsitele	Alesia Pakhuis Snoeiwerksinkels	Hannes Bester Wayne Mommsen
25 Mei 2017	Johannesburg	Borge besoeke: Bayer BASF	Hannes Bester Liezl vd Linde
26 Mei 2017	Johannesburg	Borge besoeke: Villa Crop Protection Mpact Sappi	Hannes Bester Liezl vd Linde Dawid Groenewald
29 Mei 2017	Humansdorp	Vergadering met Die Koöp: Adriaan Moolman Bertus Jacobs Gerhard van Vuuren	Hannes Bester
6-7 Jun 2017	Limpopo 1 CRI Produksie- werksinkels	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde

			Johan Joubert Werner Swiegers Jakkie Stander Hoppie Nel Hannes Coetzee
8-9 Jun 2017	Limpopo 2 CRI Produksie-werkswinkel	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Johan Joubert Werner Swiegers Jakkie Stander Hoppie Nel Hannes Coetzee
13-14 Jun 2017	Mpumalanga CRI Produksie-werkswinkel	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Johan Joubert Werner Swiegers Jakkie Stander Hannes Coetzee
14 Jun 2017	Jan Botha: Ashburton	Simposium borg	Hannes Bester
20-21 Jun 2017	Oos-Kaap CRI Produksie-werkswinkel	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Johan Joubert Werner Swiegers Jakkie Stander Hoppie Nel Hannes Coetzee
22-23 Jun 2017	Wes-Kaap CRI Produksie-werkswinkel	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Johan Joubert Werner Swiegers Jakkie Stander Hoppie Nel Hannes Coetzee
27 Jun 2017	RSG: Johan Rademan en Martelize Brink	Praatjie oor sitrus	Hannes Bester
	BFAP: Johan Boonzaaier	Projek oor ekonomie in sitrusproduksie	Hannes Bester Paul Cronje
28 Jun 2017	Dow: Johan Janse van Rensburg	Simposium borgskap	Hannes Bester
	Subtropico: Johan Smit	Nadorcott verpakking	Hannes Bester
29 Jun 2017	Franschhoek: ETP meeting	Agenda	Hannes Bester Paul Cronje

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkeses / Sprekers
5 Jul 2017	Kirkwood Studiegroep	Snoeiwerkswinkel	Hannes Bester

11 Jul 2017	Midnight Studiegroep	Snoeiwerkswinkel	Hannes Bester MC Pretorius
12 Jul 2017	Schoonbee Bdy	Brand op manderyne	Hannes Bester MC Pretorius
	Groblersdal studiegroep	Navorsings-prioriteite	Hannes Bester MC Pretorius
14-15 Jul 2017	Groblersdal	MPact strategiese sessie	Hannes Bester
17 Jul 2017	Hoedspruit studiegroep	Navorsingsprioriteite	Hannes Bester Wayne Mommsen
	Letsitele Studiegroep	Navorsingsprioriteite	Hannes Bester Wayne Mommsen
18 Jul 2017	Waterberg studiegroep	Navorsingsprioriteite	Hannes Bester Wayne Mommsen
19 Jul 2017	Tshipise Studiegroep	Navorsingsprioriteite	Hannes Bester Wayne Mommsen
24 Jul 2017	Swellendam studie-groep	Navorsingsprioriteite	Hannes Bester
25 Jul 2017	Breederivier studie-groep	Navorsingsprioriteite	Hannes Bester
26 Jul 2017	Citrusdal Skadunet-dag	Agenda	Hannes Bester Paul Cronje Jakkie Stander
	Citrusdal studiegroep	Navorsingsprioriteite	Hannes Bester
27 Jul 2017	Kirkwood: Agri SRV	Agenda	Hannes Bester Vaughan Hattingh Paul Cronje
28 Jul 2017	Katrivier Studiegroep	Navorsingsprioriteite	Hannes Bester
31 Jul 2017	Sondagsrivier studie-groep	Navorsingsprioriteite	Hannes Bester
3 Aug 2017	Benede Oranjerivier studiegroep	Navorsingsprioriteite	Hannes Bester
5 Aug 2017	Kakamas: Rian van Zyl	Boordbesoeke	Hannes Bester
8 Aug 2017	Katrivier studiegroep	Navorsingsprioriteite hersien	Hannes Bester
10 Aug 2017	Baviaans studiegroep	Navorsingsprioriteite	Hannes Bester
17 Aug 2017	Vaalharts studiegroep	Navorsingsprioriteite	Hannes Bester
	Produsente besoeke: Retha Greyling Michael van Niekerk Leon Du Preez	Snoeidemonstrasie en boordbesoeke	Hannes Bester
18 Aug 2017	Vaalharts	Oase Kwekery Boordbesoeke	Hannes Bester
23 Aug 2017	CFB	Paul Fourie en Thys Du Toit	Hannes Bester Wayne Mommsen
	Kirkwood	Inteligro: FCM beheer	Hannes Bester Wayne Mommsen
24 Aug 2017	Kirkwood	Pakhuisforum vergadering	Hannes Bester Wayne Mommsen
29-30 Aug 2017	Limpopo 1 IPM & DM werkwinkels	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Wayne Mommsen Sean Moore Aruna Manrakhan Tim Grout Martin Gilbert

			Thys Du Toit Charl Kotze Providence Moyo Jan van Niekerk Mareli Kellerman
31 Aug-1 Sep 2017	Limpopo 2 IPM & DM werkswinkels	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Wayne Mommsen Sean Moore Aruna Manrakhan Tim Grout Martin Gilbert Thys Du Toit Charl Kotze Providence Moyo Jan van Niekerk Mareli Kellerman
4 Sep 2017	Nelspruit	Voorligting beplanning	Hannes Bester MC Pretorius Liezl vd Linde
5-6 Sep 2017	Mpumalanga IPM & DM werkswinkels	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Wayne Mommsen Wayne Kirkman Elma Carstens Aruna Manrakhan Tim Grout Martin Gilbert Paul Fourie Charl Kotze Providence Moyo Jan van Niekerk
7 Sep 2017	Nelspruit	Biosecurity Coordination meeting	Hannes Bester Wayne Mommsen MC Pretorius Vaughan Hattingh Tim Grout Sean Moore Glynnis Cook Paul Fourie
12-13 Sep 2017	Oos-Kaap IPM & DM werkswinkels	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Wayne Mommsen Sean Moore Aruna Manrakhan Tim Grout Martin Gilbert Thys Du Toit Charl Kotze Providence Moyo Jan van Niekerk

			Mareli Kellerman
14-15 Sep 2017	Wes-Kaap IPM & DM werksinkels	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Wayne Mommsen Sean Moore Aruna Manrakhan Tim Grout Martin Gilbert Thys Du Toit Charl Kotze Providence Moyo Jan van Niekerk Mareli Kellerman

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkeses / Sprekers
3 Okt 2017	Nelspruit	Na-oes werksinkels beplannings-vergadering	Hannes Bester Dawid Groenewald Liezl vd Linde Keith Lesar Wilma Du Plooy Wayne Mommsen
10 Okt 2017	PE: FMS Working Group meeting	Agenda	Hannes Bester Vaughan Hattingh Sean Moore
11 Okt 2017	DAFF Citrus Coordinating meeting	Agenda	Hannes Bester Vaughan Hattingh Sean Moore
17 Okt 2017	Pretoria	Ashburton / FNB: Borgskap vir simposium	Hannes Bester Liezl vd Linde
18 Okt 2017	Johannesburg	IPM Research Committee meeting	Hannes Bester Sean Moore Tim Grout
19 Okt 2017	Johannesburg	DM Research Committee meeting	Hannes Bester Tim Grout Paul Fourie Jan v Niekerk
24 Okt 2017	Stellenbosch	CLFQ Research Committee meeting	Hannes Bester Tim Grout Paul Cronje
25 Okt 2017	Stellenbosch	Cultivar Evaluation Committee meeting	Hannes Bester Tim Grout Johan Joubert Werner Swiegers
26 Okt 2017	Stellenbosch	ETP meeting	Hannes Bester Dawid Groenewald MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage Keith Lesar
	Stellenbosch	Cooling Working Group meeting	Hannes Bester Dawid Groenewald

			MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage Keith Lesar
27 Okt 2017	Simondium	Wes-Kaap Droogtebestuur Boeredag	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage
31 Okt 2017	Nelspruit	Clean Corridor Project meeting	Hannes Bester Vaughan Hattingh Tim Grout MC Pretorius
1 Nov 2017	Nelspruit	Simposium beplanningsvergadering	Hannes Bester Liezl vd Linde MC Pretorius
7 Nov 2017	Durban	Arysta: Borgskap vir simposium	Hannes Bester Liezl vd Linde
	Durban	Umhlanga Hotel: Na-oes Werkswinkels	Hannes Bester Liezl vd Linde
	Durban	Riverside Hotel: Na-oes werkswinkels	Hannes Bester Liezl vd Linde
8 Nov 2017	CSR	Reëlings t.o.v simposium	Hannes Bester Dawid Groenewald Liezl vd Linde MC Pretorius Wayne Mommsen Christine Stoppel- Grove Jon Pinker
14 Nov 2017	Pretoria	Packaging Working Group meeting	Hannes Bester Dawid Groenewald
	Brits	Boordbesoeke	Hannes Bester
15 Nov 2017	Brits	Studiegroepvergadering	Hannes Bester Wayne Mommsen
	Brits	Boordbesoeke	Hannes Bester
16 Nov 2017	Polokwane	Elé Boeredag	Hannes Bester MC Pretorius Wayne Mommsen Johan Joubert Paul Cronje
23 Nov 2017	Johannesburg	CRI Board meeting	Hannes Bester Vaughan Hattingh Tim Grout Jon Pinker
	Johannesburg	Compac	Hannes Bester
	Johannesburg	Biosecurity Onderhoude	Hannes Bester Vaughan Hattingh Tim Grout Paul Fourie
6 Des 2017	Stellenbosch	Vaughan Hattingh Paul Cronje	Hannes Bester

