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1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens - CRI

1.1 SUMMARY

In the EU, Market Access issues remained concentrated around CBS. The citrus industry's levels of noncompliance with the EU CBS regulations declined in 2015. Additional technical CBS arguments were introduced into engagements with EU parties, in support of ongoing improvement in the area of compliance. The dispute about the failure of the EU CBS regulations to be aligned with scientific evidence, continued without resolution. The criticism of the EU CBS regulations was maintained by an international CBS Expert Panel. In Japan issues pertaining to cultivar labelling were addressed. Long outstanding requests for Japan to consider scientific data supporting improved conditions of access to the market remained unanswered by Japan. The reduced duration of cold treatment for export to USA was successfully applied in 2015. The publication of a final rule by USA, to allow citrus exports from all production areas in South Africa, remained pending publication by USDA APHIS. Official requests with supporting evidence, were lodged with China to improve conditions of access to the China market. This included, exemption of lemons from cold treatment and acceptance of bulk shipping. Bilateral engagements between South Africa and China around these proposals are ongoing. Earlier issues around interpretation of the South Korean export protocol were successfully resolved. Pursuit of access to the Vietnam market made progress through the PRA process. Indonesian officials visited South Africa and granted mutual recognition of food safety systems to South Africa, supporting improved conditions of market access. Import compliance requirements for exports to Reunion were clarified. Pursuit of access to the Philippines market continued, but progress with the PRA process was slow due to ongoing refusal by Philippine officials to accept international risk assessment norms and associated scientific data.

OPSOMMING

In die EU het die marktoegangskwessies rondom SSV gekonsentreerd gebly. Die sitrusbedryf se vlakke van nie-nakoming van die EU SSV regulasies het in 2015 afgeneem. Bykomende tegniese SSV argumente is tydens gesprekke met die EU partye voorgestel ter ondersteuning van die voortgesette verbetering om aan die vereistes te voldoen. Die geskil om die EU SSV regulasies in lyn met die wetenskap te kry, het voortgegaan sonder ŉ oplossing. 'n Internasionale deskundige paneel het voortgegaan om kritiek op die EU SSV regulasies te lewer. In Japan is die probleme wat verband hou met kultivar etikettering aangespreek. Lang uitstaande versoeke aan Japan om wetenskaplike data te oorweeg vir verbeterde marktoegang het onbeantwoord deur Japan gebly. Die korter koue behandeling vir uitvoer na die VSA is suksesvol in 2015 toegepas. Die publikasie van 'n finale wetgewing deur die VSA om sitrusuitvoere van alle produksie gebiede in Suid-Afrika toe te laat, bly hangende 'n publikasie deur USDA-APHIS. Amptelike versoeke met ondersteunende data is na China gestuur om vereistes vir toegang na die Chinese mark te verbeter. Dit sluit vrystelling van suurlemoene van koue behandeling en aanvaarding van "bulk" verskeping in. Bilaterale gesprekke tussen Suid-Afrika en China rakende hierdie voorstelle is aan die gang. Vroeëre kwessies rondom die interpretasie van die Suid-Koreaanse uitvoer protokol is suksesvol opgelos. Vordering is gemaak met toegang tot die Viëtnamese mark deur die PRA proses. Indonesiese amptenare het Suid-Afrika besoek en die wedersydse erkenning van voedselveiligheidstelsels is aan Suid-Afrika verleen wat verbeterde marktoegang ondersteun. Invoervereistes vir uitvoere na Reunion is opgelos. Prosesse om toegang tot die Filippynse mark te verkry is voortgesit, maar vordering met die PRA proses was stadig as gevolg van voortgesette weiering deur Filippynse amptenare om internasionale risikobepalingsnorme en wetenskaplike inligting te aanvaar.

1.2 EUROPE (EU)

South Africa received the first notification of a CBS interception for the 2015 season in June. Organic growers decided in August to voluntarily suspend the export of organic lemons to the EU for the remainder of the season. All citrus exports to the EU were voluntarily suspended on 25 September 2015, except for exports from the CBS pest free areas. By the end of the 2015 export season SA had received 15 notifications of CBS interceptions in the EU. Concerns were raised by the industry regarding the identification of CBS in the EU and the diagnostic procedures used especially the verification of the viability of the pathogen. Further technical arguments around the use of diagnostic procedures supporting the need to verify viability in the EU CBS regulatory inspections, were developed for use in engagements with EU regulators. In July 2015 a team of South African experts visited several laboratories in the EU to discuss their CBS testing procedures. It was confirmed that none of them conduct viability testing to confirm a positive interception. SA-DAFF provided a report to DG Sante on the visit and as part of the report they requested inclusion of viability testing as part of their routine laboratory procedure to confirm a positive CBS interception. Feedback from DG Sante in February

2016 provided no direct answer to the request to include viability testing. SA-DAFF requested DG Sante to lift the Emergency measure EC Decision 422 of 2014. DG Sante indicated that the emergency measure might be reviewed after two seasons.

In June 2015, the magisterial district of Gordonia in the Northern Cape province was accepted by the EU as a CBS pest free area in South Africa. The Commission's Decision was published on 15 July 2015 (COMMISSION IMPLEMENTING DECISION (EU) 2015/1175 of 15 July 2015 amending Decision 2006/473/EC). However, SA-DAFF had requested the EU to accept 17 magisterial districts in the Northern Cape, Free State and North West provinces of SA as CBS free. SA-DAFF was requested to follow up with the EU to determine the reason why only Gordonia was granted CBS free status.

A draft report of the visit by the EU-FVO delegation in February – March 2015 was received in June 2015. The report concluded that SA's CBS-RMS fully complies with the EU's requirements for CBS. They however identified two areas of concern namely, the availability of resources to ensure that the procedures are implemented according to the RMS and the 2014 FCM interceptions in the EU.

In the previous reporting period the IPPC launched two calls for nominations of experts to serve on the CBS Expert Committee to proceed with the CBS dispute process between the EU and SA. In May 2015 the Secretariat of the IPPC launched a third call for experts. Since then there was no further progress with the IPPC CBS dispute resolution process.

In September 2015 industry meetings took place to prepare for the next export season. In November 2015 and February 2016 inputs were made to the CBS Risk Management System for the 2016 citrus export season and the final document was distributed by SA-DAFF on 23 March 2016 for implementation. In November 2015 CRI was tasked to make recommendations on precautionary FCM measures for SA-DAFF to consider for implementation on the 2016 exports. However, historic inspection data and field monitoring data indicated that this would not be appropriate. However, it was recommended that a zero tolerance during PPECB inspection in the packhouse should be maintained.

The history of the dispute about the technical justification of the EU CBS phytosanitary regulations was recorded in the previous year's report. This included reference to the release of the European Food Safety Authority's (EFSA) pest risk assessment for CBS in 2013 and an international panel of CBS experts' complaint in 2014 that the EFSA PRA was flawed and that EFSA had failed to consider prior inputs provided by the CBS Expert Panel. In January 2015 EFSA released a document, in response to the 2014 CBS Expert Panel objections, claiming that EFSA had adequately considered all the prior inputs made by the CBS Expert Panel. In November 2015, the CBS Expert Panel released a document, refuting EFSA's claims, reiterating its objection to EFSA's disregard of the inputs provided by the expert panel and upholding the CBS Expert panel's earlier conclusion that the EFSA PRA was erroneous and had not appropriately considered relevant scientific evidence and expert opinion. In October 2015 the European Commission requested EFSA to evaluate new scientific evidence on CBS. EFSA subsequently released a statement (June 2016) claiming that the new information supported EFSA's earlier advice. However, the authors of one of main publications assessed by EFSA, objected to this claim by EFSA, stating that their research results in fact supported the CBS Expert Panel conclusions and provided further evidence that the EFSA opinion on CBS risk was flawed.

1.3 **JAPAN**

A request was submitted to Japan-MAFF in November 2009, to allow access for all mandarins (except Satsumas), under the current protocol for Clementines. Japan-MAFF responded in 2013, calling for separate disinfestation treatment data for each of the 32 mandarin cultivars. CRI provided SA-DAFF with scientific information and SA-DAFF submitted a response to Japan-MAFF in September 2014. Despite several follow up queries by SA-DAFF and Industry, a response is still pending from Japan-MAFF. The request for inclusion of all mandarin cultivars remains outstanding, now pending a technically justifiable response from Japan-MAFF for 5.5 years in total and 21 months without a response to SA's re-submission of data.

In September 2014 SA-DAFF submitted the final data package to support the adoption of a revised cold treatment condition for the export of fruit of all citrus types, compiled by CRI in accordance with Japan-MAFF requirements. Despite several follow up queries by SA-DAFF and Industry, a response is still pending from Japan-MAFF. Adoption of an improved cold treatment for all citrus types, has being ongoing between South Africa and Japan since 2009 and the latest response from Japan-MAFF has now been outstanding for 21 months.

In July 2015 cartons of SA Navels, labelled as Navelates, were rejected in Japan. The current protocol only specifies Washington and Cara Cara Navel cultivars. The consignment was released after scientific information was submitted via the Ambassador in Japan indicating that "Navelate" is a labelling name. To prevent recurrence of the problem, meetings were held with SA-DAFF and it was agreed to submit a request (provided by CRI in March 2015) to Japan-MAFF to amend the current protocol to include all navel orange cultivars.

1.4 USA

In 2014 citrus fruit was exported according to a pilot programme which entailed the following: a 22-day cold treatment with the current biometric sampling and inspection procedures and the current FCM threshold, but with an additional 200 asymptomatic fruit that needed to be cut per consignment to further determine the infestation level of FCM. No FCM were detected in 2014 in the 200 fruit that was cut per consignment. SA-DAFF submitted the information to USDA-APHIS with a request to revert back to 22d cold treatment. In March 2015 USDA-APHIS indicated that they are in the process of evaluating the data. The pilot program was however extended for the 2015 export season. No FCM were detected in 2015 in the 200 fruit per consignment that were cut. SA-DAFF submitted this information to USDA-APHIS. The USDA Federal Register has been amended, reflecting the 22d cold treatment (with the exception of consignments sent to the Port of Houston, where the 24d treatment remains). The termination of the "pilot" status of program with the additional pre-shipment fruit cutting, remains pending a response from USDA-APHIS at the end of the reporting period.

Feedback is also pending from the USDA-APHIS on the other long outstanding issues namely equivalence between USA domestic CBS regulations and USA import regulations (access for fruit to USA from the rest of SA production areas), recognition and access for CBS pest free places of production in an area of low pest prevalence, inclusion of other Western Cape magisterial districts in the export programme and the use of other ports of entry. Feedback to the USDA-APHIS on an Updated Work plan for citrus fruit exports and an updated list of actionable pests is pending from SA-DAFF.

1.5 CHINA

The current cold treatment for fresh citrus fruit to China is too harsh to allow export of lemons and Novas. Industry meetings were held to discuss the problem and it was decided that a meeting should take place between CRI-SA and CRI-China on research that can be conducted to find better shipping conditions for lemons and Novas to China. This meeting took place between the relevant researchers in June 2015. The parties, however, agreed that the request should be officially submitted by SA-DAFF to AQSIQ. A meeting was held with SA-DAFF and the data packages supporting the three requests were submitted to SA-DAFF in June 2015. SA-DAFF submitted the request for bulk shipping and exemption of lemons to China in October 2015, but not the proposed systems approach. SA-DAFF requested further information on the systems approach which CRI submitted to them in December 2015. CRI subsequently withdrew the systems approach package for replacement with an updated version.

SA-DAFF received the following feedback from AQSIQ in March 2016. AQSIQ agreed in principle with the request to allow bulk shipping but requested further technical information on temperature probes. CRI obtained this information from PPECB and provided it to SA-DAFF. SA-DAFF submitted this to AQSIQ in March 2016. AQSIQ was not supportive of the proposal to exempt lemons from the current cold treatment requirement and requested more information. CRI assessed the reasons offered by AQSIQ for its unsupportive position, but these were found to be without technical justification and not aligned with the scientific evidence supplied. CRI provided SA-DAFF with a technical analysis and proposal for response to AQSIQ.

1.6 SOUTH KOREA

For this market all the technical issues were resolved. A concerning issue for this market is, however, the high rejections for incorrect carton labelling. At the CRI Packhouse Workshops in February 2016 it was highlighted that incorrect carton labelling is a threat to retain access to this market. SA's application to export mandarins to this market remained pending.

1.7 VIETNAM

In August 2014 SA-DAFF received the first draft PRA for oranges from the Vietnamese authorities and submitted the requested information on several pests to the Vietnamese authorities in September 2014. Feedback received from Vietnam in May 2015 still listed four of the pests as being of quarantine importance, namely *Ceratitis quinaria*, *Aspidiotus nerii, Chrysomphalus pinnulifer and Pseudomonas syringae pv syringae,* despite all the information provided to them. They referenced the CABI Crop Protection Compendium to justify

their decision to keep two of the pests namely *Ceratitis quinaria and Aspidiotus nerii* on the quarantine list. This erroneous listing of *Citrus* as a host of *Ceratitis quinaria* and citrus fruit as a pathway for *Aspidiotus nerii* was addressed by approaching the Crop Protection Compendium (CPC) with a request to update their data. Scientific information was sent to the editor of the CPC to update the datasheets for *Ceratitis quinaria and Aspidiotus nerii.* Feedback received from the editor indicated that both datasheets will be updated but that this may take 3 months or longer. SA-DAFF informed the Vietnamese authorities about this route and submitted further information to support the removal of *Chrysomphalus pinnulifer and Pseudomonas syringae pv syringae.* In August 2015, the Vietnamese authorities replied that they will reconsider the status of the *Ceratitis quinaria and Aspidiotus nerii* once they received the info from CABI. They also indicated that they agreed to remove *Chrysomphalus pinnulifer* from the list. However, they still kept *Pseudomonas syringae pv syringae* on the list. Feedback received from the editor of CABI indicated that an updated datasheet for *Aspidiotus nerii* will be available in May 2016 and that the datasheet for *Ceratitis quinaria* has been completed and will be published as soon as the peer review process is completed.

1.8 INDONESIA

In August 2015 a delegation from Indonesia visited SA to approve SA's food safety control system, required to grant SA Mutual Country recognition. Feedback received from Indonesia in October 2015 indicated that there was uncertainty about the citrus types for export, pest free areas for two fruit flies that are of relevance to citrus and details about the cold treatment. CRI provided the relevant information to SA-DAFF and SA-DAFF provided a letter to Indonesia. Mutual Country Recognition was granted to South Africa on 13 April 2016.

1.9 REUNION

Information was provided to the CGA about Reunion's import conditions for fresh citrus fruit from countries where CBS is present. CBS is not present in Reunion. Although the country is part of the EU, it has its own specific import regulations pertaining to CBS and fruit flies. For CBS the requirements are the same as the standard EU 2000/29 regulations, but they did not require the additional measures that were brought in 2014 (Implementing Decision 422/2014), e.g. more intensive inspection, ethephon testing of Valencias and additional phytosanitary declarations. For fruit flies they require area freedom or an in transit cold treatment. The cold treatment options are flexible. One of the fruit fly species that is present in parts of SA and is of concern to Reunion is *B. dorsalis*. Recommendations were provided on how the CBS-RMS for 2016 pertaining to Reunion can be amended.

1.10 NEW MARKETS

1.10.1 The Philippines

In the previous reporting period it was indicated that information had been provided to the Philippine Authorities (the BPI), demonstrating that their list of quarantine pests needed to be revised as most of the listed pests did not follow the fresh fruit pathway. In July 2015, SA-DAFF requested inputs from CRI on feedback received from the Philippines as they did not remove any of the pests as requested by SA-DAFF. The BPI stated that according to them all these pests meet the requirements of the definition of a Quarantine Pest and interception data from SA indicated that some of these pests are intercepted during inspections. They also indicated that they had decided to downgrade the overall risk rating to "low" for some the pests, without clarification on what actions that would require. CRI recommended that SA-DAFF again request the removal of the inappropriately listed pests by highlighting that this is a PRA pertaining to the citrus fruit pathway. None of the pests under discussion follow the citrus fruit pathway and therefore cannot be classified as being of quarantine importance. SA-DAFF was also advised to highlight the incorrect interpretation (by Philippines) of the rejection data. CRI also requested SA-DAFF to inform the BPI that pre-shipment cold treatment will not be an option due to capacity constraints. CRI engaged in further discussion with SA-DAFF in November 2015, December 2015, February 2016 and March 2016 to finalize communication with the Philippines. SA-DAFF made a submission to the BPI in March 2016.

1.11 IMPORTS

1.11.1 Import conditions

By the end of this reporting period revision of the import conditions for Citrus vegetative propagation material remained pending. A revised set of import conditions for seed was released by SA-DAFF in August 2015.

1.12 REGULATIONS

The exotic fruit fly *Bactrocera dorsalis* (previously known as *Bactrocera invadens*) is considered to be present in specified regions, actionable and under official control in South Africa. In March 2016 a report was sent to the IPPC on new detections of *B. invadens* incursions in South Africa and new areas where the pest is present. In the areas with new incursions quarantine and eradication measures were implemented in accordance with the relevant national action plan. The Northern Cape, Western Cape, Eastern Cape and Free State provinces still remain free from *B. dorsalis*.

In November 2015 a survey was conducted in the CBS area of low pest prevalence in the far northern Limpopo province to determine whether the area still meets the requirements to be recognised as an area of low pest prevalence. A total of 203 samples were collected. At the end of the report period the laboratory report was still outstanding from SA-DAFF.

A detection survey was conducted in 2015 in the KwaZulu-Natal province for *Candidatus Liberibacter asiaticus* (HLB). None of the 363 samples collected tested positive for HLB. A detection survey for African Greening (Laf) was conducted in the buffer zone area in the Eastern Cape. Twelve samples were collected and two of the samples from the Port St John's area tested positive for Laf. Surveys were also conducted in some of the magisterial districts of the Western Cape, including the buffer zone in the Western Cape province. A total of 56 samples were collected – Ruiterbos (1), Reenendal (1), Riebeeck Kasteel (38) and Plettenberg Bay (16). Two samples, one from Plettenberg Bay and one from Riebeeck Kasteel, tested positive for Laf.