		Tarl Berry	
	Stellenbosch	Cheryl Lennox	Hannes Bester
	Stellenbosch	JBT: Jaco Theron	Hannes Bester
7 Des 2017	Durbanville	FPC: Grant & Roy Ferguson, Christo Spies	Hannes Bester
	Paarl	DOW: Johan Janse van Rensburg	Hannes Bester

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
30-31 Jan 2018	Limpopo 1 CRI Na-oes werkwinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Keith Lesar Wilma Du Plooy Wayne Mommsen Catherine Savage Sean Moore Paul Cronje
1-2 Feb 2018	Limpopo 2 CRI Na-oes werkwinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Keith Lesar Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
5-8 Feb 2018	CRI Management Meeting	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Vaughan Hattingh Tim Grout Christine Stoppel-Grove Sean Moore Paul Fourie Paul Cronje Jan van Niekerk Jon Pinker
8 Feb 2018	Scientific Committee meeting	Agenda	Hannes Bester Vaughan Hattingh Tim Grout Paul Fourie Sean Moore Paul Cronje Jan van Niekerk
9 Feb 2018	Voorligtingbeplannings-vergadering	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Dawid Groenewald Keith Lesar Catherine Savage

12-13 Feb 2018	Mpumalanga CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Keith Lesar Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
15-16 Feb 2018	KZN & Swaziland CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Keith Lesar Wilma Du Plooy Wayne Mommsen Catherine Savage Sean Moore Paul Cronje
20-21 Feb 2018	Wes-Kaap CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Keith Lesar Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
22-23 Feb 2018	Oos-Kaap CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald Liezl vd Linde Keith Lesar Wilma Du Plooy Wayne Mommsen Catherine Savage Sean Moore Paul Cronje
26 Feb 2018	Gamtoos	Besoek met Pieter Raath en Netafim	Hannes Bester Pieter Raath
28 Feb 2018	Sondagsriviervallei	CGA meeting	Hannes Bester Vaughan Hattingh
1 Mrt 2018	Gamtoos	CGA meeting	Hannes Bester Vaughan Hattingh
12-13 Mrt 2018	Nelspruit	Performance appraisals & Simposium beplanning	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl van der Linde Catherine Savage
14 Mrt 2018	CMF vergadering	Agenda	Hannes Bester
20-21 Mrt 2018	Wes-Kaap: Produsente- besoeke	Jannie Toerien At Venter Johan Mouton	Hannes Bester

		André Neethling	
22 Mrt 2018	Stellenbosch	Pieter Raath Jakkie Stander	Hannes Bester

Opsomming van aktiwiteite deur MC Pretorius vir die jaar

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
3 – 7 Apl 2017	Produsent besoeke	Groblersdal area: Charles Rossouw Koos de Wet Ista Upton Arrie v d Walt Hannes Hertzog Pieter Nel Piet Engelbrecht	MC Pretorius
10 April 2017	Nelspruit	Produksie werkswinkel borgskap vergadering Felco: Werner Felco	MC Pretorius
12- 13 April 2017	Ohrigstad	Boordbesoeke: Mahela Kobus Beetge	MC Pretorius
19 April 2017	Groblersdal	Studiegroep vergadering	Mc Pretorius Teunis Vahrmeijer
25 April 2017	Malelane	Studiegroep vergadering	MC Pretorius Johan Joubert Chris Kellerman
6 – 12 Mei 2017	KZN	Nematologie vereniging simposium	MC Pretorius Elaine Basson
15 Mei 2017	Nelspruit	Produksie werkswinkel beplannings vergadering	MC Pretorius Wayne Mommsen Liezl vd Linde James Warrington Chris Kellerman Johan Joubert
17 Mei 2017	Karino	Swartvlek bespuitings koste bepalings met Hannes Breedt	MC Pretorius
19 Mei 2017	Nelspruit	Produsente besoeke James Warrington Flip Walters	MC Pretorius
22 – 24 Mei 2017	Nelspruit	Eksperiment 1092 - studies	MC Pretorius
5 -7 Jun 2017	Hoedspruit en Groblersdal	Produksiewerkswinkels	MC Pretorius Hannes Bester Wayne Mommsen Liezl vd Linde
13 – 14 Jun 2017	Nelspruit	Produksiewerkswinkel	MC Pretorius Hannes Bester Wayne Mommsen Liezl v d Linde
19 – 23 Jun 2017	Oos-Kaap en Wes-Kaap	Produksiewerkswinkel	MC Pretorius Hannes Bester Wayne Mommsen Liezl vd Linde

27 Jun 2017	Nelspruit	Entomologie navorsings beplannings vergadering	MC Pretorius Wayne Mommsen
Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkeses / Sprekers
3 – 6 Jul 2017	Studies eksperiment 1092	Boord opnames; visueel asook deur student hulp van UP en lugopnames om Hyperspectral Imaging van decline boorde te bepaal.	MC Pretorius Prof Nico Labuschagne Barend Swart Charl Kotze
11 Jul 2017	Midnight Studiegroep	Snoei werkswinkel Studiegroep vergadering	MC Pretorius Hannes Bester
12 Jul 2017	Navorsings prioriteite – Schoonbee landgoed	Grobbersdal en Marble Hall area; Voorsitters en tegniese komitees van Loskop en Midnight studiegroepe asook Laeveld Agrochem	MC Pretorius Hannes Bester
13 Jul 2017	Navorsings prioriteite - Naranje	Burgersfort en Ohrigstad – Voorsitters en tegniese personeel en prominente produsente van areas was betrokke	MC Pretorius Wayne Mommsen
14 Jul 2017	Produsent besoeke	Neels Kok – probleem met blaarval in midnight boorde beide buite en onder net Pieter Nel – Bemesting en Gibb bespreking en boord besoek	MC Pretorius
24 Jul 2017	Navorsings prioriteite – CRI raadsaal	Nelspruit, Onderberg, Swaziland en Pongola areas	MC Pretorius James Warrington Chris Kellerman Hannes Breedt Karlien Grobler
25 Jul 2017	Navorsings prioriteite - Telefonies	Nkwaleni en Suid Natal	MC Pretorius Mike Woodburn Mike Wayfer
26 – 28 Jul 2017	Wes Kaap	Net proef terugvoer Citrusdal	MC Pretorius
31 Jul 2017	Zimbabwe besoek	Koördinering, beplanning en bespreking van Zimbabwe besoek	MC Pretorius John Perrot Tim Grout Elma Carstens Vaughan Hattingh
1-3 Aug 2017	CRI Grondvesblok – Uitenhage Boordbesoeke: Kirkwood Kwekery besoek: Paksaam	Jaarlikse Grondvesblok evaluasies Boomvrektes in Kirkwood en Sondagsrivier Voorligting aan kwekery op versoek van Paksaam	MC Pretorius Janine Joubert Michael Janse van Rensburg
7 – 11 Aug 2017	Zimbabwe besoek Admin	Zimbabwe besoek beplanning en nuwe paspoort aansoek en afhaal	MC Pretorius
15 – 18 Aug 2017	Zimbabwe biosekuriteits besoek	Biosekuriteits risiko's aan Plant Kwarantyn amptenare bekend gestel asook sitrus produksie – entomologies, vooroes en na-oes siektebestuur en kultivar en	MC Pretorius Wayne Mommsen Johan Joubert Wilma du Plooy Catherine Savage

		onderstam keuses vir die Noordelike streek spesifiek	Tim Grout
21 – 22 Aug 2017	Nelspruit area	Vergroenings boom/boord identifikasie vir Glynnis Cook vir die gebruik in die snuffel hond projek	MC Pretorius Glynnis Cook
28 Aug – 1 Sep 2017	Limpopo area	Tzaneen en Groblersdal IPM en siektebestuur werkswinkels	MC Pretorius Wayne Mommsen Hannes Bester Liezl van der Linde Catherine Savage
5 – 6 Sep 2017	Nelspruit area	Burgersfort, Ohrigstad, Nelspruit, Onderberg, Swaziland en Pongola areas; IPM en siektebestuur werkswinkels	MC Pretorius Wayne Mommsen Hannes Bester Liezl van der Linde Karin Nel
7 Sep 2017	Nelspruit – raadsaal	Biosekuriteits operasionele bestuurder - posbeskrywings bespreking Afsluitings funksie vir Dr. Fanie van Vuuren	MC Pretorius Vaughan Hatting Hannes Bester Tim Grout Sean Moore Paul Fourie Jan van Niekerk Jacolene Meyer Elma Carstens Glynnis Cook Wayne Mommsen Schalk Schoeman
11 – 15 Sep 2017	Oos Kaap en Wes Kaap	IPM en siektebestuur werkswinkels	MC Pretorius Wayne Mommsen Hannes Bester Liezl van der Linde Karin Nel
19 – 22 Sep 2017	Stellenbosch	Annual Soilborne disease interest group meeting – Climate and Soilborne disease NSSA bestuurs kommitee vergadering	MC Pretorius

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
5–6 Okt 2017	Loskop – Groblersdal/Marble Hall	Produsent besoeke - Piet Engelbrecht, Pieter Nel en Koos de Wet	MC Pretorius
10 Okt 2017	Onderberg	VKM, Parings ontwinging	MC Pretorius Chris Kellerman
16–18 Okt 2017	Nelspruit	Droogtestrategie bespreking en beplanning	MC Pretorius James Warrington Hannes Breedt
23 Okt 2017	Nelspruit	DAFF seisoen terugvoer en FCM FMS bekendstellings vergadering	MC Pretorius
24 Okt 2017	Nelspruit	Besoek moontlike venues vir Nelspruit Na-oes vergadering	MC Pretorius Liezl vd Linde
26 Okt 2017	Wes Kaap	Exporters forum meeting	MC Pretorius