2 BIOSECURITY AND REGULATIONS

The risk of introduction and spread of new pests and diseases is a critically important risk faced by the southern African citrus industry in light of both its ongoing access to export markets and sustained profitability. In 2015, the CGA allocated funding to CRI to support the post of an Industry Biosecurity Manager and in July 2015 Dr Hennie le Roux was appointed in this position. A master plan was compiled to guide the industry biosecurity programme. The greatest biosecurity threat facing the industry is Asiatic Citrus Greening also known as Huanglongbing (HLB). This destructive disease is caused by the bacterium "*Candidatus* Liberibacter asiaticus" (Las). Prior to 2015 the only reported presence of HLB and its primary vector *Diaphorina citri* in Africa was the occurrence of Las in an isolated area of northern Ethiopia. In 2015 symptomatic citrus leaf samples were collected 500km further South in Ethiopia and the presence of Las was confirmed. There were no reports of *D. citri* occurrence in the area.

Kalyebi et al. (2015) then reported that Las had been found in Uganda. In pursuit of early detection and rapid response, surveys were undertaken in Uganda and Kenya (13 – 18 March 2016). Researchers of ICIPE in Kenya were involved in the survey as well as Extension officers from Budaka and Tororo in Uganda. Orchards were visited and leaf samples with blotchy mottle symptoms were taken near the villages of Buloki, Iki-Iki and Kerekerene in the Budaka district as well as near the villages of Siwa, Malawa, Pakidama, Musasa and Tororo in the Tororo district in Uganda. Samples were also taken near Dunga and Angongo in the Kisumu district, Kiboito in the Kericho district and near Sachangwa in the Nakuru district of Kenya. Branch terminals at each site were also beaten over a white plastic tray to see whether any live psylloids were present and at the same time observations were made for any serious citrus pest. When new growth was present it was inspected for the presence of *Diaphorina citri* or *Trioza erytreae*, known vectors of Las and African greening, caused by *Candidatus* Liberibacter africanus (Laf). Laf and *Trioza erytreae* is known to be present in many African countries. Open leaf galls caused by *T. erytreae* on older foliage were also looked for. Leaf samples (under permit from DAFF) were sent for analysis by CRI (Nelspruit) and ARC (Pretoria). DNA extractions were made using a modified CTAB extraction protocol of Doyle and Doyle (1990) and subsequent Diagnostic Greening PCRs were conducted for *Candidatus* Liberibacter asiaticus and L. africanus using species specific primers and probes. For confirmation purposes, end-point PCR was performed to get an overall positive or negative HLB result. Real-time PCR was performed to distinguish between Las and Laf. Twenty of the 24 leaf samples tested positive for Liberibacter. Of these, five tested positive for Laf and 15 for Las. However, when the DNA of the 15 Las positive samples was sequenced, all the samples that showed up as Las using Real-time PCR were in fact the Laf-Cl subspecies of Laf. No *Diaphorina citri* or signs of their presence could be found during this survey and the fact that greening symptoms were not present in trees in full sunlight also indicated that *D. citri*, which is more heat-tolerant than *T. eryrtreae*, was not involved. In most orchards the symptoms (open galls) caused by *T. erytreae* on the leaves were present confirming the role of that vector. Psylloids collected in Kenya were sent to the Biosystematics Division of PPRI, Pretoria for identification.

This survey recorded the existence of Laf-Cl in Uganda in the region where earlier claims had been made that Las had been found in *Trioza erytreae,* and is the first report of Laf-Cl in citrus. It is also important to note that the trees with HLB symptoms in Uganda and Kenya were very similar in health to Laf infected trees in South

Africa. No symptoms typical of Las were observed. Therefore, the status of Las in Uganda and Kenya according to this survey can be described as: No confirmed (reliable) presence of Las; no detection of *D. citri.*

During this survey, symptoms of other citrus pests and diseases such as *Pseudocercospora angolensis*, *Phytophthora* spp., *Elsinoë fawcetti, Phyllosticta citricarpa, Eutetranychus orientalis*, rust mite, bud mite, cottony cushion scale, waxy scale, California red scale and *Bactrocera dorsalis* were also found in citrus orchards.

Talks were held with organisations/citrus growers in Mozambique, Zambia and Namibia who either wanted to start or increase citrus plantings. The dangers of bringing in exotic citrus diseases from other citrus producing countries were explained and the use of CIS planting material was emphasised. Leaf samples from a survey conducted in Angola tested negative for HLB, Citrus canker and Leprosis virus. This was extremely good news as some of the material was introduced from Brazil into Angola. Research with regard to the control of *Diaphorina citri* was also promoted. This research will be conducted in Mauritius where both Las and *D. citri* is present. The main focus will be on effective control methods and programmes to protect citrus trees the whole year round against *D. citri*.

3 PORTFOLIO: INTEGRATED PEST MANAGEMENT

3.1 PORTFOLIO SUMMARY

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

For many years now, the research priorities within the IPM Portfolio have been determined by market access issues. This pertains particularly to retention and growth of existing markets. For several years now, false codling moth (FCM) has been regarded as the number one IPM research priority and the next big market access hurdle after citrus black spot (CBS). This has not changed, other than that the urgency with which FCM research is being conducted has escalated. This is particularly relevant to post-harvest focussed research that can provide rapid answers for achieving consignment freedom in order to satisfy existing and impending market requirements. In addition to the execution of the research and rapid achievement of outcomes, it has become extremely important that these results are published in international peer-reviewed scientific journals in order to make arguments for implementation of any new protocols as compelling as possible. Consequently, researchers within the IPM Portfolio have published several high priority research papers during the last year, which present important results, such as the efficacy of the sterile insect technique (SIT), the efficacy of post-harvest irradiation, the efficacy of cold treatments and an FCM systems approach.

Other pests affecting market access that have attracted research focus and funding are fruit flies, carob moth, mealybug, Fuller's rose beetle and hitchhikers such as grain chinch bug and oribatulid beetle mites. Of these, fruit fly has received the most attention, not only because it is recognised as a potential phytosanitary issue by more markets than is the case for the other pests, but as effective pre-harvest fruit fly control is becoming more challenging throughout the country, but most particularly in the Western Cape.

In 2015 CRI appointed a Biosecurity Manager for the first time. This was to address threatening issues such as the encroaching presence of Asiatic greening disease and its vector, *Diaphorina citri*. The disease has been detected in Ethiopia and the vector in Tanzania. This threat therefore warrants some research focus within the IPM Portfolio for the first time.

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. Eight of these projects entirely or partly investigated postharvest solutions to FCM management. Of these, four were focussed on post-harvest detection and four on post-harvest control. Some exciting results that have come out of this research are the development of reduced intensity cold treatment regimes, without compromising efficacy, the development of a systems approach, which has been shown to mitigate risk at least to the same extent as a stand-alone cold treatment and the promising efficacy of a CO_2 and cold combination treatment.

The 13 pre-harvest focussed research projects within the FCM programme covered five major areas: biological control (including microbial control), sterile insect technique (SIT), pheromones (including mating disruption and monitoring), chemical control and ecology of the pest. Although many of these projects were exciting and promise a meaningful contribution to FCM control in the future, possibly the most exciting prospects at this stage are the use of entomopathogenic fungi for control of soil-dwelling life stages of FCM and the discovery

of a novel nucleopolyhedrovirus, which has demonstrated noteworthy virulence against FCM in laboratory bioassays.

The focus of the fruit fly programme remained on preharvest management of the three main fruit fly pests of citrus, Medfly, Natal fly and Oriental fruit fly. However, it is probable that in the future, some of this attention will shift back to postharvest research, as was the case a few years ago. It is now accepted that Oriental fruit fly is established in certain northern regions of the country and consequently, some of the projects within the research programme aimed to better understand the biology and behaviour of the pest. An interesting new development within the fruit fly programme is that it is now known that Natal fly consists of two distinct genotypes and morphotypes, which will probably be reclassified as two distinct species. This may have some interesting research implications in the near future.

Only three projects are reported within the mealybug and other phytosanitary pests programme. These investigated carob moth, the mealybug species *Delotococcus aberiae* and postharvest fumigation of a range of external and cryptic phytosanitary pests. All three projects are still underway.

The final two projects, which covered non-phytosanitary key pests and minor pests and mites, included five projects. The pre-harvest control of thrips, mealybug, red scale and woolly whitefly were investigated and monitoring of fruit piercing moth was also examined. Probably the most positive outcome from these projects was the demonstration of an effective mating disruption technology for red scale, which is also IPM compatible.

During the research year in question CRI entomologists collaborated well with scientists and students from other institutions. Locally these included Rhodes University, Pretoria University, Stellenbosch University, Nelson Mandela Metropolitan University, Xsit and QMS Laboratories; from other countries, these included The Royal Museum for Central Africa, Centre National de Recherche Agronomique and CIRAD. Many of the researchers and students working within the IPM Portfolio participated actively in scientific meetings both locally and internationally, emphasising to the international scientific community the quality and relevance of research coming out of this team. Additionally, a number of papers were published in top international scientific peer-reviewed journals and in our local fruit journal. CRI entomologists also participated actively in carrying the important messages emanating from their research over to the grower community – this particularly through study group meetings, pre-harvest and postharvest workshops and Cutting Edge publications.

PORTEFEULJE OPSOMMING

Vir baie jare nou is die navorsingsprioriteite binne die IPM Portefeulje deur marktoegangs sake bepaal. Hierdie het veral betrekking tot die behoud en groei van bestaande markte. Vir verskeie jare nou is valskodlingmot (VKM) as die nommer een IPM navorsingsprioriteit beskou en die volgende groot marktoegangs struikelblok na swartvlek. Hierdie het nie verander nie behalwe dat die dringendheid waarmee VKM navorsing uitgevoer word geëskaleer het. Hierdie is veral relevant tot na-oes gefokusde navorsing wat vinnige antwoorde kan gee om besending vryheid to behaal om aan bestaande en toekomstige mark vereistes te voldoen. Benewens die belangrikheid van die navorsing en vinnige bereiking van uitkomste, het dit ook baie belangrik geword om hierdie resultate in internasionale eweknie-resenseerde wetenskaplike joernale te publiseer om die redenasie vir implmentering van enige nuwe protokolle so oortuigend as moontlik te maak. Gevolglik het navorsers binne die IPM Portefeulje verskeie hoë prioriteits navorsings artikels gedurende die laaste jaar gepubliseer, wat belangrike resultate voorlê, soos die doeltreffendheid van die steriele insek tegniek (SIT), die doeltreffendheid van na-oes bestraling, die doeltreffendheid van koue behandelings en ŉ VKM stelselsbenadering.

Ander plae wat marktoegang beïnvloed en wat navorsings fokus en bevondsing gelok het is vrugtevlië, karobmot, witluis, Fuller se rooskewer en rylopers soos die graanstinkbesie en oribatulid kewermyte. Van hierdie het vrugtevlieg die meeste aandag gekry, nie net omdat dit deur meer markte herken word as ŉ potensiële fitosanitêe probleem as wat die geval is vir die ander plae, maar ook omdat doeltreffende vooroes vrugtevlieg beheer al hoe meer uitdaagend word. Hierdie word deur die land ondervind maar veral in die Wes-Kaap.

In 2015 het CRI vir die eerste keer ŉ Biosekuriteits Bestuurder aangestel. Hierdie was om dreigende kwessies soos die indringende teenwoordigheid van Asiatiese vergroening en sy vektor, *Diaphorina citri* aan te spreek. Die siekte is in Ethiopia opgespoor en die vektor in Tanzanië. Hierdie dreiging regverdig dus vir die eerste keer etlike navorsingsfokus binne die IPM Portefeulje.

IPM navorsing is in vyf progamme verdeel: VKM, vrugtevlië, witluis en ander fitosanitêre plae, sleutel nie-fitosanitêre plae en minder belangrike plae en myte.

Gedurende die laaste jaar is 21 projekte binne die VKM program uitgevoer. Agt van hierdie projekte het uitsluitlik of gedeeltelik na-oes oplossings vir VKM bestuur ondersoek. Vier van hierdie het op na-oes opsporing gefokus en die ander vier op na-oes beheer. Sekere opwindende resultate wat uit hierdie navorsing gekom het is die ontwikkeling van verminderde intensiteit koue behandelings protokolle, sonder dat doeltreffendheid benadeel word, die ontwikkeling van ŉ stelselsbenadering wat gewys is om risiko tot minstens dieselfde mate as ŉ alleenstaande koue behandeling te verminder en die belowende werking van ŉ CO_2 en koue kombinasie behandeling.

Die 13 vooroes gefokusde projekte binne die VKM program het vyf hoof areas gedek: biologiese beheer (insluitend mikrobiese beheer), die steriele mannetjie tegniek (SIT), feromone (insluitend paringsontwrigting en monitering), chemiese bestryding en die ekologie van die plaag. Alhoewel baie van hierdie projekte opwindend was en hou belofte in vir ŉ beduidende bydrae tot VKM bestuur in die toekoms, waarskynlik die mees opwindende moontlikhede op hierdie stadium is die gebruik van entomopatogeniese swamme vir beheer van grondgedraagde lewensstadiums van VKM en die ontdekking van ŉ nuwe nukleopolihedrovirus wat beduidende virulensie teen VKM in laboratorium biotoetse gewys het.

Die fokus van die vrugtevlieg program het op vooroes bestuur van die drie hoof vrugtevlieg plae op sitrus gebly, naamliks Medvlieg, Natalsevlieg en Oostersevrugtevlieg. In die toekoms sal hierdie aandag waarskynlik minstens gedeeltelik op na-oes navorsing herfokus, soos wat die geval ŉ paar jaar gelede was. Dit word nou aanvaar dat Oostersevrugtevlieg wel in sekere noordelike streke van die land gevestig is. As gevolg daarvan het sekere van die projekte binne hierdie navorsingsprogram gepoog om die biologie en gedrag van die plaag beter te verstaan. ŉ Interessante nuwe ontwikkeling binne die vrugtevlieg program is dat dit nou bekend is dat Natalsevlieg twee duidelike genotipes en morfotipes het wat heel waarskynlike as twee aparte spesies herklassifiseer sal word. Hierdie mag in die tokeoms dalk sekere interessante navorsings implikasies inhou.

Net drie projekte is binne die witluis en ander fitosanitêre plae program gerapporteer. Hierdie het karobmot, die witluis spesie *Delotococcus aberiae* en na-oes beroking van ŉ reeks eksterne en interne fitosanitêre plae ondersoek. Al drie projekte is nog aan die gang.

Die finale twee projekte, wat sleutel nie-fitosanitêre plae en minder belangrike plae en myte gedek het, het vyf projekte behels. Die vooroes beheer van blaaspootjie, witluis rooidopluis en wollerige witvlieg is ondersoek en die monitering van vrugtesteekmot is ook nagekyk. Waarskynlik die mees positiewe uitkoms van hierdie projekte is die demonstrasie van doeltreffendheid van ŉ paringsontwrigting tegnologie vir rooidopluis, wat ook IPM-verenigbaar is.

Gedurende die laaste navorsingsjaar het CRI entomoloë met wetenskaplikes en studente van ander instansies goed saamgewerk. Plaaslik het hierdie Rhodes Universiteit, Pretoria Universiteit, Stellenbosch Universiteit, Nelson Mandela Metropolitaanse Universiteit, Xsit en QMS Laboratoriums ingesluit; van ander lande het dit The Royal Museum for Central Africa, Centre National de Recherche Agronomique en CIRAD ingesluit. Baie van die navorsers en studente wat binne die IPM Portefeulje gewerk het, het aktief aan plaaslike en internationale wetenskaplike kongresse deelgeneem. Dit het die gehalte en relevansie van die navorsing wat uit dié navorsingsspan gekom het vir die internasionale wetenskaplike gemeenskap beklemtoon. Verder is ŉ hele paar artikels in top internasionale wetenskaplike eweknie-resenseerde joernale asook in ons plaaslike vrugtejoernaal gepubliseer. CRI entomoloë het ook aktief deelgeneem in die oordra van belangrike informasie wat uit hulle navorsing gekom het aan die produsente gemeenskap. Hierdie is veral deur produsentestudiegroepe, vooroes en na-oes werkswinkels en Snykant publikasies gedoen.

3.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

3.2.1 Programme summary

FCM is a regulated phytosanitary pest for several of South Africa's export markets, namely USA, China and some other Far East countries. Subsequent to a PRA on FCM being completed by the European and Mediterranean Plant Protection Organisation (EPPO) in September 2013, the status of FCM for that region has also come under review. Consequently, the importance of the FCM research programme and urgency with which promising management options are being addressed, have been elevated to the top of CRI's research priority list. This has pertained particularly to promising postharvest management options.

As was the case last year, there were 21 research projects within the IPM programme for the 2015/16 year. These have been conducted by researchers in CRI, Rhodes University, Stellenbosch University, Nelson

Mandela Metropolitan University and QMS Laboratories. Eight of these projects entirely or partly investigate postharvest solutions to FCM management. The remainder cover preharvest management research.

Of the postharvest focussed projects, four investigated detection possibilities for FCM, two investigated postharvest treatments, one investigated a combination of the two and the eighth project looked at relative FCM pressure in the different citrus producing regions in southern Africa. The purpose of this last project (3.2.7) was to determine the current ability in each region to comply with the proposed standards of the drafted FCM systems approach and to determine which areas can be regarded as areas of low pest prevalence. The latter will indicate the potential of the area for achievement of pest freedom. Data will be collected for a second season before being analysed.

A project which proposed to investigate a number of possible postharvest detection technologies, focussed strongly on X-ray (3.2.2). Scanning with a micro-focus X-ray for 1 min 26 s per fruit enabled detection of 100% of FCM penetrations on Satsuma Mandarins, 8 days after infestation. Another project aimed to evaluate existing commercially available packhouse sorting equipment (3.2.21). Two commercial companies collaborated with CRI on this, using both currently available and experimental multi-spectral technology and using preliminary results to improve algorithms for detection. A three-year project investigating the potential of a sniffer dog for detecting FCM-infested fruit came to a close, concluding that the dog was able to detect a single recently infested fruit, waxed, wrapped and buried in a carton of healthy fruit, with a success rate of almost 99%. As a result of a study conducted a few years ago, which identified a series of volatiles associated with FCM-infested fruit, a study is currently being conducted using Gas Chromatography-Mass Spectrometry and an electronic nose to reliably confirm and then detect these volatiles (3.2.16).

Of the two projects investigating postharvest treatments, one looked at the efficacy of cold (3.2.9) and the other, the efficacy of hot air (3.2.10). The following cold treatments were found to provide probit 9 disinfestation efficacy of fourth and fifth instar FCM: 16 d at -0.2 °C, 18 d at -0.4 °C and 20 d at -0.4 °C. Additionally, 19 d at 1°C was found to kill all fourth and fifth instars in a sample of around 3 000 larvae and a probit 9 disinfestation trial is now underway. A heat treatment of 46°C for 6 h against 681 third instars in fruit resulted in 100% mortality.

The final project entailing postharvest treatments actually spanned both pre- and postharvest events and involved the development of a systems approach for FCM (3.2.15). By monitoring larval infestation of fruit weekly in 33 orchards, until the time of harvest, post-picking and post-packing into export cartons, it was shown that the proportion of fruit that could be infested with FCM after application of the systems approach, including a postharvest partial cold treatment, was between 6 to 38 times less than the proportion associated with the probit 9 standard for a stand-alone cold treatment.

Of the preharvest management FCM projects, five dealt with aspects of biological control. From 2013 to 2016 a survey was conducted in the Western Cape for larval parasitoids of FCM (3.2.5). As none were found, the intention was to import and release *Agathis bishopi* in the Western Cape and to monitor for establishment. Unfortunately, the culture maintained in the Eastern Cape collapsed, so this was not possible. Another project, studying the efficacy of entomopathogenic nematodes (EPN) against FCM in large scale field trials, concluded after almost five years of research that both *Heterorhabditis bacteriophora* and *Steinernema feltiae* applied to the soil could reduce FCM infestation by up to 81% (3.2.3). However, results were variable, particularly being influenced by soil moisture. Additionally, where *H. zealandica* occurred naturally at high levels, FCM infestation of fruit could be reduced by 59%. Another soil applied pathogen group, entomopathogenic fungi (EPF), also demonstrated tremendous potential (3.2.8). Both *Beauveria bassiana* and *Metarhizium anisopliae* isolates were tested, demonstrating that a single soil applied treatment in spring could reduce FCM infestation of fruit by up to 80% and remained effective until harvest.

The final two biological control projects investigated improved control of FCM with baculoviruses. One of the major setbacks with the use of baculoviruses as biopesticides is their susceptibility to ultraviolet (UV) radiation from the sun. A project proposed to study various methods of protecting CrleGV-SA formulations against UV (3.2.20), particularly by selecting for naturally occurring UV-resistant strains. The second virus project reports on the discovery of a novel nucleopolyhedrovirus (NPV) and its virulence against FCM (3.2.22). A multiplex PCR assay has been developed to screen larval samples for the presence of either the GV or NPV and qPCR analysis has been established to accurately quantify virus occlusion bodies recovered from larval cadavers. These cadavers will be collected from a series of bioassays which are currently underway to test for any potential synergism between these viruses.

Two of the research projects deal with improving the cold tolerance of sterile FCM for enhanced performance in an SIT programme. The first aimed to expose sterile FCM to different thermal conditions and to assess the

effect of these on flight-, and mating ability (3.2.6). Cold acclimation had a greater effect on female than male moths, improving fecundity, longevity and critical thermal limit. The second SIT project investigated the use of cryoprotectants in larval diets for improving flight ability of sterile males, particularly at cool temperatures (3.2.14). Augmenting diets with cholesterol and trehalose increased male flight ability at 15° in laboratory trials by 41% and 36%, respectively. In field trials, recaptures of trehalose-treated sterile males was 3-5 times higher than untreated moths at temperatures below 20°C.

Three trials investigated mating disruption (MD) of FCM. The first aimed to establish the full potential of MD in the field, using doses well above the registered applications (3.2.12). However, where Isomate and Checkmate were used together in the same block, there was no significantly greater reduction in FCM infestation, relative to a single Isomate application. An analysis of the viability of local synthesis of FCM pheromone also indicated that it could not be done at an improved cost relative to importation. Another MD trial was conducted in the field in Letsitele to compare the efficacy of the two registered FCM MD products and the registered attract and kill product (3.2.17). FCM infestation was too low to draw conclusive results. However, trap catches were substantially lower for Isomate than the other treatments. Due to the low FCM pressure, no results were obtained in an FCM spray trial conducted simultaneously, that reviewed all registered spray options. The final MD trial was actually termed mating inhibition, as it investigated the efficacy of a novel compound, 7-vinyl decyl acetate 1 (7-VDA), rather than the FCM pheromone (3.2.11). Laboratory trials indicated that a combination of the FCM pheromone and 7-VDA reduced mating most effectively. This must now be tested in field trials.

Related to the previous three trials in their investigation of FCM pheromones, was a project which aimed at testing whether virgin female FCM from all regional populations were equally attractive to sterile males from Citrusdal, used within an SIT programme; and whether there were any differences in the attractiveness of different blends of isomers in the FCM pheromone for monitoring male moths (3.2.19). Results strongly indicate that the South African regional blend is the most attractive followed by the blends in the commercially available products and the Ivory Coast and Malawi blends being the least attractive.

The final two projects within the programme were ecological in nature. The movement of FCM and fruit fly was investigated in multi-crop systems, with the aim of establishing the influence of other susceptible crops, planted in close proximity, on citrus orchards (3.2.13). FCM male flight activity was recorded to not be related to the ripening of each fruit type, with high catches sometimes being recorded in harvested orchards. Conversely, fruit fly catches did appear to relate to the ripening of fruit in each orchard, with the exception of nectarines, where catches sometimes remained high after harvest. The final project investigated FCM population ecology in citrus orchards of different ages (3.2.18). FCM eggs were significantly higher in established orchards compared to juvenile orchards. However, egg parasitism was also higher. Bushveld sites and newly planted orchards had the highest occurrence of EPFs in soil samples. Fruit from young orchards was twice as susceptible to FCM than fruit from established orchards. This was related to differences in protein and ash content.

Programopsomming

VKM is ŉ gereguleerde fitosanitêre plaag vir veskeie Suid-Afrikaanse uitvoermarkte, naamliks VSA, China en sekere ander lande in die Verre Ooste. Nadat a PRA op VKM deur die European and Mediterranean Plant Protection Organisation (EPPO) in September 2013 voltooi is, het die status van VKM vir daardie streek onder oorsig gekom. Die belangrikheid van die VKM navorsingsprogram en die dringendheid waarmee belowende beheer opsies aangespreek word is daarbenewens verhoog tot bo aan die lys van CRI se navorsingsprioriteite. Dit geld veral vir na-oes beheeropsies.

Net soos velede jaar, was daar 21 navorsingsprojekte binne die IPM program in die 2015/16 jaar. Hulle is uitgevoer deur navorsers van CRI, Rhodes Universiteit, Stellenbosch Universiteit, Nelson Mandela Metropolitaanse Universiteit en QMS Laboratoriums. Agt van die projekte ondersoek uitsluitlik of gedeeltelik na-oes beheermaatreels vir VKM. Die oorblywende projekte behels navorsing op vooroes beheer.

Van die na-oes gefokusde projekte, het vier van hulle opsporings-moontlikhede ondersoek, twee het na-oes beheermaatreels ondersoek, een het ŉ kombinasie van die twee ondersoek, en die agste het gekyk na die relatiewe VKM-druk in die verskillende sitrusproduksiestreke in suidelike Afrika. Die doel van hierdie laaste projek (3.2.7) was om te bepaal wat die huidige vermoë in elke streek is om die voorgestelde standaarde in die VKM stelselsbenadering na te kom en om te bepaal watter areas as streke van lae plaag voorkoms beskou kan word. Laasgenoemde sal die moontlikheid aandui om plaag-vryheid in ŉ streek te bereik. Data sal vir ŉ tweede seisoen versamel word voordat dit ontleed word.

ʼn Projek wat voorgestel het om verskeie na-oes opsporingstegnologieë te ondersoek, was sterk gefokus op X-straal. Mikrofokus X-straal skandeering vir 1 min 26 s op Satsuma Mandaryne agt dae na besmetting het 100% van VKM penetrasies opgespoor. Nog ʼn projek het beoog om bestaande kommersieel beskikbare sorterings-eenhede te evalueer. Twee kommersiële maatskapye het met CRI saamgewerk, met die gebruik van tans beskikbare en eksperimentele multi-spektrale tegnologieë, en voorlopige resultate is gebruik vir die verbetering van algoritmes vir VKM opsporing. ʼn Projek wat oor drie jaar die potensiaal van ʼn snuffelhond om VKM-besmettee vrugte op te spoor, is afgehandel. Die hond kon ʼn pas-besmette vrug, wat gewaks en togedraai was, en dan binne ʼn karton gesonde vrugte begrawe is, met 99% akkuraatheid opspoor. ʼn Studie ʼn paar jaar terug het ʼn reeks vlugtigestowwe wat gepaard gaan met VKM-besmette vrugte geïdentifiseer. As gevolg hiervan is ʼn studie tans besig om met Gaskromatografie-Massaspektrometrie (GCMS) en ʼn elektroniese neus die bevindinge te bevestig, en die vugtiggestowwe op te spoor (3.2.16).

Een van die twee projekte wat na na-oes behadelinge kyk, het die doeltreffendheid van koue behandeling ondersoek (3.2.9), en die ander die doeltreffendheid van warm lug (3.2.10) Die volgende koue behandelinge het disinfestasie tot ʼn probit 9 vlak bewerkstellig: 16 d teen -0.2 °C, 18 d teen -0.4 °C en 20 d teen -0.4 °C. Daarbenewens het 19 d teen 1°C alle vierde en vyfde instars in ʼn monster van ongeveer 3000 larwes doodgemaak, en ʼn probit 9 disinfestasie proef is nou onderweg. ʼn Hitte behandeling van 46°C vir 6 ure het 100% mortaliteit van 681 derde instars in vrugte veroorsaak.

Die finale projek wat na-oes behandelinge betref, wat eintlik voor- en na-oes gebeure behels, was die ontwikkeling van ʼn stelsels-benadering vir VKM (3.2.15). Weeklikse monitering van vrugbesmetting in 33 boorde, tot en met oestyd, na oes en na pak in uitvoerkartonne, het gewys dat die verhouding van vrugte wat besmet is na toepassing van die stelsels-benadering, 6 tot 38 maal minder was as die verhouding wat verband hou met die probit 9 standaard vir ʼn alleenstaande koue behandeling.

Van die voor-oes beheer projekte het vyf biolgiese beheer behels. ʼn Opname van larwale parasitoïedes van VKM is vanaf 2013 to 2016 in die Wes-Kaap uitgevoer (3.2.5). Nadat niks givind is nie, was die doel om *Agathis bishopi* in te voer vir loslating in die Wes-Kaap, en te monitor of hulle gevestig het. Ongelukkig was loslatings nie moontlik nie, omdat die kolonie in die Oos-Kaap ineengestort het. Nog ʼn projek wat die doeltreffendheid van entomopatogeniese nematodes (EPN) teen VKM in grootskaalse veldproewe ondersoek het, het na 5 jaar se navorsing gewys dat grond toediening van albei *Heterorhabditis bacteriophora* en *Steinernema feltiae* VKM besmetting met tot 81% kan verminder (3.2.3). Resultate was wel wisselvallig, en veral deur grond vogtigheid beinvloed. Waar daar hoë natuurlike vlakke van *H. zealandica* teenwordig was, kon VKM besmetting met 59% verminder word. Entomopatogeniese swamme (EPS), wat ook aan die grond toegedien word, het ook groot potensiaal gewys (3.2.8). Albei *Beauveria bassiana* en *Metarhizium anisopliae* isolate is getoets, en kon met een enkele grondtoediening in die lente VKM besmetting met tot 80% verminder, en het tot en met pluktyd doeltreffend gebly.

Die finale twee projekte wat biologiese beheer behels het, het verbeterde beheer van VKM met bakuloviruse ondersoek. Een van die grootste tekortkominge met bakuloviruse as biologiese plaagdoders is hulle vatbaarheid vir ultraviolet (UV) bestraling van die son (3.2.20). Hierdie studie beoog om verskeie metodes te ondersoek om CrleGV-SA teen UV-bestraling te beskerm, veral om deur UV-bestande isolate wat natuurlik voorkom te selekteer. Die tweede virus projek rapporteer die ontdekking van ʼn nuwe nukleopolihedrovirus (NPV), en sy virulensie teen VKM (3.2.22). ʼn Multipleks PCR toets is ontwikkel om hierdie en enige toekomstige monsters vir die teenwoordigheid van óf die GV of NPV te skandeer en ʼn qPCR analise is ook ontwikkel om virus partikels van larwale kadawers akkuraat te kwantifiseer. Hierdie kadawers sal van ʼn reeks biotoetse versamel word wat tans aan die gang is om moontlike sinergisme tussen hierdie virusse te ondersoek.

Twee van die novorsingsprojekte behels die verhoging van koue-toleransie van steriele VKM vir verbeterde prestasie in ʼn SIT program. Die eerste het beoog om steriele VKM aan verskillende termiese omstandihede bloot te stel, en die effek hiervan op vlieg- en paringsvermoë te meet (3.2.6). Koue akklimasie het ʼn groter effek op wyfies as op mannetjies gehad. Dit het fekunditeit, langlewendheid en die kritieke termiese minimum verbeter. Die tweede SIT projek het die effek van die gebruik van kouebeskermers in larwale dieët op die vliegvermoë van steriele mannetjies ondersoek, veral teen koue temperature. (3.2.14). Die aanvulling van ʼn dieet met cholesterol of trehalose het die vliegvermoë van mannetjies by 15°C met 41% en 36% onderskeidelik onder laboratorium toestande verhoog. In vlugtoetse in die veld is hervangs van trehalose-behandelde steriele mannetjie motte 3-5 maal hoër as onbehandelde steriele mannetjies teen temperature onder 20°C.

Paringsontwrigting (PO) van VKM is in drie proewe ondersoek. Die doel van die eerste was om die volle potensiaal van PO te ondersoek deur die verhoogde gebruik (meer as wat huidig geregistreer is) van paringsontwrigting produkte (3.2.12). Die gebruik van Isomate en Checkmate saam in ʼn blok het nie VKM

besmetting beduidend verder verminder as ŉ enkele toediening van Isomate alleen nie. ŉ Ondersoek om te kyk of VKM-feromone plaaslik gesintetiseer kan word het gewys dat dit nie goedkoper sou wees as om die produkte in te voer nie. ŉ Ander PO proef is in Letsitele uitgevoer om die doeltreffenheid van twee VKM PO produkte en een geregistreerde lok-en-vrek produk te vergelyk. VKM besmetting was te laag om betekenisvolle resultate te lewer, maar lokvalvangstes was wesenlik laer vir Isomate as die ander twee produkte. ŉ Spuitproef was gelyktydig uitgevoer, waar alle geregistreerde spuitmiddels vergelyk was, en het ook geen resultate gelewer nie weens die lae VKM-vlakke. Die finale PO proef is eintlik paringsverhoeding genoem, omdat dit ŉ nuwe molekule, 7-vinieldesielasetaat 1 (7-VDA) ondersoek het, in plaas van die VKM feromoon. Laboratoriumproewe het gewys dat n kombinasie van 7-VDA en VKM feromoon, paring die meeste verminder het. Dit moet nou in veldproewe getoets word.

Nog ŉ proef, soortgelyk aan die vorige drie proewe wat VKM feromone ondersoek het, het beoog om te toets of ongepaarde VKM wyfies vanaf verskillende produksiestreke dieselfde aantreklikheid het vir steriele mannetjies van Citrusdal, wat vir die SIT program gebruik word. Daar is ook getoets of daar enige verskille in aantrekliheid vir mannetjies tussen verskillende mengsels van die isomere in die VKM feromone is vir monitering doeleindes (3.2.19). Resultate toon sterk dat die Suid-Afrikaanse mengsel die mees aantreklik is, gevolg deur kommersieel beskikbare produkte, en die mengsels van die Ivoorkus en Malawi is die minste aantreklik

Die laaste twee projekte in die program is van ekologiese aard. Die beweging van VKM en vrugtevlieg op plase waar verskillende gewasse geplant is, is ondersoek, met die doel om uit te vind watter invloed ander vatbare gewasse op sitrusboorde het waar hulle naby daaraan geplant is (3.2.13). Vliegaktiwiteit an VKM mannetjies het nie verband gehou met die rypwording van elke vrugtipe nie, en soms was daar baie hoë vangstes in boorde wat klaar geoes is. Aan die ander kant blyk dit dat vrugtevlieg vangstes wel verband hou met die rypwording van vrugte in elke boord, met die uitsondering van nektariene, waar vangstes sooms hoog gebly het na oes. Die finale projek ondersoek die VKM populasie ekologie in sitrusbooerde van verskillende ouderdomme (3.2.18). VKM eiergetalle was beduidend hoër in gevestigde boorde teenoor jong boorde, maar eierparasitisme was ook hoër. Bosveld persele en nuwe aanplantings het die meeste EPS gehad. Vrugte van jong boorde was twee maal meer vatbaar vir VKM as vrugte van gevestigde boorde. Dit hou verband met verskille in proteiën- en as-inhoud in die vrugte.

3.2.2 FINAL REPORT: Development of mechanisms for the postharvest detection of cryptic pests in citrus fruit

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

Summary

False codling moth (FCM) is considered a phytosanitary organism by certain markets, and there is therefore an urgent need to investigate methods for its postharvest detection in fruit. This study aimed to investigate options that are currently available as well as new techniques, in order to ultimately develop an affordable, practical system which could detect the presence of these pests in fruit. Degreening did not improve the detectability of FCM penetration marks, but lag time between picking and packing did. Tastetech's NIR system did not appear to be accurate enough to predict FCM infestation to a sufficiently high degree. More data would need to be acquired to augment the database, which would make it more accurate. Ultraviolet light did not highlight FCM penetration marks, unless *Penicillium* fungus had established on the fruit. X-ray tomography was very successful in detecting FCM damage inside fruit, but radiography was not. Unfortunately, the tomography process is very slow. Micro-focus X-Ray radiography was superior to normal X-ray radiography; but unfortunately, on oranges, evidence of FCM infestation was not detectable while the larva was in the rind or albedo of the fruit. This indicated that infestation would not be detected within the first few days after penetration. In Satsumas, the larvae penetrate the rind faster, and so detection is possible one day after penetration. Micro-focus Tomography (µCT) is faster than normal tomography, and the quality of images is superior, and so scans can be conducted faster. More false-positives were experienced with µCT, as the superior images show up more areas of differing density, which could be mistaken for infestation damage. The micro-focus unit was optimally set up to produce the best possible images to be used for detecting FCM infestation in fruit. The time of a tomography scan was reduced from 35 minutes to 1 minute and 26 seconds. One hundred percent of FCM penetrations were detected on Satsuma Mandarins 8, 12, 14 and 16 days after infestation, using the new settings. This shows that the ability of the technology to detect infested fruit does not appear to be compromised. Further trials were conducted on Delta Valencia oranges, where some early infestations were not detectable. It is possible that more affordable tomography units could be used to evaluate samples of incoming fruit at packhouses.