		Cooling working group	Hannes Bester Keith Lesar Liezl vd Linde Paul Cronje
27 Okt 2017	Wes Kaap	Droogtestrategie werkswinkel	MC Pretorius Hannes Bester Keith Lesar Liezl vd Linde Hugo Endeman
30 – 31 Okt 2017	Nelspruit	EU delegation to meet with CRI for assistance in compiling a proposal for the “Clean Corridor project”. Karino Orchard visits and proposal planning meeting at CRI	MC Pretorius Hannes Bester Vaughan Hattingh Tim Grout Deon Joubert Sean Moore
2 Nov 2017	Groblersdal	Besoek probleem boorde - Neels Kok Henry Pieters	MC Pretorius Charl Kotze
7 – 10 Nov 2017	CSR	Simposium beplanning	MC Pretorius Hannes Bester Wayne Momsen Liezl vd Linde Christine Stoppel-Grove Jon Pinker Dawid Groenewald
14 Nov 2017	Loskop	Studiegroep – VKM, Bemesting en Besproeiing	MC Pretorius
22 Nov 2017	Nelspruit	Bayer borgskap/ simposium vergadering	MC Pretorius Liezl vd Linde
23-24 Nov 2017	Burgersfort	Studiegroep vergadering VKM, Kultivars en onderstamme en boord besoeke	MC Pretorius Johan Joubert
28 Nov 2017	Nelspruit	Studiegroep – VKM, Parings ontwringing en Kultivar bespreking	MC Pretorius Johan Joubert
29 – 1 Des 2017	Letsitele Hoedspruit	Mahela – Boordbesoeke: Phytophthora, Midnight terugsterwings sindroom, grond pH probleem en kultivars Hoedspruit - Parma Kwekery adviseur sitruskwekery	MC Pretorius
11 – 14 Nov 2017	KZN	Nkwaleni – Mike Wafer Carrisbrooke: Mike Woodburne, Peter and Paul Button + studiegroep vergadering VKM, Kultivar en onderstam bespreking	MC Pretorius Johan Joubert

Datum	Studiegroep/Aktiwiteit	Onderwerpe/aksies	Betrokkeses / Sprekers
18–19 Jan 2018	Nelspruit Na-oes werksinkels	Finale beplanning vir werksinkels	MC Pretorius Liezl vd Linde

22-24 Jan 2018	Na-oes wekswinkels	Tzaneen werkswinkel	MC Pretorius Hannes Bester Liezl vd Linde Wayne Momsen Keith Lesar Catherine Savage Dawid Groenewald
24-26 Jan 2018	Na-oes wekswinkels	Groblersdal werkswinkel	MC Pretorius Hannes Bester Liezl vd Linde Keith Lesar Catherine Savage Dawid Groenewald
5-8 Feb 2018	CRI Bestuursvergadering	Skukuza	MC Pretorius Hannes Bester Wayne Mommsen Vaughan Hattingh Tim Grout Paul Fourie Paul Cronje Jan v Nieker Jon Pinker Christene Stoppel- Grove
9 Feb 2018	Voorligting beplannings vergadering	Voorligting jaar beplanning	MC Pretorius Hannes Bester Liezl vd Linde Wayne Momsen Keith Lesar Catherine Savage Dawid Groenewald
12-13 Feb 2018	Na-oes wekswinkels	Nelspruit werkswinkel	MC Pretorius Hannes Bester Liezl vd Linde Keith Lesar Catherine Savage Dawid Groenewald
14-16 Feb 2018	Na-oes wekswinkels	KZN werkswinkel	MC Pretorius Hannes Bester Liezl vd Linde Wayne Momsen Keith Lesar Catherine Savage Dawid Groenewald
19-21 Feb 2018	Na-oes wekswinkels	Wes-Kaap werkswinkel	MC Pretorius Hannes Bester Liezl vd Linde Keith Lesar Catherine Savage Dawid Groenewald
21-22 Feb 2018	Stellenbosch	Studies – Universiteit van Stellenbosch	MC Pretorius Adele McCloed
28 Feb 2018	Baberton	Produsent besoeke	MC Pretorius

	Nelspruit	Pakhuis voorligtingsbesoek beplanning	MC Pretorius Keith Lesar Catherine Savage
1 Mrt 2018	Ohrigstad	Produsent besoeke	MC Pretorius Mahela boerdery – Sean Colyn Kobus Beetge Smit le Roux
2 Mrt 2018	Nelspruit	Beplanning en bespreking met konsultante	MC Pretorius James Warrington Chris Kellerman
7 Mrt 2018	Nelspruit	Rayton Estate – Pakhuisvoorligting besoek CGA Roadshow	MC Pretorius Keith Lesar Catherine Savage MC Pretorius Vaughan Hattingh
8 Mrt 2018	Nelspruit	Facility transfer celebrating event beplanning	MC Pretorius Vaughan Hattingh Liezl vd Linde
9 Mrt 2018	Nelspruit	Joubert en Seuns Pakhuisvoorligting besoek	MC Pretorius Keith Lesar Catherine Savage
12-13 Mrt 2018	Nelspruit	Simposium beplanning en appraisals	MC Pretorius Hannes Bester Liezl vd Linde Dawid Groenewald Wayne Mommsen
14 Mrt 2018	Onderberg	Produsent besoeke; Chimera opvolg opname en kultivar proefblok besoek	MC Pretorius Johan Joubert Karlien Grobler GFC Komati groep
16 Mrt 2018	Karino	Karino produsent besoeke en Karino Kooperasie Pieter Raath besoek beplanning	MC Pretorius Hannes Breedt
18-19 Mrt 2018	Mookgopong/Bella Bella	NSSA symposium venue besoek en beplanning	MC Pretorius Mieke Daneel Ethnie Cameron
26 -27 Mrt 2018	Groblersdal/Marble Hall	Produsent besoeke asook Pieter Raath bekendstelling aan verskillende bemestings benaderings van produsente	MC Pretorius Pieter Raath Piet Engelbrecht Schoonbee Landgoed Schoeman Boerdery - Agron

Opsomming van aktiwiteite deur Wayne Mommsen vir die jaar

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
11 & 12 Apr 2017	Voorligtingsbesoeke	Stinkbesieskade op sagte sitrus Hoedspruit & Weipe	Wayne Mommsen
19 Apr 2017	Voorligtingsbesoek Levubu	Suurlemoen boorde uitvoer- Stef de Lange. Hannes Smit – Nuwe produsent	Wayne Mommsen

25 Apr 2017	Werkswinkel reelings Hoedspruit	Ambrosia Sitrus vir Boordbesoeke	Wayne Mommsen
4 Mei 2017	Tegniese Vergadering Hoedspruit	VKM beheer strategie en Systems approach	Wayne Mommsen Fanie Meyer Tom van der Meulen
23 Mei 2017	Studiegroep Letsitele	Snoeiwerkswinkel	Hannes Bester Wayne Mommsen
31 Mei 2017	Studiegroep Tshipise	Witluis, VKM & Karobmot bestuur	Wayne Mommsen Dr Sean Moore
1 & 2 Jun 2017	Voorligtingbesoek Zimbabwe Suid	Nottingham Estates & Cawood Citrus, CGA Direkteur ZIM	Wayne Mommsen Dr Sean Moore
6 – 9 Jun 2017	Produksiewerkswinkels	Swadini + Loskop	Hannes Bester Wayne Mommsen MC Pretorius Jakkie Stander Dr Hoppie Nel Johan Joubert Werner Swiegers John Roberts Dr Hannes Coetzee Liezl vd Linde
13 & 14 jun 2017	Produksiewerkswinkels	Nelspruit	Hannes Bester Wayne Mommsen MC Pretorius Jakkie Stander Dr Hoppie Nel Johan Joubert Werner Swiegers John Roberts Dr Hannes Coetzee Liezl vd Linde
20 – 23 Jun 2017	Produksiewerkswinkels	Addo + Citrusdal	Hannes Bester Wayne Mommsen MC Pretorius Jakkie Stander Dr Hoppie Nel Johan Joubert Werner Swiegers John Roberts Dr Hannes Coetzee Liezl vd Linde
27 Jun 2017	CRI Voorligting en Navorsing Vergadering	IPM navorsingsprojekte bespreking	Aruna Manrakahn Claire Love John Henry Daneel Martin Gilbert MC Pretorius Sean Moore Tim Grout Vaughn Hattingh Wayne Kirkman Wayne Mommsen