Opsomming

Valskodlingmot (VKM) word deur sekere markte beskou as ŉ fitosanitêre organisme, dus is daar ŉ ernstige behoefte om metodes vir na-oes opsporing te ondersoek. Die studie poog om alle beskikbare metodes te ondersoek, asook nuwe tegnieke, om uiteindelik ŉ bekostigbare praktiese sisteem te ontwikkel wat insekte in vrugte kan opspoor. Ontgroening het nie die opspoorbaarheid van VKM penetrasiemerke verbeter nie, maar ŉ verlengde tydperk tussen pluk en pak het wel. Tastech se NIR sisteem was nie akkuraat genoeg om VKM besmetting te voorspel nie. Meer data word benodig om die databasis aan te vul, wat die sisteem meer akkuraat sal maak. Ultaviolet lig het nie VKM penetrasiemerke meer sigbaar gemaak nie, tensy *Penicillium* swam infeksie teenwoordig was. X-straal tomografie het VKM skade binnekant vrugte suksesvol opgespoor, maar nie radiografie nie. Die tomografie proses was ongelukkig te stadig. Beeldkwaliteit van mikrofokus radiografie was beter as gewone radiografie, maar ongelukkig kon larwes in lemoene nie opgespoor word terwyl hulle in die skil of albedo was nie. Dit het aangedui dat besmetting nie binne die eerste paar dae na penetrasie opgespoor kan word nie. Larwes penetreer die skil van Satsumas vinniger, en besmetting kon een dag na penetrasie opgespoor word. Mikrofokus tomografie (µCT) is vinniger as gewone tomografie, en beeldkwaliteit is beter, daarom kan skandeerings vinniger gedoen word. Meer vals positiewes is ervaar met µCT omdat die beter beelde meer areas met verskillende digthede wys, wat dan met VKM skade verwar kan word. Die mikrofokus eenheid is optimaal opgestel vir die beste beeldkwaliteit om VKM op te spoor. Mikrofokus skanderingstyd is verminder tot 1 minuut 26 sekondes. Met die nuwe instellings is 100% van VKM penetrasies op Satsuma Mandaryne opgespoor, 8, 12, 14 en 16 dae na besmetting. Dit het gewys dat die vermoë van die tegnologie om VKM op te spoor nie benadeel is nie. Verdere proewe op Delta Valencias het gewys dat sommige vroeë besmettings nie opgespoor kan word nie. Dit is moontlik dat meer bekostigbare tomografie eenhede gebruik kan word om monsters van inkomende vrugte by pakhuise te evalueer.

Introduction

False codling moth (FCM) is considered a phytosanitary organism by certain markets, and there is therefore an urgent need to investigate methods for its postharvest detection in fruit. Modern markets, such as the USA, Korea, China and Iran have been far more sensitive to these issues than the traditional markets (Western Europe). However, the EU, which is by far the largest market for South African citrus, is becoming increasingly strict in its perspective on the phytosanitary risks of FCM and fruit flies A PRA was recently completed by EPPO. Cold sterilisation to these markets would not be economically viable. Several effective pre-harvest control measures exist for FCM, but there is no ‘silver bullet’ which can ensure that no infested fruit reach the packhouse. Such control measures are inadequate for a pest for which there is zero tolerance. There is an urgent necessity to investigate methods for postharvest detection of cryptic pests, in particular FCM, but also fruit flies, on an individual fruit basis. The aim of this study is to thoroughly investigate all options, those currently available as well as new techniques, in order to ultimately develop an affordable, practical system which could detect the presence of these pests in fruit in the packhouse, and so avoid phytosanitary interceptions and their potentially devastating consequences. Techniques being investigated include, X-ray, near infra-red, ultraviolet light and volatile emissions.

Stated objectives

- Intensive literature search on all methods to detect the presence of cryptic pests.
- Revisit the older and existing technologies, such as dyes. These would only be limited to testing samples of fruit as an indication of FCM infestation levels.
- Revisit ultra-violet and fluorescent light technologies to indicate surface penetration marks. Repeat certain treatments where insufficient data are available, and where the technologies have not been applied specifically to citrus.
- Investigate Infra-red and sonar scanning. Initially screen existing systems at universities and packhouses where they are installed, to assess potential. Mafroda and Greefa currently manufacture such systems. If any of these systems show great potential, it may be necessary to acquire equipment for extensive testing.
- Thoroughly investigate the use of X-ray technologies. NECSA have the equipment and have indicated willingness to co-operate with CRI.
- Investigation of markers/metallic compounds which could be fed to larvae to make them easily detectable in X-rays
- Thoroughly investigate the use of Sonar scanning technologies.
- Thoroughly investigate the use of volatile measuring.

Materials and methods

<u>Degreening and lag time after picking</u>

Several hundred Newhall Navel oranges were harvested, and each was inoculated with 5 neonate FCM larvae. Thirty fruit were used per treatment (Table 3.2.2.1). One treatment was inspected for FCM infestation 2 days later, simulating fruit which was newly infested, and packed on the day of delivery to the packhouse. Other treatments were inspected 5 and 8 days after infestation, with and without degreening. Degreening took place over 48 hours at the SRCC Summerville packhouse. Fruit is normally picked one day, drenched the following day, and then degreened for two days, and packed the day after that. If a weekend falls in between, the fruit stands for another 2-3 days after degreening (Russell Stead, personal communication). The treatments were designed to simulate these processes – the inspection dates being the dates that the fruit would have been packed.

The fruit were inspected for 5 seconds, to simulate a packhouse situation. Visible penetration marks were recorded and marked with a Marker pen. After inspection, the fruit was left for 2 weeks, and then dissected and inspected for FCM infestation to verify the accuracy of the earlier inspections.

Table 3.2.2.1. Navel oranges inspected at different intervals after inoculation with neonate FCM larvae, in an attempt to simulate a packhouse sorting process

Treatment		**Test**
1	Inspect 2 days after infestation (simulate packing on day of delivery at packhouse)	The effect of lag time after picking on FCM detectability
2	Inspect 5 days after infestation (simulate 3 days standing before packing)	
3	Inspect 8 days after infestation (simulate 6 days standing before packing)	
4	Inspect 5 days after infestation – degreening* (simulate 5 days standing and degreening before packing)	The effect of degreening on FCM detectability
5	Inspect 8 days after infestation – degreening (simulate 6 days standing and degreening before packing)	

Near infra-red spectroscopy (NIR)

Collaborative research was conducted with Tastetech, a commercial manufacturer of packhouse grading equipment, from New Zealand. Their equipment has various uses, such as determining sugar content in citrus fruit, internal defects in apples, and ripeness of avocados. There are three means of measuring a sample's NIR absorption spectrum: reflectance, transmission and interactance. Their T1 head, which utilizes transmission mode, was used for the trials. The two light sources are located on opposite ends of the sample, and the sensor above it (Fig 3.2.2.1.). With the T1 head the horizontal line of light is used to get a leading and trailing edge of the fruit. A dark spot was left on top surface of the fruit, so avoiding any surface reflection back into the sensor.

Several thousand fruit were collected from the SRCC Kirkwood packhouse. Approximately half of the fruit were chosen as they appeared to be infested by FCM, and the other half were chosen randomly.

NIR trial 1

Acquisitions (scans) were done on 40 clean and 40 seemingly infested fruit. Four scans were done per fruit, with stem end up, stem end down, and from 2 opposite sides. With this unit, 31 spectra are used per acquisition. Each spectrum has 256 values, one per wavelength. The average of these spectra would give a value for the fruit, which was stored in a data base. Each fruit was dissected and given a damage/destructive value of between 0 and 10. Nought represented no infestation, 1 - 3 represented little internal damage, usually by young FCM, and 8-10 was extensive damage caused by 5th instar larvae. A test/validation was then done by scanning/grading fruit, and then dissecting them, to see if the system would recognise infested fruit.

NIR trial 2

A second method of grading was tested – a simple yes/no test for infestation. If the grading gave a predictive value of less than 25, it was assumed that the fruit was healthy, and any fruit which gave a value greater than 30 would be assumed to be infested. A second validation was done by facing the FCM penetration point directly at the sensor. The fruit was then dissected and inspected for signs of FCM infestation.

NIR trial 3

Several hundred more fruit acquisitions were done, and the fruit was cut up and given a destructive value. This was done in order to add more information into the database, and so hopefully make the system more accurate. All the data were then used to construct a three-way model. For a destructive rating of 0, the predictive value should be less than 20 (Table3.2.2.2), and the model rating would be 0. For a destructive rating of between 1 and 5, the predictive value should be between 20 and 60, and the model value 1. For a destructive rating of between 6 and 10 the predictive value should be between 20 and 60, and the model value 2. Fruit were then graded, and according to the model they were given a rating of 0, 1 or two. The fruit were then dissected to give the destructive (actual) value, thereby testing the accuracy of the model.

Table 3.2.2.2. Corresponding NIR model ratings for destructive and predictive values

Model Rating	**Destructive rating**	**Predictive value**
0	0	< 20
1	1 - 5	20 – 60
2	6 - 10	60 - 100

NIR trial 4

Several hundred more fruit were dissected and the information fed into the database; as more information is fed into the system, it becomes more accurate. A new model was designed with 5 grades instead of the 3 in the previous model (Table 3.2.2.3.). This was to determine how closely the 'beefed-up' model could predict the level of FCM damage. Fruit were then graded and dissected to give the destructive (actual) value, thereby testing the accuracy of the model.

Table 3.2.2.3. Corresponding NIR model ratings for destructive and predictive values in an attempt to refine the model

Model Rating	**Destructive rating**	**Predictive value**
0	0	< 30
1A	1 – 3	31 – 50
1	3 – 6	51 – 70
1B	6 – 8	71 - 90
2	8 – 10	> 90

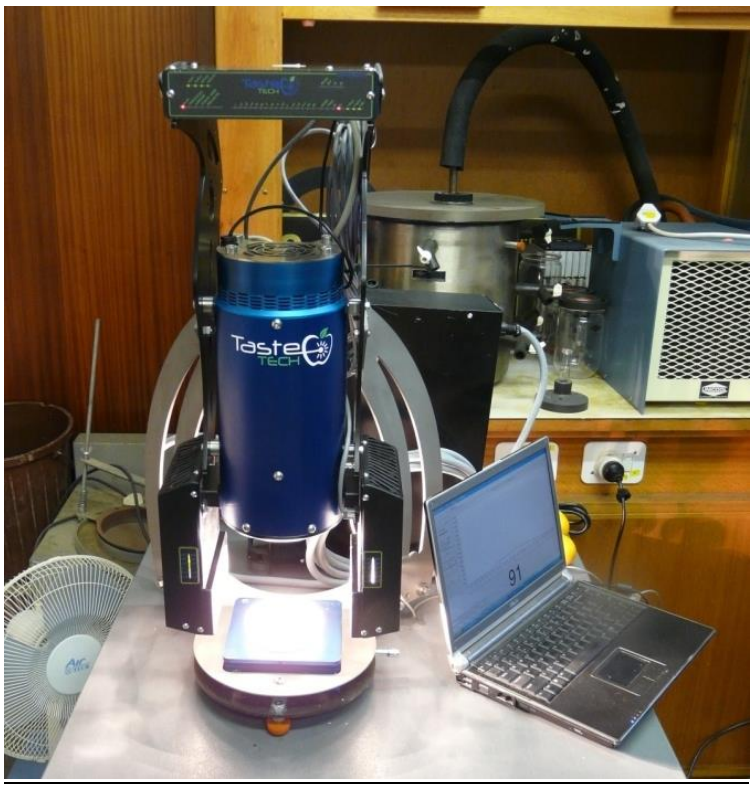

Figure 3.2.2.1. Tastetech T1 transmittance NIR spectrometer

Ultra Violet light

Several Navel oranges, at various stages of FCM infestation, were viewed under ultraviolet (UV) light in a UV-chamber belonging to Greefa, a commercial company which manufactures fruit packing and grading equipment. The aim was to determine if FCM penetration marks were more visible under UV light than conventional light.

X-ray

X-ray bioassays – X-ray trial 1

It was evident from our trials that larvae were not visible on X-rays. Bioassays were conducted to test whether Iodine and Barium Sulphate were toxic to neonate FCM larvae. These substances, if ingested by neonate larvae, could be used as 'tracers' to help track the larvae in the fruit by X-ray technologies. Iodine was bought as a 0.05% solution. A five-fold series dilution was made (5 dilutions), with 0.05% being the strongest concentration. Once the droplets of the iodine solutions were fed to the larvae, they were put onto plugs of artificial diet, containing agar, in plastic bacterial count bottles. Twenty-five larvae were used per treatment. It was not possible to see the iodine inside the larvae, so they were observed drinking by using an optical visor (head loupe), and those which were seen to have ingested the solutions were placed onto the diet.

Literature reviews showed that Barium sulphate is used in Barium meals and enemas at a concentration of 12% w/v. A five-fold series dilution was prepared (5 dilutions), with 12% being the strongest suspension. These suspensions were then fed to neonate larvae, and they were placed on artificial diet as explained in the previous section. Polypots were used in this trial, with 5 larvae placed onto artificial diet in each Polypot; 4 Polypots per treatment. This gave a total of 20 larvae per treatment. Barium sulphate does not dissolve, so the suspension had to be well shaken each time the droplets were placed in the honey jar lids. It is possible that the compound may have settled to the bottom of the droplet, and that some of the larvae may not have ingested any particles. The Polypots were kept at 25˚C for 10 days, and evaluated for larval survival.

X-ray trial 2

The first visit involved a training session on how to use the equipment, and to see if certain compounds, such as Iodine and Barium sulphate, would show up on X-rays if ingested by larvae. Navel oranges were harvested in the Hankey area, and inoculated with 10 FCM larvae each, 5, 8 and 14 days prior to X-ray, to ensure the availability of different age levels of infested fruit for scanning. Polypots with artificial diet were also inoculated

at various intervals with larvae fed with the compounds. Navel oranges infested with Medfly eggs 7 days prior were also X-rayed. We were introduced to the techniques of radiography (X-ray) and tomography (multiple X-rays). The NECSA CCD camera, recorded 1024 pixels, 10 micron per pixel. This has been doubled to 2048 pixels, 2000 pixels per 10mm, = 200 per mm, = 0.005 mm/pixel, which improves spatial resolution. The greater the focal spot (1 mm in the case of this camera), the greater the distortion. The detector turns X-rays into photons, which can be captured. A new micro-focus radiography unit, which NECSA will acquire in June 2011, will have a focal spot of 1-2 micron, and will deliver clearer images.

Fruit were mounted on a pot-plant sponge, which did not show on the X-rays. The fruit was positioned so that the penetration mark was on the outer perimeter of the X-ray image – to avoid overlap with any other penetrations. A piece of Prestik was placed on the penetration mark to ensure correct positioning and to view it on the X-ray image. A 1 mm focal spot was used, but nothing was visible on the image. 100 KV (Energy) was used, and 8 milliamps.

Eppendorf vials with water, iodine + Brilliant Blue dye and Barium sulphate + Brilliant Blue dye were X-rayed. Ten neonate FCM larvae were places onto a glass surface and also X-rayed.

X-ray trial 3

Several Lane Late Navel oranges were harvested from an orchard in the Sundays River Valley. Approximately 20 oranges were inoculated with 8 neonate FCM larvae 4, 8 and 15 days prior to X-ray. At NECSA, 6 fruit from each date of inoculation were inspected under a magnifying lens to look for penetration marks. They were X-rayed, using both radiography and tomography, to see what was visible. The fruit were then dissected and inspected for signs of FCM infestation.

X-ray trial 4

A similar trial to the previous one was conducted, using Satsuma mandarins harvested from an orchard in the Sundays River Valley. About 20 Satsumas were inoculated with 8 neonate FCM larvae on 5, 7 and 10 days prior to X-ray. At NECSA, 6 fruit from each date of inoculation were inspected under a magnifying lens to look for penetration marks. They were X-rayed using both radiography and tomography, to see what was visible. The fruit were then dissected and inspected for signs of FCM infestation. At NECSA, several fruit were inoculated with neonate FCM larvae one day before X-ray, to determine if FCM damage could be detected one day after infestation.

X-ray trial 5

Several FCM infested fruit were X-rayed at X-Sight, a commercial company which supplies micro-focus X-ray equipment. Hiring of the equipment was expensive, so only radiography was conducted on several FCM infested fruit. The machine had a focal point of 1 micron, 1000 time smaller than the normal X-ray unit. It was hoped that the greater resolution of micro-focus images would make it possible to detect lesser levels of damage, caused by smaller larvae, than the normal X-Ray unit at NECSA. Further scans were done two days after infestation, in an attempt to track the development of the larvae, and to determine at what stage the infestation could be detected.

X-ray trial 6 - Preparation of the micro-focus system

Preliminary studies showed that images from the micro-focus X-ray unit were clearer, but it was still not possible to detect FCM infestation while the larva was in the albedo of the fruit. A full evaluation of the micro-focus machine and its ability to detect FCM infestation was conducted at NECSA.

Evaluating targets and filters

Micro-focus X-ray systems operate on the basis on a filament emitting electrons. These electrons are aimed at a spot on a target, which then reflects the electrons through the fruit, and onto a detector where the image is formed (Fig 3.2.2.2).

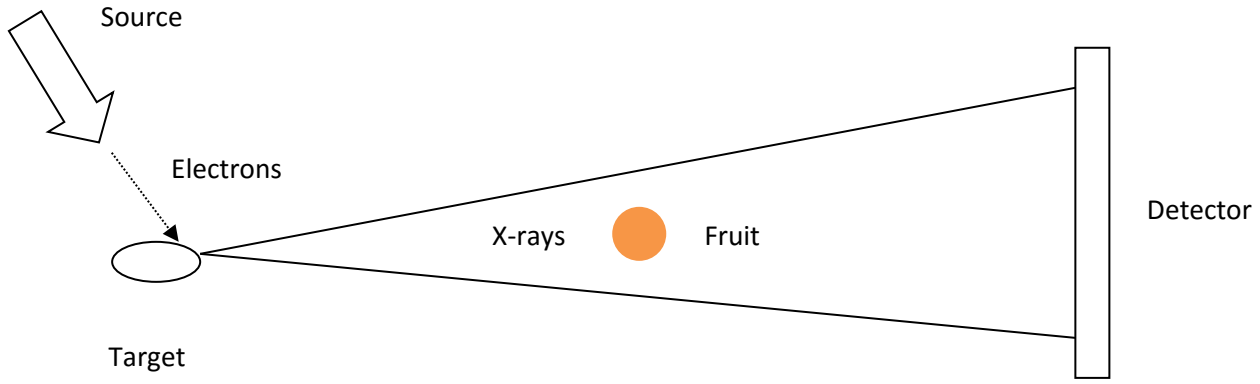

Figure 3.2.2.2. Diagrammatic representation of a micro-focus X-ray system.

The target influences the quality of the image, so trials were conducted to evaluate tungsten, copper, silver and molybdenum targets, to see which would produce the clearest image. Copper, aluminium, silver and selenium filters were compared to see which delivered the best image.

Determining the optimum voltage

Trials were conducted to determine the optimum voltage for the X-rays. This was done by creating images using a range of voltages.

Determining the optimum number of projections for micro-focus tomography

Tomograms were created using various numbers of projections to determine the optimum number of projections required to create the best images.

<u>X-ray trial 7</u>

Several Delta Valencia oranges were harvested from an orchard in the Sundays River Valley. About 20 oranges were inoculated with 8 neonate FCM larvae 1, 3, 10 and 13 days prior to X-ray. At NECSA, 6 fruit from each date of inoculation were inspected under an illuminated magnifying lens to look for penetration marks. They were X-rayed with the micro-focus X-ray machine, using both radiography and tomography, to see what was visible. The fruit were then dissected and inspected for signs of FCM infestation.

<u>X-ray trial 8 - Image Analysis and development of an algorithm to automatically detect FCM infestation</u>

X-ray cross-sections from tomograms were analysed. The different Gray (Gy) values of FCM damage, natural vacuoles and healthy fruit segments were recorded, in an attempt to develop an algorithm which could automatically detect FCM infestation from X-ray images.

<u>X-ray trial 9 - preparation and calibration of the micro-focus system</u>

The micro-focus X-ray machine at NECSA (Fig 3.2.2.3) has a focal point of 1 micron, 1000 time smaller than the normal X-ray unit. A full evaluation of the micro-focus machine and its ability to detect FCM infestation was conducted at NECSA in the previous period, and these investigations were continued.

Figure 3.2.2.3. The micro-focus X-ray unit at NECSA.

Evaluating targets and filters

Micro-focus X-ray systems operate on the basis of filament emitting electrons. These electrons are aimed at a spot on a target, which then reflects the electrons through the fruit and onto a detector where the image is formed. The target influences the quality of the image, so trials were conducted to re-evaluate various targets, to see which would produce the clearest image. Images were captured using copper, silver and tungsten targets. These images were then visually assessed to determine which target delivered the clearest images. The process was repeated using various filters to see which could improve the quality of images.

Determining the optimum voltage

Further trials were conducted to determine the optimum voltage for the X-rays. This was done by creating images using a range of voltages.

Determining the optimum number of projections for micro-focus tomography

Tomograms were created using various numbers of projections to determine the optimum number of projections required to create the best images.

<u>X-ray trial 10</u>

Several Midknight Valencia oranges were obtained from an orchard in the Sundays River Valley, on 13 October 2012. These were divided into three groups. The fruit from one group was inoculated immediately with 6 to 8 neonate FCM larvae, using a grade 000 paint brush. The second group was inoculated in a similar way on 16 October, and the third on 19 October 2012. At NECSA, 6 fruit from group were inspected under a 2X illuminated magnifying lens to look for penetration marks. They were X-rayed with the micro-focus X-ray machine, using both radiography and tomography, to see what was visible. The fruit were then dissected and inspected for signs of FCM infestation.

<u>X-ray trial 11</u>

Six Delta Valencia oranges were infested with 6-8 neonate FCM larvae at NECSA. These were then X--rayed with the micro-focus X-ray machine, using both radiography and tomography, one and two days after infestation, to see what was visible. The fruit were then dissected and inspected for signs of FCM infestation.

<u>X-ray trial 12 - Image Analysis and development of an algorithm to automatically detect FCM infestation</u>

Six Delta Valencia oranges were X-rayed prior to infestation. These fruit were then infested with 4 neonate larvae, and X-rayed again at 1, 2 and 3 days after infestation, positioned in the same way as before. This was done in an attempt to detect differences in Gy values between healthy and infested fruit, using the healthy fruit

as a base/template. The Gy values of various transects of the fruit were registered, in an attempt to develop an algorithm which could automatically detect FCM infestation from X-ray images.

X-ray trial 13 – creating faster tomography scans

At the beginning of this study, tomography scans took 35 minutes to complete. In later studies this was reduced to 16 minutes using the micro-focus X-ray unit. The parameters for these scans were as follows: Voltage 100 kV, current 100 mA, binning of 1, and 500 projections, with an exposure time of 2000 milliseconds (ms).

Reduction of Exposure time

The exposure time is the time needed to acquire one projection (all the images that were taken in 360 degrees). Overall time of a scan can be reduced by reducing the time of exposure, i.e. the time for each individual image. Exposure times tested were 2000 ms, 1000 ms, 500 ms, 250 ms, 125 ms, and 67 ms.

Accumulation

The accumulation is the amount of images that were taken to form one projection. This is a method whereby detector noise is minimized. For all previous scans, the accumulation rate was set at 1. Scans were taken with an exposure rate of 67 ms, with accumulation rates of 4, 16, 64 and 256, in an attempt to minimise the scan length.

Binning

The detector has 2048 pixels (horizontal and vertical) and each one has one signal readout, so 2048 x 2048 different signal readouts per projection. By using a binning of 2, 4 pixels are grouped together to form one signal. Binning, or the readout rate, can therefore affect the speed of scans. Previously a binning of 1 had been used. If the binning is changed to 2, then the time is reduced fourfold. The increase in the speed of the scan can be detrimental to the quality. A range of exposure times were tested with a binning of 2.

Number of projections

The projections are the amount of images that are taken in a 360 degree rotation. The more projections, the smaller the angular increment between successive projections and the better quality of the scan. Previously scans were done using 500 projections. Quality of images was assessed for 50, 150, 200 and 250 projections per scan, using the original exposure time of 2000 ms.

Comparison of images

Signal to Noise Ratio (SNR) and Contrast Ratio (CR) and Michelson's Contrast Ratio are objective methods to compare the quality of images (Griot 2007).

SNR = mean pixel value/standard deviation
CR = highest pixel value/lowest pixel value
Michelson CR = highest pixel value – lowest pixel value/ pixel value + lowest pixel value.

In order to make these comparisons, the images must first me normalised, using the following formula"

Normalisation = Image/flat field x C, where C is a factor calculated by Image J

Final choice of settings

All the different parameters were reviewed, so as to select a final combination to use for scans.

X-ray trial 14 - Evaluation of new scan settings on Satsuma Mandarins

Satsuma mandarins, harvested from an orchard in the Sundays River Valley, were infested in the laboratory 8, 12, 14 and 16 days prior to X-ray (6 fruit for each). Radiography and tomography scans were done on fruit infested

X-ray trial 15 - Evaluation of new scan settings on Delta Valencia oranges

Approximately 100 Delta Valencia oranges were infested with neonate FCM larvae at intervals of 11, 6 and 4 days prior to scanning. These fruit were then evaluated using the fast micro-focus X-ray scans.

Results and discussion

Degreening and lag time after picking

It appeared that degreening did not make FCM penetration marks more visible, but lag time before packing did (Table 3.2.2.4). Once fruit had been degreened, it was more difficult to detect the penetration marks, unless the fruit had begun to decay. On the fruit that was not degreened, the penetration marks were surrounded by an orange patch, which was visible against the slightly green background of the fruit. However, it is possible that the fruit would have been too green to pack unless degreened.

Table 3.2.2.4. Detection of FCM infestation in Navel oranges, inspected at different intervals after inoculation with neonate FCM larvae, in an attempt to simulate a packhouse sorting process

Treatment		**FCM penetrations detected correctly (%)**	**FCM not detected (%)**
1	Inspect 2 days after infestation	65.0	55.0
2	Inspect 5 days after infestation (simulate 3 days standing before packing)	87.9	20.0
3	Inspect 8 days after infestation (simulate 6 days standing before packing)	80.5	19.5
4	Inspect 5 days after infestation – degreening* (simulate 5 days standing & degreening before packing)	64.7	31.6
5	Inspect 8 days after infestation – degreening (simulate 6 days standing & degreening before packing)	65.4	29.6

Near Infra Red (NIR)

NIR trial 1
The validation showed no correlation between infested and uninfected fruit.

NIR trial 2
There was some agreement between the validation by the unit and infestation as evaluated by dissection of the fruit. In the second validation, where the penetration point was faced at the sensor, the unit managed to classify 30 out of 34 fruit correctly, but the reading did not correspond to the level of FCM damage.

NIR trial 3

Twenty fruit were rated 0 (Table 3.2.2.5). Twelve were correctly rated according to the model (60% accurate). Ninety-six fruit were rated 1. Forty were correctly rated according to the model (42% accurate). Forty-one fruit were rated 2. Nineteen were correctly rated according to the model (46% accurate).

Table 3.2.2.5. Number of fruit and percentage accuracy of grading in a trial to determine the accuracy of an NIR model in detecting FCM infested fruit

Grading	**Number of fruit**	**Number of fruit correctly graded**	**% fruit correctly graded**
0	20	12	60
1	96	40	42
2	41	19	41

NIR trial 4

The results were disappointing, as accuracy within groups ranged from 36% to 58% (Table 3.2.2.6). However, when any predictive value higher than 30 was taken as infested, and we did not attempt to quantify the amount of damage in each group, the accuracy ranged between 74% and 88%. Unfortunately, there was also a high percentage of false positives.

Table 3.2.2.6. Number of fruit and percentage accuracy of grading in a trial to determine the accuracy of a modified NIR model in detecting FCM infested fruit

Model rating	Destructive rating	Predictive value	Number of fruit	Number of fruit correctly graded	% fruit correctly graded	% fruit correctly graded as infested (predictive value > 30)
0	0	< 30	9	5	56	
1A	1 – 3	31 – 50	31	12	39	74
1	3 – 6	51 – 70	62	36	58	77
1B	6 – 8	71 - 90	32	13	41	88
2	9 – 10	> 90	8	4	50	75

Ultra Violet light

It was determined that FCM penetration marks were not more visible under UV light than under conventional light. The UV light was only beneficial once the fruit had decayed to the point that *Penicillium* fungus was growing on it. The fungus flouresced under UV light (Fig 3.2.2.4.). This would not be much use for detecting FCM infestation, as the fruit does not decay quickly. However, UV light could highlight fruit fly infestation, as fruit decays quickly when infested by this pest. Further trials will be conducted with UV light.

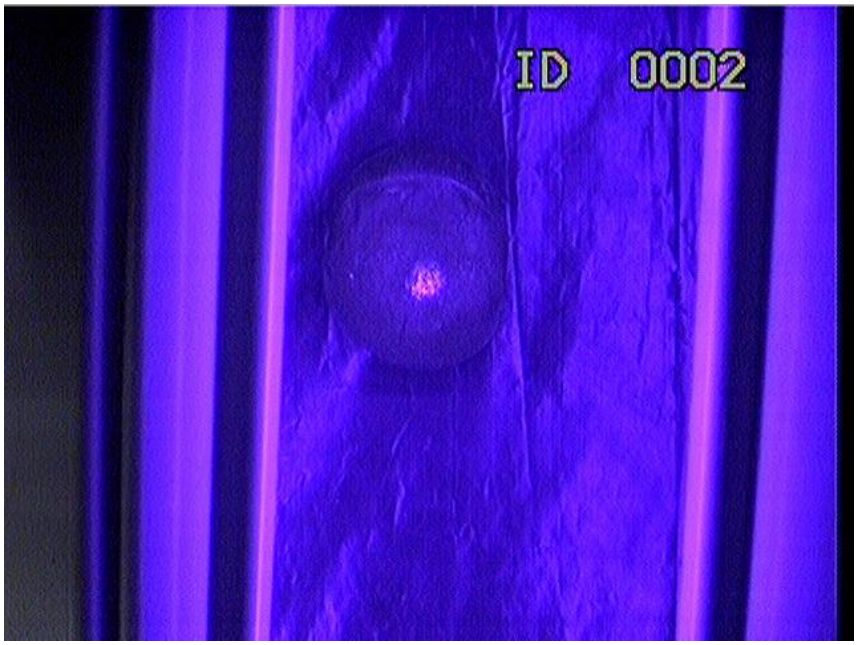

Figure 3.2.2.4. An FCM infested Navel orange viewed under UV light, where *Penicillium* fungus has established on the decaying fruit.

X-Ray

X-ray trial 1 - X-ray bioassays

Although the results were variable and unclear (Table 3.2.2.7. and 3.2.2.8), it was decided to use treatment 4 concentrations for both products to feed to larvae and inoculate them onto fruit and artificial diet in preparation for radiography and tomography scans. Larval mortality showed that the products did have some effect on the larvae, so it was decided to inoculate a large number (10) of larvae onto each fruit to ensure some penetration and survival.

Table 3.2.2.7. Survival of larvae fed droplets of several concentrations of iodine solutions prepared in a five-fold dilution series

Treatment	Concentration iodine (%)	No of larvae survived (n = 25)
1	Distilled water	6
2	0.00008	9
3	0.0004	13
4	0.02	8
5	0.10	5
6	0.05	No ingestion by any larvae

Table 3.2.2.8. Survival of larvae fed droplets of several concentrations of barium sulphate suspensions prepared in a five-fold dilution series

Treatment	Concentration barium sulphate (% w/v)	No of larvae survived (n = 20)
1	Distilled water	15
2	0.02	14
3	0.10	15
4	0.48	13
5	2.40	12
6	12.00	8

X-ray trial 2

Radiography: Previous trials had shown that FCM larvae were not visible on X-rays, but lower densities, or loss of matter in the fruit due to the feeding of FCM larvae could be detected. Radiographs of fruit inoculated 5 days prior to X-ray showed very little, and doubtful signs of infestation. Radiography on a fruit inoculated on 8 and 14 days after inoculation clearly showed some tunnelling and loss of material where the larva had fed. It was becoming increasingly apparent that radiography does not show penetration through the albedo, but only loss of matter in the flesh.

Radiography of a fruit infested with fruit fly eggs seven days prior to X-ray showed very little or nothing at the stem end. When enlarged, it looked like there might possibly be some loss of matter, but this was not clear enough to confidently diagnose it as with FCM damage.

Tomography: Tomography of a fruit inoculated 5 days prior showed a penetration mark, a tunnel through the albedo and some small signs of disturbance in the flesh. When dissected, penetration and tunnelling through albedo was found, with very little disturbance in the flesh, but no larva was found. Tomography of a fruit inoculated on 8 days prior showed four obvious entry points, with tunnelling and feeding damage. Dissection confirmed four second instar FCM larvae in the fruit. Tomography of a fruit inoculated 14 days prior showed an obvious hole in the rind and albedo. Penetration was not very deep, and the larva appeared to leave the fruit before entering the flesh. This was confirmed by dissecting the fruit.

Tomography of a fruit infested with fruit fly eggs on 15 October 2011 (7 days prior to X-ray), showed very clear damage and loss of material (Fig 3.2.2.5.).

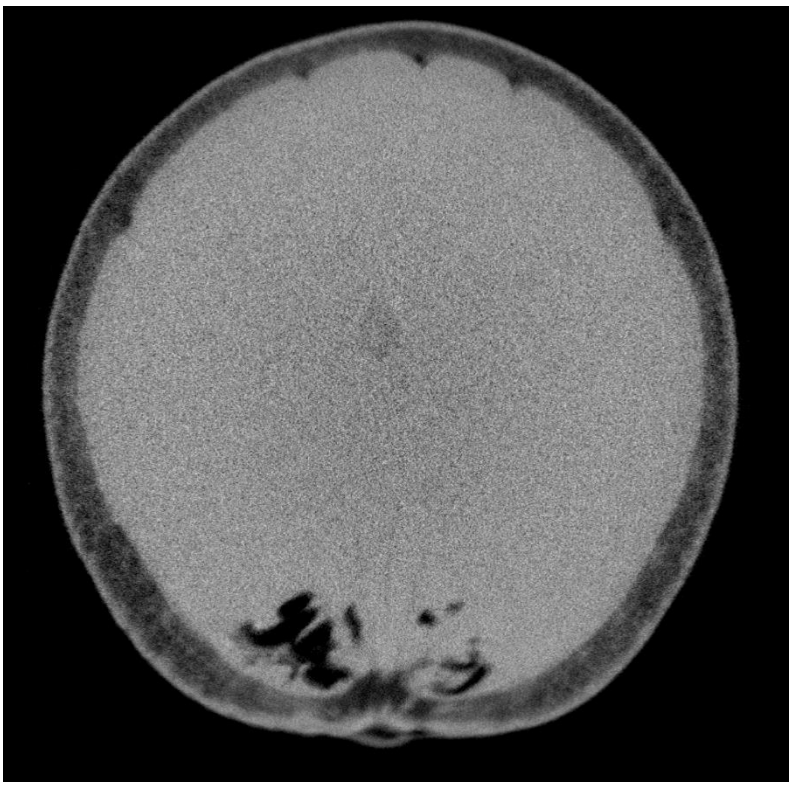

Figure 3.2.2.5. Tomogram of a Navel orange infested with fruit fly eggs 7 days earlier.