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
5 Jul 2017	Kliente Besoek Letsitele	Bespreek Blaaspooitjie en Witluis beheer program Laeveld Sitrus	Wayne Mommsen Niek Grobler
6 Jul 2017	Vergadering Weipe	CGA Direkteur Musina. Reel vir TTG vergadering en Navorsingsprioriteite.	Wayne Mommsen Bertus Dillman
11 Jul 2017	Boeredag Letsitele	Maxi Yield nadering tot bemesting met vis emulsie. Heelwat hektare wat inkoop op dit.	Wayne Mommsen
13 Jul 2017	Vergadering Burgersfort	Navorsings prioriteite met Origstad en Burgersfort produsente.	MC Pretorius Wayne Mommsen Andrew Cooper Smit Le Roux Kobus Beetge Gustav Mallo Albert Winterbach Frans Winterbach
14 Jul 2017	Vergadering Studiegroep Voorsitter en Borge	Studiegroep reëlings en borge vir werkswinkel	Hannes Meintjies Andries Burger van Rolfes Group
17 Jul 2017	Navorsing prioriteite vergadering	Hoedspruit	Wayne Mommsen Hannes Bester Hannes Meintjies Tom Van der Meulen Fanie Van Vuuren
17 Jul 2017	Navorsing prioriteite vergadering	Letsitele	Wayne Mommsen Hannes Bester Ben Vorster Eddie Vorster Henk Van Rooyen Gerhard Vorster Pierre Smit Desi Fourie
18 Jul 2017	Navorsing prioriteite vergadering	Waterberg StudieGroep	Wayne Mommsen Hannes Bester Danie Janse Van Vuuren Abe Devilliers
19 Jul 2017	Navorsing prioriteite vergadering	Tshipise	Wayne Mommsen Hannes Bester Peter Nicholson Willie Nel Christo Vorster
19 Jul 2017	Navorsing prioriteite vergadering	Weipe	Wayne Mommsen Bertus Dillman Francois Dillman Danie Erasmus Pietman Pieterse
26 Jul 2017	CRI Inligtingsdag	Navorsing terugvoer op skadu net	Wayne Mommsen Hannes Bester MC Pretorius CRI & Produsente
31 Jul 2017	Boeredag Letsitele	Philagro	Wayne Mommsen

			Ian Garden
5 Aug 2017	StudieGroep Waterberg	Gee terugvoer by Studiegroep oor Navorsing prioriteite	Wayne Mommsen Dewalt Eksteen Jan Odendaal
7 Aug 2017	Pakhuis Besoeke Hoedspruit	Ambrosia Sitrus Moriah Sitrus	Wayne Mommsen Catherine Savage Keith Lesar
8 Aug 2017	Pakuis Besoek Hoedsp. Studiegroep	Landman Vars Produkte Constantia Studiegroep	Wayne Mommsen Catherine Savage Keith Lesar
9 – 11 Aug 2017	Pakhuis Besoeke	Novelle La Cotte, Rooister Bdy Letaba Estates, CPJ Erasmus Bdy, Mahela Bdy	Wayne Mommsen Catherine Savage Keith Lesar
10 Aug 2017	Papier Mulch Proef	Novengila Letsitele Groep 91	Wayne Mommsen Dawid Groenewald Frikkie Van Wyk Wimpie Mostert
14 Aug 2017	Tshipise Studie Groep	Hoof fokus op Witluis beheer en Blaaspooitjie program	Wayne Mommsen BioBee SA Produsente
15-18 Aug 2017	N. Zimbabwe Besoek	Besoek plase in Umvurwi, Mazoe en Chigutu. Biosekuriteits vergadering om af te sluit	Wayne Mommsen Johan Joubert Tim Grout MC Pretorius Catherine Savage MC Pretorius Wilma Du Plooy
23 Aug 2017	Kirkwood Boeredag	FCM bestuur aanbieding	Wayne Mommsen Hannes Bester Wayne Kirkman
24 Aug 2017	Pakhuisforumvergadering	SRCC	Wayne Mommsen Hannes Bester
29-30 Aug 2017	IPM & DM Werkswinkel	Fairview Tzaneen	Wayne Mommsen Hannes Bester MC Pretorius CRI Team
31 Aug – 01 Sept 2017	IPM & DM Werkswinkel	Loskop Groblersdal	Wayne Mommsen Hannes Bester MC Pretorius CRI Team
5-6 Sept 2017	IPM & DM Werkswinkel	Nelspruit	Wayne Mommsen Hannes Bester MC Pretorius CRI Team
7 Sept 2017	Biosekuriteit vergadering	Nelspruit kantoor	Wayne Mommsen Hannes Bester Sean Moore Schalk Schoeman Tim Grout Vaughn Hattingh Elma Carstens MC Pretorius Aruna Manrakhan

11-15 Sept 2017	IPM & DM Werkswinkel	J-Bay & Franschoek	Wayne Mommsen Hannes Bester MC Pretorius CRI Team
19 Sept 2017	Studiegroep Tegniiese komitee vergadering	Mahela raadsaal oor FCM strategie 2018	Wayne Mommsen Eddie Vorster Hugo Endemann Gerhard Vorster Desi Fourie Deon Koekemoer Johan Botma Henk Van Rooyen Johan Gubitz
29 Sept 2017	Vergadering	Groep 91 oor FCM beheer opsies en strategie	Wayne Mommsen Henk Van Rooyen

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
4 Okt 2017	Tshipise Studiegroep	FCM bestuur & FMS	Wayne Mommsen (CRI)
10 Okt 2017	Letsitele Studiegroep Tegniiese Komitee	FCM bestuur strategie bespreking	Ben Vorster Eddie Vorster Hugo Endemann Henk Van Rooyen Johan Gubitz Deon Koekemoer Johan Botma Desie Fourie Pierre Smit Gerhard Vorster Wayne Mommsen
	Constantia Studiegroep	FMS en Area wye FCM bestuur strategie	Wayne Mommsen Chris Hendriks Cor Greyling (Wenkem)
12 Okt 2017	Weipe Besoek CGA Direkteur	Beplanning vir Studiegroep en Swartvlek spuitprogram bespreking	Francois Dillman Bertus Dillman Wayne Mommsen
13 Okt 2017	Letsitele Boeredag	Laeveld Agrochem FCM bestuurs-opsies	Otto Friedlingsdorf (Chem pak), Chris Hendriks (RB), Wayne Mommsen (CRI), Deon Marais (LAC)
18 Okt 2017	Hoedspruit Studiegroep	FMS en FCM bestuur	
24 Okt 2017	Daff/CGA Roadshow	Hoedspruit	Tankiso Mpholo Vaughn Hattingh Wayne Mommsen
25 Okt 2017	Daff/CGA Roadshow	Letsitele	Tankiso Mpholo Vaughn Hattingh Wayne Mommsen

26 Okt 2017	Tshipise Studiegroep	FCM paaringsontwrigting, Water kwaliteit	At Pelser (Aquamat), Martin V Niekerk (Insect Science) Wayne Mommsen
26 Okt 2017	Limpopo River Studiegroep	FCM paringsontwrigting, Water kwaliteit	At Pelser Martin Van Niekerk Wayne Mommsen
27 Okt 2017	Daff/CGA Roadshow	Musina	Tankiso Mpholo Paul Hardman Wayne Mommsen
1 – 3 Nov 2017	Zimbabwe North Citrus	Zimtrade Aanbiedings op FCM bestuur en FMS vir Zimbabwe uitvoer	John Perrot Pete Breitenstein Dr Mguni (DR&SS)
14 Nov 2017	Plase Besoek Brits	Jong bome bestuur, Mariaan Le Roux	Wayne Mommsen, Hannes Bester
15 Nov 2017	Noord-Wes Studiegroep	Swartvlek Bestuur en FMS	Charl Kotze Wayne Mommsen, Hannes Bester Ryno Prins
16 Nov 2017	ELE Boeredag	Algemene Boeredag vir produsente in die Noorde	Wayne Mommsen, Hannes Bester Johan Joubert Dr. Paul Cronje Marlene Calitz
17 Nov 2017	Tshipise Boord Besoeke	Witluis bestuur Strategie met Biologiese beheer (BioBee) Alicedale en Maswiri	Wayne Mommsen Peter Nicholson Dawie Smuts Eldad Peer Rami Friedman Arthur Lilford Willie Nel
24 Nov 2017	Letsitele Boordbesoek	Witluis skade op pomelos	Brian Trollip Wayne Estman Wayne Mommsen
1 Dec 2017	Pakhuis besoek Letsitele	Christie Landman pakhuis	Niel Jacobs Wayne Mommsen
4 Dec 2017	Plaasbesoek	Swartvlek Onderzoek	Carel Minnaar Wayne Mommsen
5 Dec 2017	Letsitele Studiegroep	LAC FCM Moniteering en FCM Lugbespuitings	Wayne Mommsen Chris Hendriks Johan Visagie (LAC)
6 Dec 2017	Letsitele Proefperseel	Mulching Proef met Papier	Dawid Groenewald Frikkie Van Wyk Wayne Mommsen
13 Dec 2017	Sitrus Kwekery besoek Hartebeespoort	Nuwe kwekery Stig en nuwe sitrus boorde plant	Wayne Mommsen David Heuer
14 Dec 2017	Waterberg Studiegroep	FCM bestuur 2018	Danie JV Rensburg Dewalt Eksteen Allan Penney(BASF), Dennis vd Walt(BASF)

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
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9 Jan 2018	Produsent Besoek Letsitele	Inspekteer pomelo boorde onder water stres	Wayne Mommsen Harry Grove
10 Jan 2018	Produsent Voorligting Letsitele	Advies oor Snoei van jong suurlemoen bome.	Dirk Denyschen Ockert Denyschen Wayne Mommsen
12 Jan 2018	Produsent Besoek Tom Burke	Advies oor Witluis uitbraak op 50 Ha Suurlemoene	Wayne Mommsen Arthur Lilford Thinus Adrianse
16 Jan 2018	Ontwikkeling Produsent Besoek Mooketsi (Makapesi Trading)	Algemene besoek en advies oor Woolly whitefly uitbraak op pomelos en valentias	Victor Munyai Wayne Mommsen
17 Jan 2018	Besoek met Navorsing Nelspruit CRI	Bespreek CBS en VV aksies.	Aruna Manrakhan Wilma Du Plooy Wayne Mommsen
22 Jan 2018	Voorligting met Uitvoer Agent	Phytclean Voorligting met Alliance Fruit Letsitele	Ivan Crocker Corrie Labuschagne Wayne Mommsen
30-31 Jan 2018	CRI Na-oes werkswinkel Tzaneen	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
1 - 2 Feb 2018	Monster naming vir CBS proewe	Kumquat monsters by Rooister Boerdery Letsitele	Tommie Rautenbach Wayne Mommsen
5 - 7 Feb 2018	Bestuursvergadering	CRI Beplanning en aksies te Skukuza	Tim Grout Vaughn Hattingh Sean Moore Hannes Bester MC Pretorius Christine Stoppel-Grove Jon Pinker Paul Fourie Paul Cronje Jan Van Niekerk Wayne Mommsen
8 Feb 2018	Studiegroep vergadering	Voorligting oorsig van hoofpunte van FMS	Eddie Vorster Henk Van Rooyen Ben Vorster Wayne Mommsen Harry Grove
9 Feb 2018	Voorligting Beplanning vergadering	2018 beplanning en Simposium	Hannes Bester Wayne Mommsen Dawid Groenewald Liezl van der Linde Keith Lesar Catherine Savage MC Pretorius