Where the Eppendorf vials were X-rayed (radiography), there was no visible difference between the samples which we had fed to larvae. Only when Barium sulphate settled to the bottom of the vial, was it visible (white). Surprisingly, even pure water looked exactly the same as the other solutions/suspensions.

Where 10 neonate FCM larvae were places onto a glass surface, nothing was visible on the radiogram at all.

X-ray trial 3

Penetration marks were more visible and easier to detect on the fruit which was infested on 15 days prior to X-ray (Table 3.2.2.9). With radiography 75% of penetration damage could be detected and 100% with tomography. The results were expressed as the percentage of visible FCM penetrations (sometimes more than 1 per fruit) that could be detected by the X-ray techniques. With newly infested fruit (infested 4 days prior), tomography showed 87% more FCM presence than could be seen under a magnifying lens. When fruit were dissected, it was determined that no penetrations escaped detection by tomography, while several escapeddetection with radiography. Unfortunately, tomography is a slow process, with each scan taking 25 minutes to complete.

Table 3.2.2.9. Signs of FCM infestation on Navel oranges (six fruit per treatment), detected with a magnifying lens, radiography and tomography

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography
15	16	12 (75%)*	16 (100%)
8	7	3 (42.9%)	7 (100%)
4	8	5 (62.5%)	15 (187.5%)

* Percentage taken as damage detected vs visual penetration marks - under illuminated magnifying lens.

X-ray trial 4

Penetration marks were not as easily detected on Satsuma Mandarins as on Navel oranges. Once again tomography was able to identify all damage associated with FCM infestation (Table 3.2.2.10). There were also a few other defects which were visible on tomograms, which were mistaken for FCM damage. This

resulted in false positives, especially in the fruit which contained the youngest larvae, which had very low levels of damage.

Signs of FCM penetration were detected with tomography one day after infestation, but not with radiography. A day later, the there was no increase in the amount of infestations detected with tomography (Table 3.2.2.11), but the signs were clearer and easier to detect, as the damaged areas due to feeding were larger.

Table 3.2.2.10. Signs of FCM infestation on Satsuma Mandarins, detected with a magnifying lens, radiography and tomography

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography	FCM detected in dissection
10	12	12	18	18
7	6	6	13	13
5	7	4	15	12

*Percentage taken as damage found vs visual penetration marks - under illuminated magnifying glass.

Table 3.2.2.11. Signs of FCM infestation on Satsuma Mandarins detected with a magnifying lens and X-Ray radiography and tomography at various intervals after infestation

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography
1	17	0	29
2	16	0	29

X-ray trial 5

Images from the micro-focus radiography unit were much clearer than the standard X-ray images. Smaller areas of damage could be detected, but unfortunately where FCM larvae were in the albedo, penetration could not be detected.

X-Ray trial 6

a) Evaluating targets and filters

Each target has a different histogram peak, which would make a certain target more suitable than others for a separate substrate. X-rays were conducted at 130 KV, and it was determined that silver was the most appropriate target for X-raying citrus fruit. The silver target resulted in the clearest images, and the histogram peaks were more defined than with the other targets. The histogram also had a long dynamic range, which resulted in clearer images.

The copper filter resulted in clearer images than any of the other filters tested.

b) Determining the optimum voltage

Various voltages were tested, and it was determined that at 150 KV, maximum Gy scale values of approximately 55 000 Gy were recorded. The maximum gray scale value which the NECSA detector can read is 65 000 Gy. If the Gy scale value exceeds this maximum, the detector will be permanently damaged. It was therefore decided to use the 150 KV voltage, as it delivered Gy scale values 10 000 lower than the maximum. This would produce high quality images, and allow for some pixel malfunction without damaging the detector.

c) Determining the optimum number of projections for micro-focus tomography

The machine was set at 0.5 frames per second (2 second exposure), voltage at 150 KV and 100 mA. When set at 1000 projections per scan, the images were of extremely high quality, and took 34 minutes to complete. One scan was done at 4000 projections, which took about 2 hours to complete, and the image quality was not substantially better. When 500 projections were used, image quality was only fractionally worse than with 1000

projections, and the scan took 16.5 minutes to complete. It was decided to use 500 projections per scan for the trials.

X-ray trial 7

Micro-focus tomography was once again highly successful as a means to detect damage caused by FCM, correctly identifying 100% of infestations (Table 3.2.2.12). This technique did however, deliver a few false positives, as seen in the higher number of infestations recorded for tomography than actual FCM larvae found in the destructive dissection. This was probably due to the fact that the images are so clear that small natural vacuoles/defects were interpreted as FCM damage. Micro-focus radiography was less effective, detecting 71% of infestations after 13 days, 70% after 10 days, 50% after 3 days and 33% 1 day after infestation. Infestation was not detectable while the larvae were in the rind or albedo.

Table 3.2.2.12. Signs of FCM infestation on Satsuma Mandarins, detected with a magnifying lens, micro-focus radiography and tomography

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography	FCM detected in dissection
13	12	10	16	14
10	16	12	21	17
3	11	8	23	16
1	11	3	10	10

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X-ray trial 8 - Image Analysis and development of an algorithm to automatically detect FCM infestation

Image analysis was done in collaboration with NECSA. After the tomograms were assessed for quality, 2D slice images from the tomogram were analysed for infestation defection using ImageJ software. The differences between infested and clean fruits were visualized in ImageJ with a naked eye. Seen from the axial position of the orange, the clean oranges don't have extra big pores close to the edge, apart from the centre pore, while the slice images of the infested oranges have extra pores close to the edge (Fig 3.2.2.6).

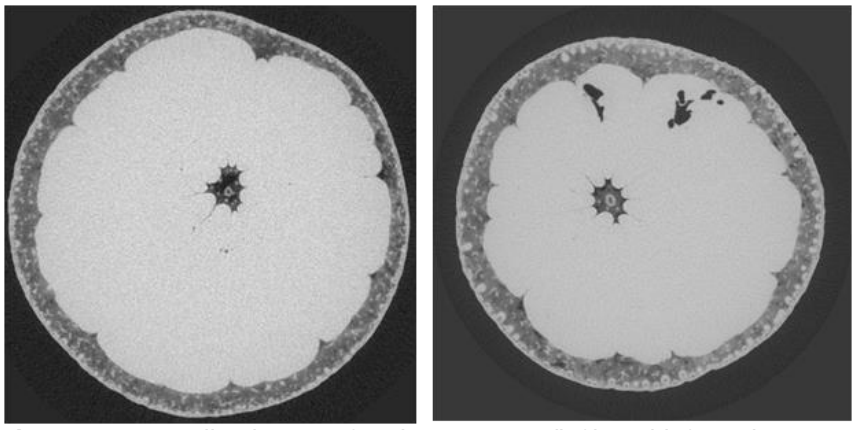

Figure 3.2.2.6. Slice image of a clean orange (left) and infested orange (right), the infestation is revealed by µCT as dark spot close to the edge within inner flesh of the fruit.

Results showed that different parts of the orange were represented by different Gy values in the tomogram (Table 3.2.2.13). The Gy values of natural pores and infested regions were close to each other, and as shown in Table 3.2.2.13, the similarities of natural pores and infested region pose a big challenge to detect the infestation on the basis of their Gy values in the image alone.

Table 3.2.2.13. Gy values of different sections of the orange fruit. The material (rind and segments) were different from the porous region (natural orange pores and infested region)

Section of orange	Gy values			
	Minimum	**Maximum**	**Mean**	**Standard deviation**
Rind	17721	27542	23163	2221
Segment (edible fresh)	26888	30343	28988	412
Natural pores	13641	23485	15824	1274
Infested region	15482	17045	16276	395

On each cross-sectional image, different parts of fruit are represented by different Gy values except for the natural pores (normally situated at the core of the image and between segments) and the infested region (Fig 3.2.2.7). But it was noted that the infestation regions in the cross section images were located close to the rind of the orange; while the natural pores/vacuoles were located at the centre of the orange. The localization of the infested regions made it possible to write detection algorithms based not only on the Gy values that represent different parts of the orange but also on the location of the pores inside the orange. The detection algorithm is therefore based on the location of infested regions with respect to the natural pores within the oranges.

The detection algorithm will be applied on each slice image. The orange fruit will be virtually cut into radial cross sections and each section image will be tested for infestation. If the infestation is found in any of the cross section images of the sample, then the sample will be declared as infested.

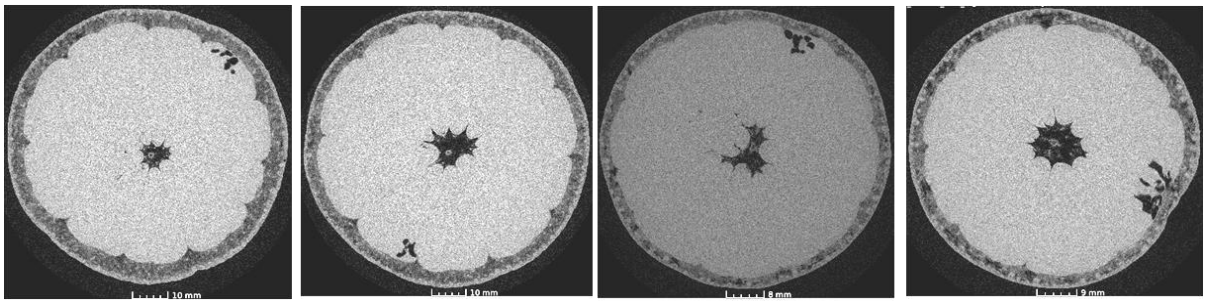

Figure 3.2.2.7. Cross section images from different orange samples. The infested area (dark spot at the edge of the segments area) is located close to the rind (skin of the orange. The detection algorithm will used for the location of the pores to separate an infested orange from a non-infested one.

Below is the pseudo code, in standard format, of the algorithm which could be used to separate the infested oranges from the clean ones. The algorithm will be applied on each reconstructed cross-section image of the fruit.

While *scanning orange* ***do***
    Reconstruct cross section image
    Identify pores
    Classify pores as natural or infested regions using their locations as criteria
      If *pores are natural*
      Mark cross section as clean
      Else if *pores are infested region*
      Mark cross section as infested
End of while
  If *infested cross section image is found*
    Send orange left
  Else
    Send orange right

The algorithm above works in correlation with the set up in Figure 3.2.2.7, where the position of the orange (base situated on top or bottom) makes it possible for the natural pores to be at the centre of each axial cross section image. In the current form, the algorithm won't be able to detect the infestation inside the apex or the base of the orange (since they will be marked as natural vacuoles).

X-ray trial 9 - preparation and calibration of the micro-focus system

The greater resolution of micro-focus images makes it possible to detect lesser levels of damage, caused by smaller larvae, than the normal X-Ray unit. Preliminary studies showed that images were clearer, but it was still not possible to detect FCM infestation while the larva was in the albedo of the fruit.

Evaluating targets and filters

Targets

As the NECSA staff became more experienced using the micro-focus X-ray unit, new opinions on a suitable target were voiced. Each target has a different histogram peak, which would make a certain target more suitable than others for a separate substrate. It was previously determined that silver was the most appropriate target for X-raying citrus fruit. However, tungsten has subsequently been shown to be the best target for most materials, so it was compared to silver. Both tungsten and silver targets delivered transmission penetration of 23 percent, and the image qualities were similar. The histogram peaks were more clearly defined for both targets. The histogram for both had a long dynamic range, which results in clearer images. It was decided to use tungsten as the target, instead of silver, as tungsten was generally accepted as the best target for most materials, and it would not pit/erode as easily as a silver target. This would result in safer usage and more consistent images.

Filters

The aim of the filter is to remove the lowest energy photons which do not reach the target (referred to as noise), and to concentrate the higher energy photons evenly. It was previously determined that a copper filter resulted in clearer images than any of the other filters tested. However, the latest evaluation showed aluminium to be the most effective filter. It is best practice to use a filter with a density as close to the material being X-rayed (citrus fruit). Aluminium was the least dense of the filters tested, making it more suitable, as oranges have a relatively low density.

Determining the optimum voltage

Previously it was determined that at 150 KV, maximum gray scale values of approximately 55 000 Gy were recorded. It was believed that this would produce high quality images, and allow for some pixel malfunction without damaging the detector. However, recent studies had shown that the lower the energy, the greater the contrast in the images. It was determined that settings of 70 KV and 40 W delivered the clearest images with the most contrast

Determining the optimum number of projections for micro-focus tomography

In the previous study, the machine was set at 0.5 frames per second (2 second exposure). A 4 second exposure time was tested, and this resulted in clearer images. It was decided to use 500 projections per scan (i.e. per fruit) for the trials, with a 4 second exposure time. This resulted in scans taking 33 minutes to complete the scan of a fruit.

X-ray trial 10

One hundred percent of larval infestation was detected using micro-focus tomography 10, 7 and 4 days after infestation (Table 3.2.2.13). With radiography, 60%, 77% and 44% of infestations were detected 10, 7 and 4 days after infestation respectively. It is clear that micro-focus tomography is a very accurate technique to detect FCM infestation in fruit. However, the process took between 15 and 33 minutes per fruit.

Table 3.2.2.13. Signs of FCM infestation on Midknight Valencia oranges, detected with a 2X magnifying lens, micro-focus radiography and tomography

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography	FCM detected in dissection
10	10	6	10	10
7	16	10	14	13
4	13	4	9	9

X-ray trial 11

Micro-focus radiography was not able to detect signs of FCM damage one or two days after infestation (Table 3.2.2.14). Where tomography was used, 40% and 83% of FCM infestation was detected one and two days after infestation respectively. In previous studies conducted on Satsuma Mandarins, radiography detected 30% and tomography for 100% of FCM infestation after one day. The greater success in Satsumas is because the larvae penetrated through the soft, thin rind and albedo faster than with oranges, and after one day there were already signs of damage in the flesh of the fruit. The major shortcoming of radiography appears to be that FCM damage is not detectable in the rind and albedo, but only once the flesh is damaged.

Table 3.2.2.14. Signs of FCM infestation on Delta Valencia oranges, detected with a magnifying lens, micro-focus radiography and tomography

Days after infestation	Visible penetration marks	FCM damage visible with radiography	FCM damage visible with tomography	FCM detected in dissection
2	13	0	10	12
1	9	0	4	10

X-ray trial 12 - Image Analysis and development of an algorithm to automatically detect FCM infestation

Unfortunately, the oranges could never be positioned in exactly the same positions before and after infestation, so the data/images could not be used for algorithm development.

X-ray trial 13 – creating faster tomography scans

Reduction of Exposure time
The images taken with exposure rates of 67 ms and 125 ms were not clear enough. Five hundred ms provided a useable image.

Accumulation
Increasing the accumulation rate with scans using a 67 ms exposure time did not improve the quality of scans. Therefore, it was decided to continue with an accumulation rate of 1.

Binning
The quality of the images taken with a binning of 2, and exposure rates of 67 ms, 125 ms, and 250 ms ware not satisfactory. At 500 ms the quality was better, and satisfactory at 1000 and 2000 ms.

Number of projections
The quality of scans taken with 50 and 100 projections was not satisfactory. One hundred and twenty-five projections delivered a useable image. The quality improved with 150, 200 and 250 projections per scan, but this increased the time (Table 3.2.2.15).

Table 3.2.2.15. Time of scans for different number of projections, using an exposure time of 2000 ms.

Number of projections	Time per scan (minutes)
50	1
150	5
200	6
250	7.56

Comparison of images

The Contrast Ratios for various exposure times were calculated (Fig. 3.2.2.8).

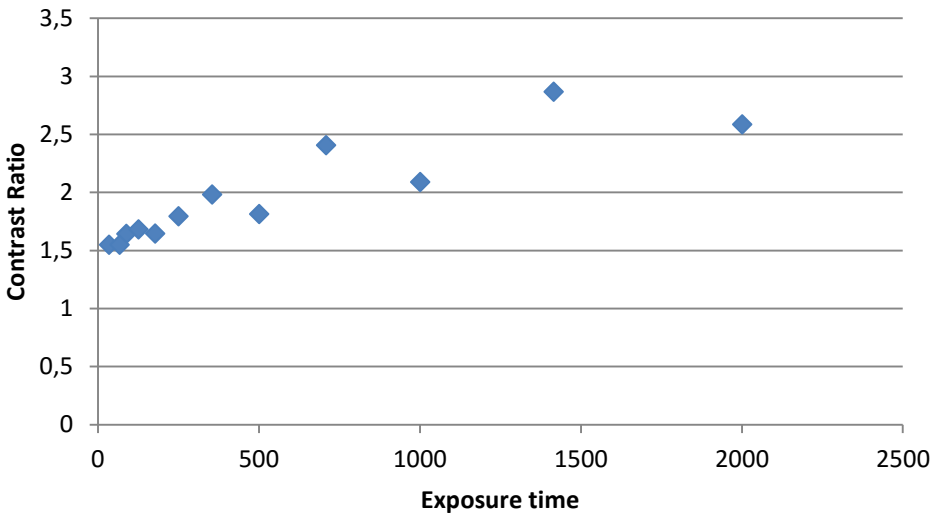

Fig 3.2.2.8. Contrast Ratios for various exposure times of corrected image values

An exposure time of 708 ms, with a binning of 2 was chosen, as the quality was satisfactory, and the scan was faster than at 1412 ms and 2000 ms or 2500.

Final choice of settings
After considering all the variables, it was decided to use an exposure time of 708 ms, binning of 2, and 125 projections. This scan took 1 minute and 26 seconds to complete.

X-ray trial 14 - Evaluation of new scan settings on Satsuma mandarins

Micro-focus X-ray tomography, using the new settings, resulting in a scan lasting 1 minute and 26 seconds, was able to detect 100% of FCM infestations on Satsuma mandarins, 8, 12, 14 and 16 days after infestation (Table 3.2.2.16).