13 Feb 2018	Produsent besoek Letsitele	Letaba Estates sitrus insetkoste versameling	Deon Koekemoer Wayne Mommsen
14-16 Feb 2018	CRI Na-oes werksinkels Durban	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore
20 Feb 2018	Scouting Kursus	Marble Hall / Groblersdal bestuurders en Scouts	Wayne Mommsen
21-23 Feb 2018	CRI Na-oes werksinkels J-Bay	Agenda	Dawid Groenewald Keith Lesar MC Pretorius Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore Karin Nel
27 Feb 2018	Produsente Besoek Letsitele	Vergader met Direksie van Groep 91 Uitvoer en CGA Direkteur	Jan-Louis Pretorius Henk Van Rooyen Burgert Van Rooyen Wayne Mommsen
28 Feb - 02 Mrt 2018	Voorligting Suid Zimbabwe Produsente en CGA Direkteur.	FMS Voorligting en Ontwikkeling Plaas besoek. Cawood, Bishopstone en Knottingham. Shashi sitrus projek.	Paul Bristow Clive Pountney Rodon Tourle Andrew Knott Martin Van Niekerk Andrew Mbedzi
6 – 7 Mrt 2018	CGA Roadshow	Teenwoordig saam CGA span in Hoedspruit en Letsitele	Justin Chadwick Deon Joubert Tim Grout Lukhanyo Nkombisa Wayne Mommsen Clint Lawson Jacomien De Klerk
7 Mrt 2018	Produsente Besoek Hoedspruit	Besoek Suurlemoen Plaas Ambrosia en inligting oor uitvoere en Pryse	Coenie Scheepers Wayne Mommsen
7 Mrt 2018	Palette Proewe Letsitele	Defleksie toetse op Plastiek palette en 11 Slat palette vir VSA uitvoere	Dawid Groenewald Wimpie Mostert Frikkie Van Wyk Wayne Mommsen
12 Mrt 2018	Performance Appraisals	Nelspruit CRI	Hannes Bester Wayne Mommsen
13 Mrt 2018	Simposium Beplanning	Nelspruit CRI Raadsaal	Hannes Bester Liezl Van der Linde MC Pretorius Wayne Mommsen
15 Mrt 2018	Produsent Besoek Letsitele	Advies oor Phytophthora en Fusarium verwelk by Beli Trust. Chemiese brand op vrugte	Wayne Eastman Wayne Mommsen

16 Mrt 2018	Pakhuis besoek Letsitele	Nuwe Pakhuis by Denyschen Broers	Jacques Nel Dirk Denyschen Wayne Mommsen Andrew Jackson
19 Mrt 2018	Pakhuis besoek	Moletete Pakhuis Degreening Ambrosia Sitrus Pakhuis	Wayne Mommsen Keith Lesar Catherine Savage
20 Mrt 2018	Produsent en CGA direkteur besoek Pont Drift	CBS afgekeurde plaas en Nuwe ontwikkeling	Guy Whittaker Justin Whittaker Pietman Pieterse Wayne Mommsen
23 Mrt 2018	DAFF en PPECB Roadshow	Teenwoordig by Roadshow	Letsitele en Noord Limpopo produsente Wayne Mommsen
29 Mrt 2018	Produsent Besoek Letsitele	Advies oor snoei van jong suurlemoen bome by Gubitiz Laparisa	Johan Gubitiz Ian Pienaar Wayne Mommsen

Opsomming van aktiwiteite deur Dawid Groenewald vir die jaar

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
4 Apr 2017	Sappi Technology Centre	Akkrediasie toetse en papier kombinasie probleme	Dawid Groenewald
26 Apr 2017	Schoeman Boerdery	Pallet Warehouse ondersoek om palette by pakhuis te vervaardig en voorlopige werk op nawel verpakking. Ter voorbereiding vir "robot" palettisering	Dawid Groenewald
26 Mei 2017	Irene Shopping Mall, Mpac Hoofkantoor en Sappi Hoofkantoor	Verseke om borgskap vir CRI 2018 Navorsings Simposium	Hannes Bester Liezl vd Linde Dawid Groenewald
30 Mei 2017	Sappi Technology Centre	Nuwe besigheid, nuwe "pulp" vir vrugtevlieg lokvalle en swaar "mulching" papier,	Dawid Groenewald
8 Junie 2017	JP Landgoed	"Robot" palettisering onder-soek	Dawid Groenewald
14 Junie 2017	Wilhelm le Roux se plaas in Brits distrik	Mulching proef.	Dawid Groenewald
27 Junie 2017	Schoeman Boerdery	NZZ Pallet Netting proef	Dawid Groenewald

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes/ Sprekers
3 Jul 2017	Noordchem Direkteur Letsitele.	Borgskap vir 2018 CRI Simposium Gholfdag.	Gerhard en Eddie Vorster en Dawid Groenewald.
3 Jul 2017	Houers en CRI Letsitele.	Beplanning van Mulching proef en beskikbaarheid van papier.	Frikkie v Wyk, Wayne Mommsen en Dawid Groenewald.
28 Jul 2017	APL Cartons.	Proef met nuwe ontwerp A15C binnestuk.	Roché Kenny en Dawid Groenewald.
10 Aug 2017	Groep 91, CRI en Houers.	Mulching proef by Groep 91.	Wimpie Mostert, Frikkie van Wyk,

			Wayne Mommsen en Dawid Groenewald.
21 Aug 2017	Thandaza Pallets.	Swamgroeï op palette en toekomstige beskikbaarheid van palette.	Malcolm Easton en Dawid Groenewald
21 Aug 2017	CRI Nelspruit, Hamilton Lodge, Malelane/ Pallet Netting.	Swamme op palette. Voorbereiding vir netting werkswinkel	Jacques Coetzee, AC Koch, (NNZ) Dawid Groenewald.
22 Aug 2017	Riverside Pakhuis/Pallet Netting.	Netting werkswinkel en demonstrasie.	Cornel v.d Merwe, Gerhard Greeff en Deon Swarts plus bogenoemde mense.
24 Aug 2017	Schoeman Boerdery.	Hemingway Plastiese palet proef.	Christo de Jonge. André Potterill, Dawid Groenewald.
1 Sep 2017	Innovation Hub.	Palogix vergadering oor plastiese palette en "bins"	André Kruger en Dawid Groenewald.
14 Sep 2017	Sappi Technology Centre.	Toets van eksperimentele A15C binnestukke.	Dawid Groenewald.
21 Sep 2017	Pretoria mark.	Verpakking van eksperimentele A15C binnestukke en studie van toedraai metodes.	Dawid Groenewald
28 Sep 2017	Letsite NG Kerk en Bosveld Sitrus.	Oorlede Christie Landman se begrafnis en werk op verskillende maniere van toedraai van vrugte.	Frikkie van Wyk en Dawid Groenewald.

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkes/Sprekers
3 Okt 2017	Beplanning vir 2018 CRI-Na-oes Werksinkels. CRI kantore Nsp.	Opstel van agendas. Venues ens.	Beplannings komitee o.l.v. Hannes Bester.
4 Okt 2017	Sappi Technology Centre.	Oorsig oor 2017 Sitrusseisoen. Akkreditasie toetse.	Dawid Groenewald en verskeie senior Sappi personeel .
10 Okt 2017	Innovation Hub, Pta.	NNZ Pallet Netting Proewe.	Dawid Groenewald Jacques Coetzee van Nederland.
11 Okt 2017	Sappi Technology Centre.	Nuwe besigheid.	Dawid Groenewald en verskeie senior Sappi personeel.
17 Okt 2017	Lynwood Bridge Pta.	Vorderingsvergadering met Hannes Bester.	Hannes Bester Liezl v.d. Linde Dawid Groenewald.
7-10 Nov 2017	Champagne Sports Resort.	Beplanning van 2018 CRI navorsings simposium.	Hele CRI span o.l.v. Hannes Bester.
14 Nov 2017	Sappi Technology Centre.	Verpakkingswerk-groep vergadering.	Dawid Groenewald Hannes Bester.
29 Nov 2017	Pretoria Mark.	Moontlikheid om palette daar te toets.	Dawid Groenewald Johan du Toit (Subtropico).
6 -7 Des 2017	Letsitele-Houers, Groep 91 en CRI.	Samesprekings met Houers en Wayne Mommsen.	Dawid Groenewald Wimpie Mostert Frikkie van Wyk

			Wayne Mommsen.
13 Des 2017	Sappi Technology Centre.	Opvolg oor moontlike nuwe besigheid.	Dawid Groenewald en verskeie Sappi senior personeel.