Table 3.2.2.16. Evaluation of FCM infestation of Satsuma Mandarins X-rayed at various intervals after infestation

Days after infestation	Tomography	Radiography	Destructive	Description of larval instars (L) from destructive evaluation
8	9	6	9	1 x L3, 8 x l2
12	9	7	9	2 x L4, 4 x L3, 3 x L2
14	12	9	12	2 Exited, 2 x L5, 5 x L4, 3 x L3
16	13	7	13	1 Exited, 6 x L5, 3 x L4, 1 x L3

X-ray trial 15 - evaluation of new scan settings on Delta Valencia oranges

The X-ray study was completed by evaluating the success of new faster scans on Delta Valencia oranges, which proved successful. All infestations 5 days and older could be detected. All second instar (L2) and larger larvae were detected (damage). Larvae which had entered the flesh could be detected. Damage in the rind and albedo (blind penetrations) cannot be detected. At 4 days after infestation 86% of the infestations (L1) could be visually detected (Table 3.2.2.17).

Table 3.2.2.17. Evaluation of FCM infestation of Delta Valencias X-rayed at various intervals after infestation

Days after infestation	Tomography	Destructive	Description of larval instars (L) from destructive evaluation
4	32	37	All L1
6	57	57	37 x L1, 20 x L2
11	43	43	2 x L1, 18 x L2, 19 x L3, 4 x L4

Conclusion

Degreening did not improve the detectability of FCM penetration marks, but lag time between picking and packing did. These trials should be repeated, and it must be determined if the fruit is too green to be packed without degreening. X-ray tomography was very successful in detecting FCM damage inside fruit, but radiography was not. Unfortunately, the tomography process is very slow. Tastetech's NIR system does not appear to be accurate enough to predict FCM infestation to a sufficiently high degree. However, more data would need to be acquired to augment the database, which would make it more accurate. Ultraviolet light did not highlight FCM penetration marks, unless *Penicillium* fungus had established on the fruit. Micro-focus X-ray radiography was superior to normal X-ray radiography, but unfortunately, on oranges, evidence of FCM infestation was not detectable while the larva is in the rind or albedo of the fruit. This indicated that infestation would not be detected within the few days after penetration. In Satsumas, the larvae penetrate the rind faster, and so detection is possible one day after penetration. Micro-focus Tomography (µCT) is faster than normal tomography, and the quality of images is superior. More false-positives were experienced with µCT, as the superior images show up more areas of differing density, which could be mistaken for infestation damage. The micro-focus unit was optimally set up to produce the best possible images to be used for detecting FCM infestation in fruit. Micro-focus tomography (µCT) can be faster than normal tomography, and the quality of images is superior. The time of a tomography scan was reduced from 35 minutes to 1 minute and 26 seconds. One hundred percent of FCM penetrations were detected on Satsuma Mandarins 8, 12, 14 and 16 days after infestation, using the new settings. This shows that the ability of the technology to detect infested fruit does not appear to be compromised. Further trials were conducted on Delta Valencia oranges, where some early infestations were not detectable.

Although tomography scans are not fast enough to be used online in a packhouse, they could be used for non-destructive evaluation of 300 fruit samples as suggested by the FCM Systems Approach (Moore et. al. 2016). It is possible that cheaper units, which do not have as good clarity of images as the micro-focus unit at NECSA, could be used with similar success to do non-destructive sampling.

Future research

No future research is planned.

Technology transfer

CRI Research Symposium 2010. Post-harvest detection of cryptic pests – an introduction: Wayne Kirkman and Sean Moore.
ESSA Conference 2011. Post-harvest detection of cryptic pests – an introduction: Wayne Kirkman and Sean Moore.
CRI Research Symposium 2010. Seeing the invisible: X-ray for post-harvest detection of FCM**:** Wayne Kirkman, Sean Moore, Frikkie de Beer, Kobus Hoffmann and Lunga Bam.

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MOORE, S, KIRKMAN, W & HATTINGH, V. (2016). Verification of Inspection Standards and Efficacy of a Systems Approach for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) for Export Citrus from South Africa. *Journal of Economic Entomology.* In print.

3.2.3 FINAL REPORT: Large scale field trials with entomopathogenic nematodes for control of FCM, fruit fly and thrips

Project 1042 (Sep 2011 – March 2016) by Sean Moore (CRI), Ralf-Udo Ehlers (e-nema), Aruna Manrakhan, Martin Gilbert, Wayne Kirkman and John-Henry Daneel (CRI)

Summary

As a result of promising fundamental work in the laboratory with entomopathogenic nematodes (EPNs) against FCM, a field study was initiated to test the efficacy of EPNs in controlling the soil-dwelling life stages of FCM in citrus orchards. Simultaneously, control of fruit flies and thrips was recorded in a few of the trials. A total of 16 trials were conducted in three provinces over a four year period. EPNs were applied underneath tree canopies, either with a spray machine or through the microsprinkler system. A single spring application of *Heterorhabditis bacteriophora* infective juveniles (IJ) at 10 or 20 IJs/cm^2 succeeded in reducing FCM infestation of fruit by up to 81%. A single winter application of *Steinernema feltiae* at 20 IJs/cm^2 also reduced FCM infestation by 81%. EPNs survived and persisted in the soil for up to five months. However, this appeared to be dependent on soil moisture and good results were therefore not achieved under drip irrigation. Where monthly applications at 10 IJs/cm^2 were compared to a single spring application, the repeated applications fared better, reducing FCM infestation by 72% compared to 28%. Late season corrective treatments against moderately high levels of FCM did not appear to be very effective. Although high levels of natural occurrence of EPNs in orchards appeared rare, where *H. zealandica* was present at high levels, it was shown to reduce FCM infestation of fruit by 59% compared to a treatment where it was excluded from the soil by applying a nematicide. No thrips control was recorded where EPNs were applied, but a dramatic reduction in fruit fly trap catches was recorded for all EPN treatments in at least one trial. As a result of these promising results, particularly against FCM, EPNs have subsequently been registered for use in citrus.

Opsomming

As gevolg van belowende fundamentele navorsing met entomopatogeniese nematodes (EPN) teen VKM in die laboratorium, is ŉ veldstudie begin om die werking van EPN vir die beheer van grondgedraagde lewensstadiums van VKM in sitrusboorde te toets. Beheer van vrugtevlië en blaaspootjie is gelyktydig in ŉ paar van die proewe ook getoets. ŉ Totaal van 16 proewe is in drie provinsies oor vier jaar uitgevoer. EPN is onder boom kappies toegedien met of ŉ spuitmasjien of deur die mikrobesproeiing stelsel. ŉ Enkele lente bespuiting van *Heterorhabditis bacteriophora* infektiewe onvolgroeides (IJ) teen 10 or 20 IJs/cm^2 het VKM besmetting van vrugte met tot 81% verminder. ŉ Enkele winter toedieining van *Steinernema feltiae* teen 20 IJs/cm^2 het ook VKM besmetting met 81% verminder. EPN het vir tot vyf maande in die grond oorleef en aktief gebly maar die blyk hierdie is afhangend van grond vogtigheid en dus is goeie resultate nie onder dripbesproeing gekry nie. Waar maandelikse toedienings teen 10 IJs/cm^2 met ŉ enkele lente bespuiting vergelyk is, het die herhaalde toedienings beter gevaar en het VKM besmetting met 72% verminder in vergelyking met 28%. Laat seisoen korrektiewe behandelings teen redelike hoë vlakke van VKM was nie baie doeltreffend nie. Al was die natuurlike voorkoms van hoë vlakke van EPN in boorde redelik vreemd, waar *H. zealandica* wel teen ŉ hoë vlak teenwoordig was, het dit VKM besmetting van vrugte met 59% verminder. Dit was in vergelyking met ŉ aalwurmdoder behandeling op die grond wat die EPN uitgeluit het. Geen blaastpootjie beheer is aangeteken waar EPN toegedien is, maar ŉ dramatiese vermindering in vrugtevlieg vangste is vir alle EPN behandelings in minstens een van die proewe gekry. As geveolg van hierdie belowende resultate, veral teen VKM, is minstens een EPN produk vir gebruik op sitrus daarna geregistreer.

Introduction

Eight years of research was conducted by Stellenbosch University on identifying and testing entomopathogenic nematodes (EPNs) for control of FCM on citrus (Malan & Moore, 2005, 2006, 2008, 2009, 2010, 2011, 2012; Malan et al, 2011). This work demonstrated a high level of sensitivity of FCM larvae and pupae to EPNs and hence tremendous potential for control of FCM. Targeting of the subterranean life stages of FCM (prepupae and pupae) is something which has never been done within an FCM control programme. This was therefore considered to be a potentially valuable additional tool in the armoury of products used against FCM, particularly at a time of the year when nothing else is or can be done to suppress this pest.

As this study proposed to test the efficacy of EPNs against FCM in fairly large blocks, it made sense to simultaneously examine any possible suppression of other pests which also undergo subterranean life stages, such as fruit flies and citrus thrips. In support of this, laboratory trials showed that EPNs exhibited good potential against both Mediterranean fruit fly and Natal fruit fly, albeit at higher concentrations than those shown to be effective against FCM (Manrakhan et al., 2012).

Objectives

To determine the efficacy of soil-applied EPNs in suppressing FCM and fruit fly in citrus orchards in the Eastern Cape, Western Cape and Mpumalanga.

Materials and methods

General methodology

Source of EPNs

The EPN species used in most cases was *Heterorhabditis bacteriophora*. In some trials a *Steinernema feltiae* treatment was also used. Both species were obtained as semi-desiccated commercial formulations of infective juveniles (IJs) from e-nema, Germany.

Quality control of EPNs

Before application of EPNs, quality control tests were conducted. This entailed enumeration of the EPNs using a light microscope and dose-response bioassays against mealworm larvae. Only EPNs which passed the predetermined quality standards were used for the trials.

Trial sites

Trials were conducted in the Eastern Cape, Western Cape and Mpumalanga. Orchards with a history of a conspicuous level of FCM infestation were selected. Additionally, it was ascertained that there was no recent history of nematicide usage.

Application of EPNs

EPNs were always applied in the late afternoon or evening to avoid rapid desiccation of the soil and the detrimental effect of exposure of EPNs to direct UV-irradiation. Before application of EPNs, treatment blocks were irrigated to ensure EPNs were applied to a moist soil. After application, orchards were subjected to a full irrigation cycle. EPNs were either sprayed onto the soil using a pesticide spray machine, directing the bottom three nozzles on both left and right horizontal booms underneath tree canopies; or through the microsprinkler irrigation system.

Monitoring of EPNs

Before application of EPNs, monitoring was conducted to determine whether there was any natural occurrence of EPNs in the soil. During the first two years' trials, monitoring was conducted as follows. Small polytop vial-sized cages were made, consisting of a wire mesh structure (40 mesh/425 µm aperture size) and stoppered on each end by plastic lids. At least one of the lids was sealed using a glue gun. The other lid was either sealed in the same way or using tape. This was only done once the cage was filled. The cage was filled with sieved soil from the trial orchard and 20 5th instar FCM larvae. Six cages were planted per treatment – one cage under each of 6 trees, positioned in regular formation towards the middle of the treatment block. Cages were planted in either a vertical or horizontal position, close to the trunk of the tree under a very thin layer of soil. Cages were removed after one week and FCM larvae were counted, recorded as live or dead and dead larvae were dissected to inspect for infestation with EPNs. Immediately after application of EPNs, fresh cages were again planted. This was repeated at various intervals after application.

During the last two seasons' trials, the method of monitoring was changed to the following. Fifty soil samples were collected from each treatment block. Soil samples were collected from throughout each block and each sample was approximately 35 g. In the laboratory, each sample was placed into a petri dish, two 5th instar FCM larvae were placed onto the surface of the soil and petri dishes closed and sealed. After seven days, petri dishes were opened and FCM larvae were recorded as alive or dead. Dead larvae were dissected and inspected for EPN infection. This was conducted in each treatment block shortly after application of EPNs.

Evaluation of pest control

FCM control was evaluated in all trials. To monitor FCM populations, one FCM trap was hung in the middle of each treatment block and monitored weekly until termination of the trial. To monitor FCM control more directly, 10 data trees were marked in the middle of each treatment block. Each week on the same day, all

fruit which had dropped from the data trees were retrieved and assessed (dissected and inspected) for FCM infestation.

Citrus thrips were monitored by hanging six sticky yellow traps per each treatment block shortly after application of treatments. Traps were replaced each week. This was continued for several weeks. This was only conducted during the 2011/12 season.

Fruit fly traps were hung a few weeks before the projected harvest date and monitored weekly. Three Capilure-loaded Sensus traps and three Questlure-loaded Sensus traps were hung in the middle of each treatment block. This was only done at some trial sites during the first two years.

2011/12

Trial sites

Details of trial sites are given in Table 3.2.3.1. The second trial in the Eastern Cape (Sitrusoewer Farm) was conducted as no survival of EPNs was recorded at the first trial site (Boerboon Farm). A second trial was conducted at Crocodile Valley Estates in the same orchards as the first trial. This trial is explained under the sub-heading, *Impact of naturally occurring EPNs*.

Table 3.2.3.1. Details of EPN trial sites

Region	Farm	Coordinates	Cultivar	Soil type (description)[2]	Irrigation type
Sundays River Valley, E.Cape	Boerboon	33º27'50"S 25º34'95"E	Navels	Clay	Drip
	Sitrusoewer	33º25'71"S 25º21'86"E	Palmer Navels	Sandy-loam	Microjet
Clanwilliam, W.Cape	Danie Smit	32º21'26"S 18º55'82"E	Palmer navels	Sandy	Microjet
Nelspruit, Mpumalanga	Crocodile Valley	25º28'39"S 31º03'59"E	Washington navels[1]	Sandy	Microjet

[1]Interspersed with some Bahianinha navels, but only Washingtons were used for evaluations of FCM and fruit fly infestation.
[2]Exact clay percentage of soil will be measured and reported.

Application of EPNs

Heterorhabditis bacteriophora was used in all but one trial and was always applied in spring. At Sitrusoewer (Eastern Cape) *Steinernema feltiae* was also used and was applied in mid-summer. Details of application are provided in Table 3.2.3.2.

As the orchard soil at Boerboon (Eastern Cape) was very dry and irrigation was applied through drippers, thus wetting a very limited area underneath tree canopies, EPNs were applied in a water volume of 7000 L per ha using the spray machine.

Table 3.2.3.2. Details of application of EPNs at trial sites

Farm	Application date	Mode of application	Rate of application (IJs/cm^2)	Volume of water applied (L per ha)	Spray machine calibration
Boerboon	26 Sep 11	Spray machine	10 & 20	7000	Pressure 10 Bar, no nozzles, tractor gear 1st low
		Drip irrigation	10	150 L + 2 h pre-wetting & 4 h post-wetting	
Sitrusoewer*	23 Dec 11	Spray machine	20	870	Pressure 5 Bar, no nozzles, tractor gear 3rd low
Danie Smit	27-28 Sep 11	Spray machine	10 & 20	1450 L	Pressure unknown, no nozzles, tractor gear = 3rd low
		Microjet irrigation	20	200 L; orchard irrigated for 6 h	
Crocodile Valley	29 Sep 11	Spray machine	10 & 20	2000	Pressure 6 Bar, no nozzles, Tractor gear 3rd low

**Steinernema feltiae* and *Heterorhabditis bacteriophora* used at Sitrusoewer; only *H. bacteriophora* used at all other sites.

Evaluation of pest control

FCM and citrus thrips control were evaluated in trials in all three regions. Fruit fly control was only evaluated in the second trial in Mpumalanga.

Impact of naturally occurring EPNs

In the Crocodile Valley (Mpumalanga) trial orchards, the incidence of naturally occurring EPNs was so high that any possible effect of the applied EPNs was masked. It was consequently decided to examine the impact of these naturally occurring EPNs. Rugby (cadusafos) (100 g/L ME (EW)) was applied to one block of approximately 1 ha and an adjacent block of the same size was left untreated and used as a control. Rugby was applied to the soil underneath trees through a herbicide applicator at a rate of 20 ml (a.i.) per m^2. This equated to 560 ml per Washington navel tree (28 m^2 surface area underneath the canopy). Natural occurrence of EPNs and the impact of the cadusafos application on these EPNs was determined as described above under Monitoring of EPNs. This was done before application and 2, 4 and 8 weeks post-application. FCM monitoring was also initiated immediately after application, as described above.

2012/13

Trial sites

Details of trial sites are provided in Table 3.2.3.3.

Table 3.2.3.3. Details of EPN trial sites

Region	Farm	Coordinates	Cultivar	Treatment block sizes (ha)
Sundays River Valley, E.Cape	Huguenot	33º27'50"S 25º34'95"E	Palmer Navels	1.4 ha
	Ronnivale	33º25'71"S 25º21'86"E	Newhall Navels	1 ha
Citrusdal, W.Cape	Hexrivier	32º26'21"S 18º58'02"E	Robyn Navels	1.5 ha
	Klawervlei	32º21'26"S 18º55'82"E	Robyn Navels	0.84 (control) – 2.30
Nelspruit, Mpumalanga	Montana	25º26' 25.68"S 30º50'22.78"E	Turkey Valencia	9.4 (Including untreated block of 2.58 ha)

Application of EPNs

Full details of EPN application are provided in Table 3.2.3.4. EPNs were applied in autumn (May) or in spring (September/October) and in one case in winter (July).

Table 3.2.3.4. Details of application of EPNs at trial sites

Farm	Application date	EPN sp.*	Mode of application	Rate of application (IJs/cm^2)	Volume of water applied (L per ha)	Spray machine calibration
Huguenot	22 May 12	Hb	Microsprinklers	20		na
			Spray machine	20	1040	Bakkie gear 4L, 1000 rpm, pressure 20 bar, 2.3mm nozzles
Ronnievale	21 May 12		Spray machine	20	800	Bakkie gear 4L, 1000 rpm, pressure 20 bar, 2.3mm nozzles
	19 Sep 12		Spray machine	10 & 20	800	Bakkie gear 4L, 1000 rpm, pressure 20 bar, 2.3mm nozzles
Hexrivier	17 May 12	Hb	Microspinklers	10	200	na
			Spray machine	10	1000	Gear Low 2, 2.36 km/h, 5.5 bar, 15 revs, mixed DT4.0 & D5 nozzles
	20 Sep 12	Hb	Microspinklers	10	200	na
				20	200	na
Klawervlei	16 May 12	Hb	Spray machine	10	774	Gear Low 3, 4.28 km/h, no pressure gage, D4 nozzles
		Sf	Spray machine	10	774	Gear Low 3, 4.28 km/h, no pressure gage, D4 nozzles
	19 Sep 12	Hb	Spray machine	10	621	Gear Low 3, 4.28 km/h, no pressure gage, D4 nozzles

		Sf	Spray machine	10	609	Gear Low 3, 4.28 km/h, no pressure gage, D4 nozzles
Montana	27 July 12	Hb	Microspinklers (EPN applied using Dosatron at operating flow of 50 L/hour)	20	150	na
		Sf	Microspinklers (EPN applied using Dosatron at operating flow of 50 L/hour)	20	50	na
	12 October 2012	Hb	Microspinklers (EPN applied using Dosatron at operating flow of 50 L/hour)	20	100	na

*Hb=*Heterorhabditis bacteriophora*; Sf=*Steinernema feltiae*

Evaluation of pest control

FCM traps were hung from September or October and FCM infestation was monitored from January or February.