Datum	Studiegroep/Aktiwiteit	Onderwerp/Aksies	Betrokkenes/Sprekers
25 Jan 2018	Sappi Technology Centre	Lignosulphonate Voorlegging	Eric Schubert George Rautenbach (Sappi) Wayne Mommsen Dawid Groenewald
29 Jan 2018	Tzaneen	Beplanning vir 2018 verpakkingsnavorsing.	Wimpie Mostert (Houers) Hannes Bester Dawid Groenewald.
30 & 31 Jan 2018	Fairview Hotel	Limpopo 1 CRI Na-oes werkwinkel	Alle betrokke CRI personeel
1 & 2 Feb 2018	Loskopdam	Limpopo 2 CRI Na-oes werkwinkel	Alle betrokke CRI personeel
8 & 9 Feb 2018	Nelspruit CRI kantore en Beetleloop Gastehuis	CRI Voorligtings-beplanning vergaderings en navorsings prioriteite	Hannes Bester MC Pretorius Keith Lesar Wayne Mommsen Liezl vd Linde Catherine Savage Dawid Groenewald
12 & 13 Feb 2018	Nelspruit Mbombela Stadium	Mpumalanga CRI Na-oes werkwinkel	Alle betrokke CRI personeel
15 & 16 Feb 2018	Durban Gateway Hotel	KZN/Swaziland CRI Na-oes werkwinkel	Alle betrokke CRI personeel
19 Feb 2018	Innovation Hub, Pretoria	Samesprekings i.v.m. toetse op PalletPlast plastiese palette	Eugene Cuyler Johan Botha Dawid Groenewald.
20 & 21 Feb 2018	Allee-Bleue	Wes Kaap CRI Na-oes werkwinkel	Alle betrokke CRI personeel
22 & 23 Feb 2018	Mentorskraal, Jeffreysbaai	Oos Kaap CRI Na-oes werkwinkel	Alle betrokke CRI personeel
1 Mrt 2018	Sappi Technology Centre	Inligting-sessie oor swamgroei op hout en beskikbaarheid van dennehout	Francois Wolfaardt (Sappi) Dawid Groenewald
2 Mrt 2018	Innovation Hub, Pretoria	Opvolg gesprekke oor PalletPlast plastiese palette	Johan Botha Dawid Groenewald
7 Mrt 2018	Innovation Hub, Pretoria	Eerste gesprek oor moontlike proewe met OptiFlo Freezer Spacers	Mike Cohen Dawid Groenewald
8 Mrt 2018	Houers, Letsitele	Lugvloei toetse. Standaard- vs 11 Slat palet en deurbuiging toets op PalletPlast palet	Wimpie Mostert Frikkie van Wyk (Houers) Wayne Mommsen Dawid Groenewald
9 Mrt 2018	Houers, Letsitele	Verpakkings-navorsings prioriteite vir 2018	Wimpie Mostert

			Frikkie van Wyk (Houers) Dawid Groenewald.
12 & 13 Mrt 2018	Kry Hannes Bester by OR Tambo. Ry na CRI kantore in Nelspruit	Voorligting samesprekings en beplanning vir die 2018 CRI simposium	Hannes Bester MC Pretorius Keith Lesar Wayne Mommsen Liezl vd Linde Catherine Savage Dawid Groenewald
14 Mrt 2018	Birchwood Hotel	CMF vegadering	Hannes Bester Dawid Groenewald
16 Mrt 2018	Innovation Hub, Pretoria	Opvolg gesprek oor Palogix plastiese palet	André Kruger Dawid Groenewald

Opsomming van aktiwiteite deur Keith Lesar en Catherine Savage vir die jaar

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
26 Apr 2017	Orchards View pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Wilma du Plooy
26 Apr 2017	MarxPak pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Wilma du Plooy
4 Mei 2017	Voorligting Vergadering	Voorligting sake	Hannes Bester MC Pretorius Keith Lesar Wayne Mommsen Liezl vd Linde Wilma du Plooy Catherine Savage
22 Mei 2017	Suncape (Cape Citrus) pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
	Panzi pakhuis Kirkwood		Keith Lesar Catherine Savage
	Wicklow pakhuis Kirkwood	Eerste CRI "drench" proef	Keith Lesar Catherine Savage
23 Mei 2017	Golden Ridge pakhuis Kirkwood (Rian Ehlers)	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
23 Mei 2017	2Rivers pakhuis Kirkwood (Hennie Ehlers)	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
24 Mei 2017	Wicklow pakhuis Kirkwood	Tweede CRI "drench" proef	Keith Lesar Catherine Savage
24 Mei 2017	Atwell pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
24 Mei 2017	Son Sitrus Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
25 Mei 2017	Wicklow pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
5 Jun 2017	Kaspersnek (nou Lona) pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
5 Jun 2017	Waterval (Bosveld) pakhuis Burgersfort	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
6 Jun 2017	PLM pakhuis Burgersfort	Pakhuis besoek/konsultasie	Keith Lesar

			Catherine Savage
6 Jun 2017	Morone sitrus pakhuis Burgersfort	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
7 Jun 2017	J P Landgoed (Cobus & Pieter Beetge) pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
7 Jun 2017	OHR sitrus pack Ohrigstad (Le Roux's)	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
21 Jun 2017	Sitrus Rand pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
21 Jun 2017	Riverland pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
21 Jun 2017	Wicklow pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
22 Jun 2017	Sunriver pakhuis Kirkwood	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
22 Jun 2017	Pakhuis Forum Vergadering SRV	Pakhuis sake	Keith Lesar Catherine Savage Wilma du Plooy
23 Jun 2017	Pakhuis Forum Vergadering Patensie	Pakhuis sake	Keith Lesar Catherine Savage Wilma du Plooy

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
06 Jul 2017	Orchards View pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Wilma du Plooy
06 Jul 2017	MarxPak pakhuis Ohrigstad	Pakhuis besoek/konsultasie	Keith Lesar Wilma du Plooy
18 Jul 2017	Afrifert Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
18 Jul 2017	Engelbrecht Trust	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
19 Jul 2017	Marble Hall / Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
19 Jul 2017	Schoonbee pakhuis Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
19 Jul 2017	Schoeman Bdy pakhuis Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
20 Jul 2017	Diphale pakhuis Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
20 Jul 2017	Roslé pakhuis Groblersdal	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
27 Jul 2017	ALG Pakhuis Citrusdal	Pakhuis besoek/konsultasie	Wilma du Plooy Catherine Savage
31 Jul 2017	Oorlogspoort Pakhuis Patensie	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
31 Jul 2017	Endulini "Blou" Sitrus Pakhuis Patensie	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
31 Jul 2017	Endulini "Wit" Sitrus Pakhuis Patensie	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
31 Jul 2017	Venstershoek Pakhuis Patensie	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage

01 Aug 2017	Patensie Ko-op pakhuis	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
02 Aug 2017	Wagendrift Pakhuis Hankey	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
02 Aug 2017	Mandaryn Pakhuis Hankey	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
02 Aug 2017	Patensie Raadsaal Patensie	Gamtoos Pakhuis Forum Vergadering	Keith Lesar Catherine Savage
03 Aug 2017	Sonop Pakhuis Patensie	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
03 Aug 2017	Kwaggaskloof Pakhuis	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
07 Aug 2017	Ambrosia Pakhuis Hoedspruit	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
07 Aug 2017	Moriah Pakhuis Hoedspruit	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
08 Aug 2017	Landman Pakkers Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
08 Aug 2017	Christie Landman Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
08 Aug 2017	CP Minnaar	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
08 Aug 2017	CONSTANTIA Studie Gp vergadering Letsitele	Produksie/Pakhuis sake	Keith Lesar Catherine Savage
09 Aug 2017	Novelle La Cotte Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage

09 Aug 2017	Rooister Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
09 Aug 2017	Groep 91 Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
10 Aug 2017	Letaba Est Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
10 Aug 2017	Merite Pakhuis	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
11 Aug 2017	CPJ Erasmus (Callie Erasmus) Pakhuis Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
12 Aug 2017	Mahela Boerdery Letsitele	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
16 Aug 2017	Mvurwi CitriFresh Pakhuis Zimbabwe - Mvurwi	Pakhuis besoek/konsultasie	Wilma du Plooy Catherine Savage
16 Aug 2017	Mazoe Citrus Zimbabwe - Mazoe	Pakhuis besoek/konsultasie	Wilma du Plooy Catherine Savage
17 Aug 2017	Dodhill Citrus Zimbabwe - Chetgutu	Pakhuis besoek/konsultasie	Wilma du Plooy Catherine Savage

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
3 Okt 2017	CRI	Voorbereiding vir 2018 Pakhuis Werkswinkel vergaderings	Keith Lesar

19 Okt 2017	SRV Pakhuis Forum Vergadering Africanos Addo OosKaap	Pakhuis/Na-oes besprekings	Keith Lesar
26 Okt 2017	Uitvoerders Tegnieese Paneel Vergadering Agri- Hub Stellenbosch	Mark terugvoering markverwante probleme, uitvoer probleme en vruggehalte probleme en kontak met uitvoerders vir 2017.	Keith Lesar
26 Okt 2017	CRI Verkoeling Werkersgroep Vergadering	Verskeppings temperature, Verkoeling, Navorsings prioriteite ens.	Keith Lesar
27 Okt 2017	Droogte Bestuur Werkswinkel (WesKaap) Stellenbosch	Uitsigte vir die kort en langtermyn in die landbou bedryf.	Keith Lesar
11 – 17 Nov 2017	Citrosol Spanje uilnodiging	Vergaderinge en besoeke aan Citrosol fabriek en sitruspakhuisbesoeke	Keith Lesar Wilma du Plooy Catherine Savage

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes / Sprekers
9 Feb 2018	Beetleloop Nelspruit	Voorligting Beplanning Vergadering	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage
7 Maart 2018	Ryton Pakhuis Ngodwana	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage MC Pretorius
9 Maart 2018	Joubert & Seuns Pakhuis	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage MC Pretorius
15 Maart 2018	Twycross Pakhuis	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage
19 Maart 2018	Moletele Pakhuis Hoedspruit	Pakhuis besoek/konsultasie	Keith Lesar Catherine Savage Wayne Mommsen

7.2 OTHER MEANS OF TECHNOLOGY TRANSFER

7.2.1 SA Fruit Journal by Tim G Grout (CRI)

Table 7.2.1.1. S A Fruit Journal articles in 2017-18 besides Extension Briefs.

Issue	Pages	Title	Author/s
April/May 16(2)	60-64	Snoei van Sitrus – 'n Winsgewende Praktyk	Hannes Bester and James Warrington
	56-58	Soil fumigation in citrus replant situations – making informed decisions	Jan M. van Niekerk
Jun/Jul 16(3)	79-81	Effect of netting on aerial fruit fly bait spray deposition in citrus orchards- a preliminary investigation	Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, MC

			Pretorius and Tom Van Der Meulen
	82-83	Citrus Viroids: A re-emerging problem	Chanel Steyn and Glynnis Cook
	84-86	Nuwe Formaat vir CRI Na-oes Werkswinkels	Hannes Bester, Dawid Groenewald and Wilma du Plooy
Aug/Sep 16(4)	53	Postgraduate qualifications in agricultural entomology and microbiology	Sean Moore
	102-107	An investigation of excessive fruit drop in the Eastern and Western Cape during the 2016/17 season	Jakkie Stander
	108-109	Citrus segments	Liezl van der Linde
Oct/Nov 16(5)	105-106	Important considerations for application of wax on citrus and other fruit	Wilma du Plooy
	108-109	Citrus segments	Liezl van der Linde
Dec/Jan 16(6)	102-106	CRI IPM & Disease Management Workshops on Raising the Standards of Citrus production in South Africa	Wayne Mommsen and Hannes Bester
Feb/Mar 17(1)	64-65	A new parasitoid species attacking carob moth in South Africa	Sean Thackeray, Cornelis van Achterberg, Sean Moore and Martin Hill
	66-69	Citrus production in the Central Valley of California	Jakkie Stander
	75-77	Planning your new citrus planting: availability of propagation material	Paul Fourie, Jacolene Meyer, Thys du Toit and Michelle le Roux

7.2.2 CRI website by Tim G Grout

Table 7.2.2.1. Visits and page requests on www.cri.co.za since April 2017.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2016/7	30 979	56 157	175 368	602 324	11.20 GB
Apr 2017	2 727	5 051	16 687	54 936	1.04 GB
May 2017	3 090	6 208	26 532	109 917	1.89 GB
Jun 2017	3 539	6 546	22 258	78 396	1.75 GB
Jul 2017	3 676	6 216	20 107	68 592	1.69 GB
Aug 2017	4 367	8 493	23 449	93 950	2.18 GB
Sep 2017	4 158	9 193	20 243	70 713	1.62 GB
Oct 2017	3 870	8 071	23 405	78 462	1.59 GB
Nov 2017	3 583	5 870	16 198	66 010	1.55 GB
Dec 2017	3 961	6 922	14 850	47 842	1.06 GB
Jan 2018	5 344	8 982	23 871	116 210	2.68 GB
Feb 2018	4 033	6 604	25 752	107 300	2.76 GB
Mar 2018	4 542	7 492	29 704	103 747	2.67 GB
Total 2017/8	46 890	85 648	263 056	996 075	22.48 GB

7.2.3 CRInet by Tim G Grout

CRInet provides a good opportunity for growers to share opinions or ask questions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. The 43 messages sent

during the 2017 calendar year are fewer than the average of 52 per annum for the last 9 years but in January 2018 there were 18 messages so it does fluctuate with the topic being discussed (Table 7.2.3.1). There are currently 490 CRInet members.

Table 7.2.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2018	18	3	2										23
2017	0	0	4	7	0	4	2	14	3	5	3	1	43
2016	6	3	0	3	1	8	10	3	1	6	3	6	50
2015	5	2	3	3	2	3	12	4	4	1	3	1	43
2014	4	3	4	1	12	6	13	1	0	1	3	1	49
2013	1	15	0	7	3	0	2	4	6	13	1	6	58
2012	5	1	19	4	5	2	4	3	1	0	2	0	46
2011	14	3	5	2	8	24	2	3	3	2	2	2	70
2010	0	1	5	3	2	0	6	12	9	4	9	3	54
2009	1	7	3	6	11	0	6	8	4	2	1	2	51
2008	3	6	1	8	5	2	7	3	3	5	3	4	50

7.2.4. Cutting Edge by Tim G Grout (CRI)

Some growers consider the Cutting Edge to be the most valuable means of communication from CRI, perhaps because it always contains urgent information and is to the point. Past issues of the Cutting Edge can be downloaded from the member area of the CRI website. Topics covered in 2017/8 are given in Table 7.2.4.1.

Table 7.2.4.1. Cutting Edge issues during 2017-18.

No.	Title	Month	Author
229	Export recommendations for Citrus Fruit to the EU in 2017	Apr	Paul Cronje, Sean Moore, Elma Carstens and Vaughan Hattingh
230	Carob moth in citrus orchards	Apr	Sean Moore and Vaughan Hattingh
231	Consumer Assurance and MRL Update	Apr	Paul Hardman
232	Update: Export recommendations for citrus fruit to the EU in 2017	Jun	Paul Cronje, Sean Moore, Elma Carstens and Vaughan Hattingh
233	Managing Phytophthora root, collar and brown rot following pre-harvest rain	Jun	Jan van Niekerk, M.C. Pretorius and Paul Hardman
234	Registration of Fludioxonil in postharvest wax	Jun	Wilma du Plooy and Keith Lesar
235	CIS Nursery Advisory service	Jul	Paul Fourie
236	Increased Incidence of Citrus Viroid Diseases	Sep	Chanel Steyn, Paul Fourie and Glynnis Cook
237	Updated Citrus Black Spot Spray Programmes 2017 – 2018	Oct	Charl Kotze, Providence Moyo and Paul Fourie
238	The false codling moth risk management system (FMS) for export of citrus to the EU	Oct	Vaughan Hattingh, Sean Moore and Paul Hardman
239	Emulsifiers and Available Spray Oils	Oct	Tim G. Grout
240	Surveillance of the Asian Citrus Psyllid in citrus production areas in Southern Africa	Nov	Aruna Manrakhan, Peter Stephen, MC Pretorius, Tim Grout and Paul Fourie

241	Citrus false codling moth risk management system (FMS) alert	Dec	Sean Moore, Paul Hardman, Vaughan Hattingh and Hannes Bester
242	CRI extension – FMS notification Jan 2018	Jan	Extension
243	Update on status of Oriental fruit fly (OFF) and regulatory measures on the pest in South Africa January 2018	Jan	Aruna Manrakhan, Elma Carstens and Vaughan Hattingh
244	Okuleerhout aanvraag en aanbod: tekort aan sekere kultivars	Feb	Paul Fourie, Jacolene Meyer, Michelle le Roux, Thys du Toit and Scott McKenzie
245	Sampling procedure for <i>Phytophthora</i> and citrus nematode analysis and latest price list for services rendered by the Diagnostic Centre	Mar	Jan van Niekerk and Elaine Basson
246	Postharvest pathogen resistance monitoring in the packhouse and orchard	Mar	Wilma du Plooy, Catherine Savage and Elaine Basson

8 PUBLICATIONS IN 2017

8.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

BOARDMAN, L., J.G. SØRENSEN, T.G. GROUT, J.S. TERBLANCHE. 2017. Molecular and physiological insights into the potential efficacy of CO₂-augmented postharvest cold treatments for false codling moth. *Postharvest Biology & Technology* 132: 109-118.

CARSTENS, E., C. C. LINDE, R. SLABBERT, A. K. MILES, N. J. DONOVAN, H. LI, K. ZHANG, M. M. DEWDNEY, J. A. ROLLINS, C. GLIENKE, G. C. SCHUTTE, P. H. FOURIE, A. MCLEOD. 2017. A Global Perspective on the Population Structure and Reproductive System of *Phyllosticta citricarpa*. *Phytopathology* 107(6): 758-768

COOMBES, C.A., HILL, M.P., MOORE, S.D., & DAMES, J.F. 2017. Potential of entomopathogenic fungal isolates for control of the soil-dwelling life stages of *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in citrus. *African Entomology*, 25(1): 235-238

CRONJE, P.J.R., J. JOUBERT, H. MARAIS, E.W. HOFFMAN, L. ZACARIAS. 2016. Influence of late nitrogen soil applications on mandarin fruit quality. *Citrus Research & Technology* 37(1): 56-65

CRONJÉ, P.J.R., ZACARÍAS, L., ALFÉREZ, 2017. Susceptibility to postharvest peel pitting in Citrus fruits as related to albedo thickness, water loss and phospholipase activity. *Postharvest Biology & Technology* 123: 77–82

DEFRAEYE, T., W. WU, K. PRAWIRANTO, G. FORTUNATO, S. KEMP, S. HARTMANN, P. CRONJE, P. VERBOVEN, B. NICOLAI. 2017. Artificial fruit for monitoring the thermal history of horticultural produce in the cold chain. *Journal of Food Engineering* 215: 51-60

DOHINO, T., G.J. HALLMAN, T.G. GROUT, A.R. CLARKE, P.A. FOLLETT, D.R. CUGALA, D. M. TU, 8.W. MURDITA, E. HERNANDEZ, R. PEREIRA, S.W. MYERS. 2017. Phytosanitary Treatments Against *Bactrocera dorsalis* (Diptera: Tephritidae): Current Situation and Future Prospects. *J. Econ. Entomol.* 110 (1): 67-79

FOURIE, P.H., G.C. SCHUTTE, E. CARSTENS, V. HATTINGH, I. PAUL, R.D. MAGAREY, T.R. GOTTWALD, T. YONOW, D.J. KRITICOS. 2017. Scientific critique of the paper "Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*. A historical analysis of disease spread in South Africa" by Martínez-Minaya et al. (2015). *Eur. J. Plant Pathol.* 148: 497-502

- GUARNACCIA, V., J.Z. GROENEWALD, H. LI, C. GLIENKE, E. CARSTENS, V. HATTINGH, P.H. FOURIE, P.W. CROUS. 2017. First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe. *Studies in Mycology* 87: 161-185
- JOOSTE, T.L., VISSER, M., COOK, G., BURGER, J.T., MAREE, H.J. 2017. In Silico Probe-Based Detection of Citrus Viruses in NGS Data. *Phytopathology* 107:988-993
- KELLERMAN, M., A. MCLEOD, I. BEUKES, L.J. ROSE, A. ERASMUS, P.H. FOURIE. 2017. Classification of imazalil resistance in an international collection of *Penicillium digitatum* isolates. *Can. J. Plant Pathol.* 39(2): 133-137
- KHAMIS, F.M., RWOMUSHANA, I., OMBURA, L.O., COOK, G., MOHAMED, S.A., TANGA, C.M., NDERITU, P.W., BORGEMEISTER, C., SETAMOU, M., GROUT, T.G., EKESI, S. 2017. DNA Barcode Reference Library for the African Citrus Trioqid, *Trioza erytrae* (Hemiptera: Triozidae): Vector of African Citrus Greening. *J Econ Entomol* 110:2637-2646
- KREJMER-RABALSKA, M., RABALSKI, L., DE SOUZA, M.L., MOORE, S.D., SZEWCZYK, B. 2017. New method for differentiation of granuloviruses (Betabaculoviruses) based on multitemperature single stranded conformational polymorphism. *International Journal of Molecular Sciences*, 19, 83; doi:10.3390/ijms19010083
- LLOYD, M., KNOX, C.M., THACKERAY, M.P., HILL, M.P. & MOORE, S.D. 2017. Isolation, identification and genetic characterisation of a microsporidium isolated from carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae). *African Entomology* 25(2): 529-533.
- MAKUMBE, L.D.M., MANRAKHAN, A. AND WELDON, C.W. 2017. Marking *Bactrocera dorsalis* (Diptera: Tephritidae) with fluorescent pigments: effects of pigment colour and concentration. *African Entomology* 25(1): 220-234
- MANRAKHAN, A., DANEEL, J-H., BECK, R.B., VIRGILIO, M., MEGANCK, K. AND DE MEYER, M. 2017. Efficacy of trapping systems for monitoring Afrotropical fruit flies. *Journal of Applied Entomology* 141(10): 825-840
- MANRAKHAN, A., DANEEL, J-H., VIRGILIO, M. AND DE MEYER, M. 2017. Sensitivity of an enriched ginger oil based trapping system for Ceratitis fruit fly pests (Diptera: Tephritidae). *Crop Protection*; 99: 26-32
- NEPGEN, E.S, MOORE, S.D. & HILL, M.P. 2018. Integrating chemical control with sterile insect releases in an integrated pest management programme for *Thaumatotibia leucotreta*. *Journal of Applied Entomology*, 142: 421-427
- ROBERTS, R., COOK, G., GROUT, T.G., KHAMIS, F., RWOMUSHANA, I., NDERITU, P.W., SEGUNI, Z., MATERU, C.L., STEYN, C., PIETERSEN, G., EKESI, S., LE ROUX, H.F. 2017. Resolution of the Identity of 'Candidatus Liberibacter' Species From Huanglongbing-Affected Citrus in East Africa. *Plant Disease* 101:1481-1488
- RWOMUSHANA, I., KHAMIS, F.M., GROUT, T.G., MOHAMED, S.A., SÉTAMOU, M., BORGEMEISTER, C., HEYA, H.M., TANGA, C.M., NDERITU, P.W., SEGUNI, Z.S., MATERU, C.L., EKESI, S. 2017. Detection of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in Kenya and potential implication for the spread of Huanglongbing disease in East Africa. *Biological Invasions* 19(10): 2777-2787
- STANDER, O.P.J., G.H. BARRY, P.J.R. CRONJÉ. 2017. Fruit-load-induced starch accumulation causes leaf chlorosis in "off" 'Nadorcott' mandarin trees. *Scientia Horticulturae* 222: 62-68.
- STANDER, O.P.J., M.J. GILBERT, S.D. MOORE, W. KIRKMAN, G.C. SCHUTTE. 2017. Benefits of reducing the size of the navel-end opening in 'Navel' sweet oranges (*Citrus sinensis*). *Crop Protection* 96: 123-129.

- STEYN, C., COOK, G., BURGER, J.T., MAREE, H.J. 2016. Construction and application of infectious citrus viroids for biological indexing. *Journal of Citrus Pathology* 3
- THACKERAY, S.R., MOORE, S.D., KIRKMAN, W. & HILL, M.P. 2017. Biology and rearing of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), carob moth, a pest of multiple crops in South Africa. *African Entomology* 25(2): 474-480.
- THERON, C.D., MANRAKHAN, A. AND WELDON, C.W. 2017. Host use of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), in South Africa. *Journal of Applied Entomology*; 141(10): 810-816
- VAN ACHTERBERG, C., S.R. THACKERAY, S.D. MOORE, M.P. HILL. 2017. A new species of *Phanerotoma wesmael* (Hymenoptera: Braconidae: Cheloninae) parasitoid of the carob moth in South Africa. *Zootaxa* 4227(1): 127–134.
- VAN DER MERWE, M., JUKES, M.D., RABALSKI, L., KNOX, C., OPOKU-DEBRAH, J.K., MOORE, S.D., KREJMER-RABALSKA, M., SZEWCZYK, B., HILL, M.P. 2017. Genome analysis and genetic stability of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) after 15 years of commercial use as a biopesticide. *International Journal of Molecular Sciences*. 18, 2327; doi:10.3390/ijms18112327
- VIRGILIO, M., MANRAKHAN, A., DELATTE, H., DANEEL, J-H., MWATAWALA, M.W., MEGANCK, K., BARR, N.B., DE MEYER, M. 2017. The complex case of *Ceratitis cosyra* (Diptera: Tephritidae) and relatives. A DNA barcoding perspective. *Journal of Applied Entomology*; 141(10): 788-797
- VISSER, M., COOK, G., BURGER, J.T., MAREE, H.J. 2017. In silico analysis of the grapefruit sRNAome, transcriptome and gene regulation in response to CTV-CDVd co-infection. *Virology Journal* 14:200

9 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

- ALBERTYN, S., HILL, M.P. & MOORE, S.D. 2017. False codling moth population ecology in citrus orchards: the influence of orchard age. ESSA and ZSSA Combined Congress 2017, 3-7 July 2017, CSIR Pretoria.
- ACHEAMPONG, M., COOMBES, C.A., HILL, M.P. & MOORE, S.D. 2017. Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological traits and effect of formulation. ESSA and ZSSA Combined Congress 2017, 3-7 July 2017, CSIR Pretoria.
- DANEEL, J-H., MANRAKHAN, A. AND VAN DEN BERG, J. 2017. Natal fly versus the Cape fly (Diptera: Tephritidae) in the Limpopo and Mpumalanga provinces, South Africa. Combined Congress of the Entomological Society of Southern Africa (ESSA) and the Zoological Society of Southern Africa (ZSSA), 3-7 July 2017, Pretoria.
- GROUT, T.G. 2017. Undercover complications: The trend for growing citrus under net provides new opportunities for pest management research. Combined Congress of the Entomological Society of Southern Africa (ESSA) and the Zoological Society of Southern Africa (ZSSA), 3-7 July 2017, Pretoria.
- JUKES, M.D., RABALSKI, L., KNOX, C.M., HILL, M.P., MOORE, S.D. and SZEWCZYK, B., 2017. Baculovirus synergy: mixed *Alphabaculovirus* and *Betabaculovirus* infections for the control of *Thaumatotibia leucotreta* in South Africa. Proceedings of the 16th Meeting of the IOBC-WPRS Working Group: Microbial and Nematode Control of Invertebrate pests, Tbilisi, Georgia, 11-15 June 2017, vol. 129, pp 170-174.

- KIRKMAN, W., TANDLICH, R., KRAUSE, R., MOORE, S.D. & HILL, M.P. 2017. Postharvest detection of *Thaumatotibia leucotreta* in citrus fruit in South Africa. ESSA and ZSSA Combined Congress 2017, 3-7 July 2017, CSIR Pretoria.
- MANRAKHAN, A., WELDON, C.W., ADDISON, P., MAKUMBE, L.D., PIETERSE, W., THERON, C.D., SIBIYA, X. AND DANEEL, J-H. 2017. The Oriental fruit fly: Four years after first establishment in the northern areas of South Africa. Combined Congress of the Entomological Society of Southern Africa (ESSA) and the Zoological Society of Southern Africa (ZSSA), 3-7 July 2017, Pretoria.
- MANRAKHAN, A., DANEEL, J-H., DE MEYER, M., VIRGILIO, M., DUYNCK, P.F., DELATTE, H. AND HALA, F.N. 2017. Developing effective detection tools for Afrotropical fruit fly pests- The ERAfrica 'FRUIT FLY' project. Third FAO-IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques. 22-26 May 2017, IAEA Headquarters, Vienna, Austria.
- MARSBERG, T., JUKES, M., CHAMBERS, C., HENDRIKS, C., OPOKU-DEBRAH, J., KNOX, C., HILL, M., MOORE, S., 2017. The isolation of a novel alphabaculovirus and its potential for microbial control of key tortricid moth pests. Proceedings of the 16th Meeting of the IOBC-WPRS Working Group: Microbial and Nematode Control of Invertebrate pests, Tbilisi, Georgia, 11-15 June 2017, vol. 129, pp 175-178.
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