Fruit fly traps were hung a few weeks before the projected harvest date and monitored weekly. Three Capilure-loaded Sensus traps and three Questlure-loaded Sensus traps were hung in the middle of each treatment block. This was not done at the Klawervlei trial site.

2013/14

Trial sites

Details of trial sites are given in Table 3.2.3.5.

Table 3.2.3.5. Details of EPN trial sites

Region	**Farm**	**Coordinates**	**Cultivar**	**Orchard/s**	**Treatment block sizes (ha)**
Sundays River Valley, E.Cape	Waverley	33º25'71"S 25º21'86"E	Newhall Navels	50 & 51	1 ha
Citrusdal, W.Cape	Ou Werf	32º38'36.52" 19º38'19.88"	Washington Navel	Block 6	1 ha
Nelspruit, Mpumalanga	Montana	25º26' 25.68"S 30º50'22.78"E	Turkey Valencia	T1	2.16 - 2.58 ha

Application of EPNs

Full details of EPN application are provided in Table 3.2.3.6.

Table 3.2.3.6. Details of application of EPNs at trial sites

Farm	Rate of application (IJs/cm^2)	Application dates	Mode of application	Volume of water applied (L per ha)	Spray machine calibration
Waverley	10	18 Sep 2013	Microsprinklers	9250	na
	10	18 Sep, 16 Oct, 20 Nov & 12 Dec 2013	Microsprinklers	9250	na
	5	18 Sep, 16 Oct, 20 Nov & 12 Dec 2013	Microsprinklers	9250	na
Ou Werf	10	3 Oct, 31 Oct, & 4 Dec 2013	Spraycart & Hand lances	1000L	Tractor – 3.4 km/hr Rev – 1500 Appl – 34L / min.
	10	3 Oct 2013	Spraycart & Hand lances	1000L	Tractor – 3.4 km/hr Rev – 1500 Appl – 34L / min.
Montana	10	30 Sep 2013	Microspinklers (EPN applied using Dosatron at operating flow of 50 L/hour)		na
	10	30 Sep, 28 Oct, 26 Nov, 17 Dec 2013	As above		na
	5	30 Sep, 28 Oct, 26 Nov, 17 Dec 2013	As above		na

Pest control evaluations

FCM traps were hung from October or November and fruit infestation was monitored from January.

Hot-spot trials

A late corrective trial was initiated on 6 March in an orchard of Newhall Navels on Ronnievale Farm in the Sundays River Valley. FCM infestation in the orchard was at a relatively high level and the purpose of the trial was to determine whether an EPN application is capable of having a knock-down effect. *Heterorhabditis bacteriophora* was applied at both 10 and 20 IJs/cm^2 – each to a 1 ha block within the orchard and a third hectare was left untreated. EPNs were applied using a hand gun spray machine. Hand guns were aimed underneath the canopy of the trees using a wide spray plume and the following calibrations were used: Bakkie gear 4x4 Low and 1st gear, 1000 rpm, pressure 20 bar, 2.3mm nozzles.

2014/15

A hot-spot (late corrective) trial was initiated on 11 March 2015 in two similar orchards of Washington Navels on Stenhope Farm in the Sundays River Valley. *Heterorhabditis bacteriophora* was applied at both 10 and 20 IJs/cm^2 – each to a 1 ha block within the orchards and a third hectare was left untreated. EPNs were applied through the microjet irrigation system.

Fifty soil samples were collected from each treatment block. Soil samples were collected from throughout each block and each sample was approximately 35 g. In the laboratory, each sample was placed into a petri dish, two 5th instar FCM larvae were placed onto the surface of the soil and petri dishes closed and sealed. After seven days, petri dishes were opened and FCM larvae were recorded as alive or dead. Dead larvae were dissected and inspected for EPN infection. This was conducted in each treatment block shortly after application of EPNs. FCM traps were hung and monitored weekly. Ten data trees were marked in the middle

of each treatment block. Each week on the same day, all fruit which had dropped from the data trees were retrieved and assessed (dissected and inspected) for FCM infestation.

Results and discussion

2011/12

Monitoring of EPNs

No EPN-induced mortality of sentinel FCM larvae was recorded before and after application of EPNs at the Boerboon trial site. The soil was simply too dry to facilitate any survival of EPNs. It became clear that drip irrigation makes an orchard unsuitable for use of EPNs in the soil, unless adequate rainfall can be guaranteed.

At Sitrusoewer, no EPNs were detected in the soil before application (Fig. 3.2.3.1). Good recovery of *S. feltiae* was recorded immediately after application. Recovery of *H. bacteriophora* was not as good. Four weeks later, recovery of EPNs had dramatically declined. However, what was peculiar is that EPNs were now detected in the control. As these results were questioned, the planting of caged sentinel FCM larvae was repeated a further two weeks later (6 weeks after application). Again EPNs were detected in the control, albeit at a low level, as was the case with the two treated blocks (Fig. 3.2.3.1). It was subsequently concluded that the once-weekly irrigation applied to the orchard was not sufficient for good survival of EPNs.

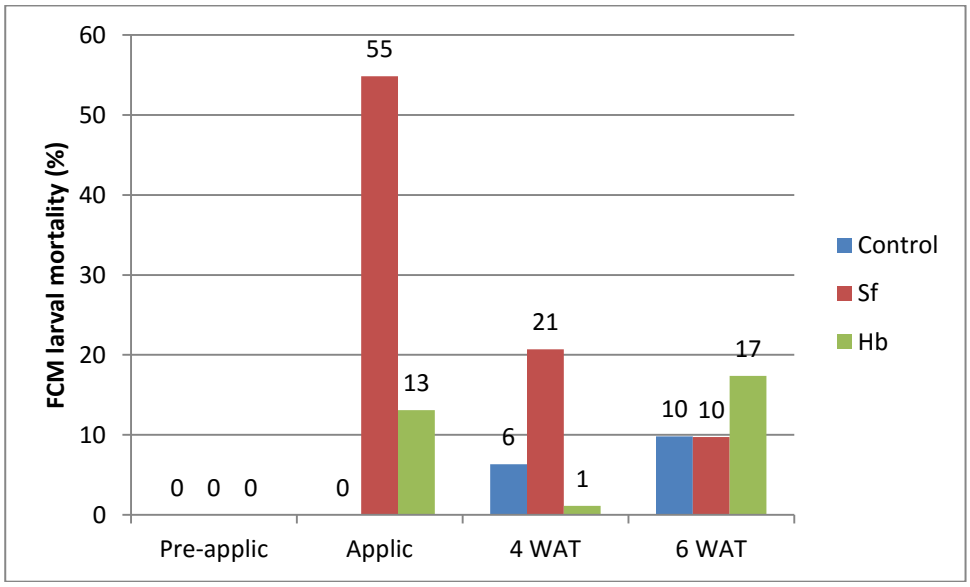

Figure 3.2.3.1. EPN induced mortality of caged planted sentinel FCM larvae at Sitrusoewer

Survival and recovery of EPNs at the Danie Smit trial site was impressive, up to eight weeks after application (Fig. 3.2.3.2). This was particularly so with application through the microjet irrigation system. Surprisingly, there was no survival of EPNs beyond one week after treatment where the same higher concentration had been applied using the spray machine. It was subsequently discovered that this orchard had only been converted from drip irrigation to microjet irrigation one year previously and that the water pressure in this orchard had not been adjusted accordingly. Hence wetting of the soil in this orchard was almost certainly inferior to that in the other orchards.

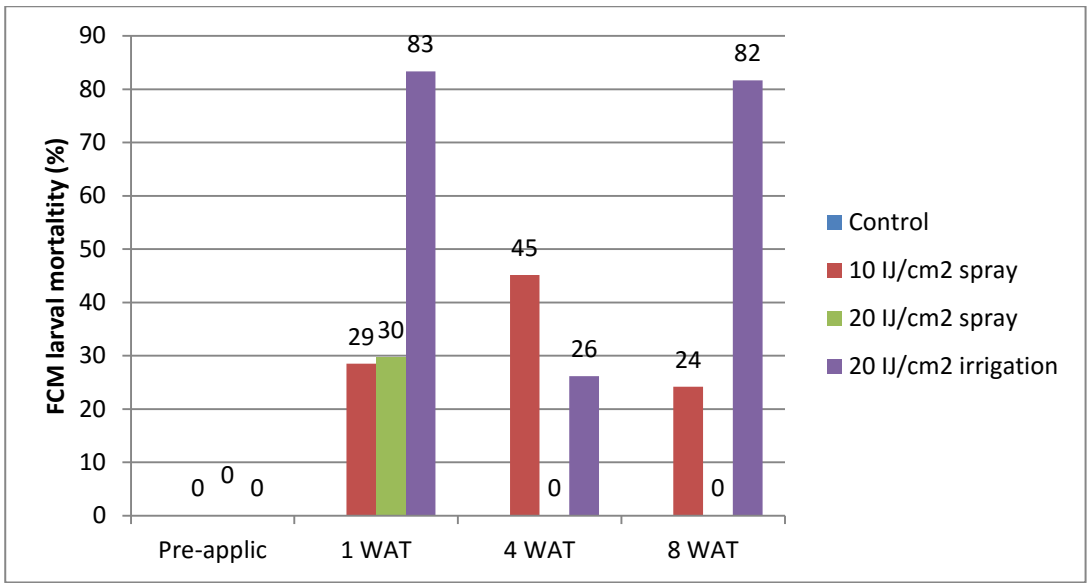

Figure 3.2.3.2. EPN induced mortality of caged planted sentinel FCM larvae at Danie Smit

At Crocodile Valley a high level of natural EPN infestation in the soil was detected before application of EPNs (Fig. 3.2.3.3). This should have indicated that this site was unsuitable for the trial. However, sentinel larvae were only removed from the orchard shortly before application commenced and results from this pre-application baiting were only available after the trial had already been applied. The naturally occurring species was identified as *H. zealandica*, thus differing from the species applied (*H. bacteriophora*). There was a notable increase in EPN recovery after application, particularly in the block which had previously shown the lowest level of natural occurrence of EPNs (the 10 IJ/cm^2 treatment). However, this high level of natural occurrence prevented development of any differences in pest control between the treated and control blocks. The trial was thus halted and instead an examination of the impact of the naturally occurring EPNs was investigated, as described under the sub-heading *Impact of naturally occurring EPNs*.

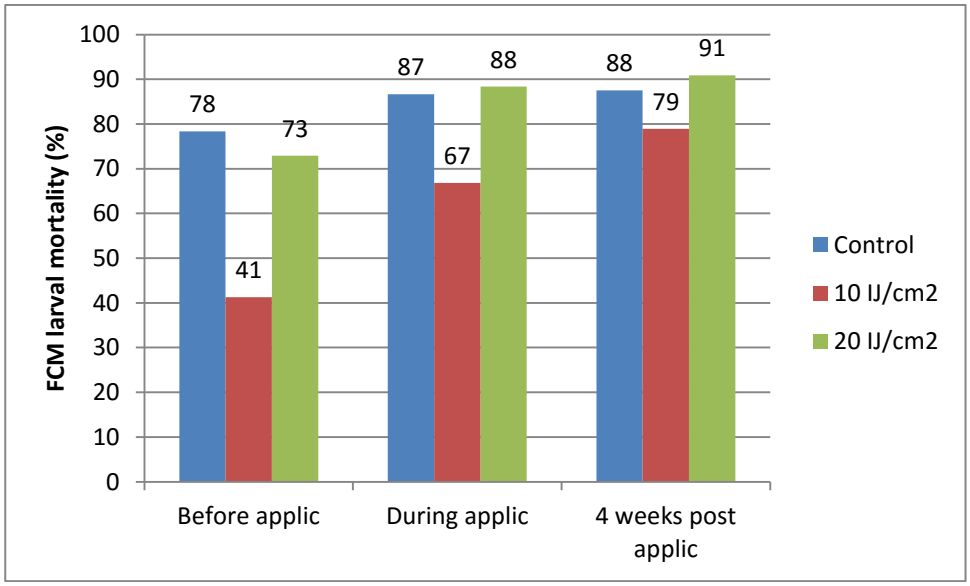

Figure 3.2.3.3. EPN induced mortality of caged planted sentinel FCM larvae at Crocodile Valley

Evaluation of pest control

As there was no persistence of the EPNs applied at Boerboon Farm, there would have been no effect on pest levels. As sticky yellow traps for monitoring thrips levels were hung before this lack of persistence was know, results are available for a five-week period. As expected, there was no statistically significant difference in citrus thrips levels between the different treatments (Fig. 3.2.3.4). Surprisingly, there was a significant difference in numbers of blossom thrips caught between the control and the drip-applied treatment. However, this difference cannot be associated with the treatments (apart from being the reverse of what would happen if the treatment was effective).

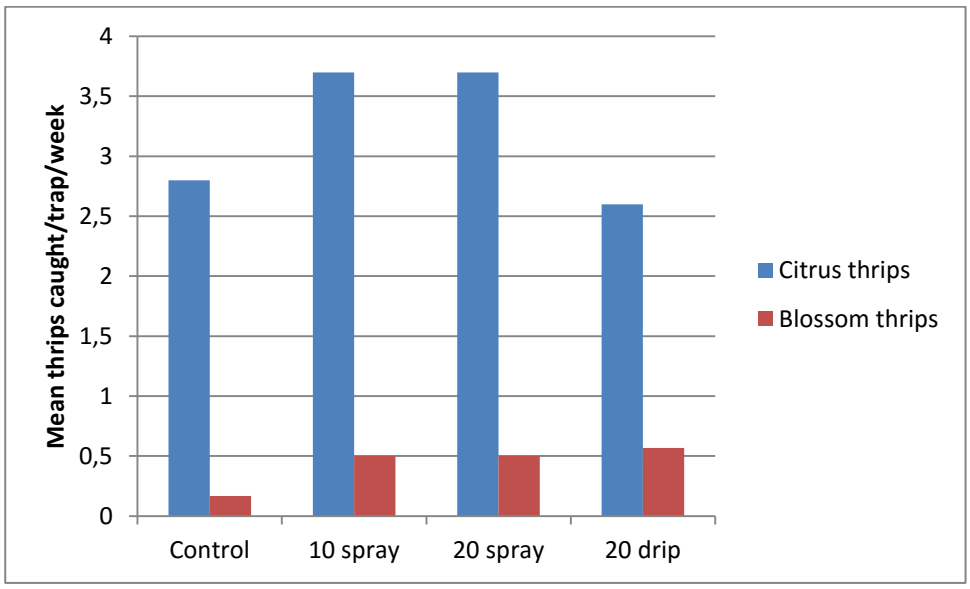

Figure 3.2.3.4. Catches of citrus and blossom thrips on sticky yellow traps in different treatment blocks at Boerboon Farm over a 5-week period from 26 September to 26 October 2011. (The only significant difference recorded was that between blossom thrips caught in the control block and the drip block; α=0.05, LSD multiple range test).

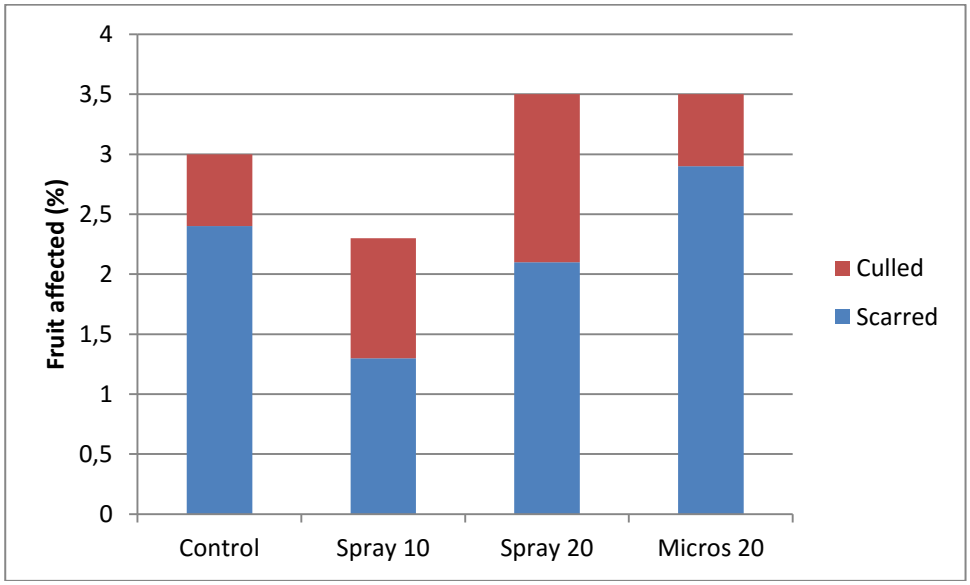

Figure 3.2.3.5. Fruit damaged by thrips at Danie Smit, evaluated on 12 April 2012. (The only significant difference recorded was that between fruit scarred in the 10 IJ/cm^2 treatment and the microjet applied treatment; α=0.05, LSD multiple range test).

Thrips damage evaluations at Danie Smit revealed a significantly lower level of damage to fruit in the 10 IJ/cm2 treatment compared to the 20 IJ/cm2 microjet treatment (Fig. 3.2.3.5). However, this did not make sense as

these were the two effective treatments i.e. the two in which EPNs persisted well (Fig. 3.2.3.2). It is thus concluded that it is most probably that the EPNs provided no meaningful thrips control.

At Crocodile Valley there were no significant differences between thrips catches on traps in the different treatments over a 9-week period (Fig. 3.2.3.6). However, this was to be expected, as even if the EPNs had an effective on thrips, the high level of natural occurrence of *H. zealandica* at the trial site, was have obscured this.

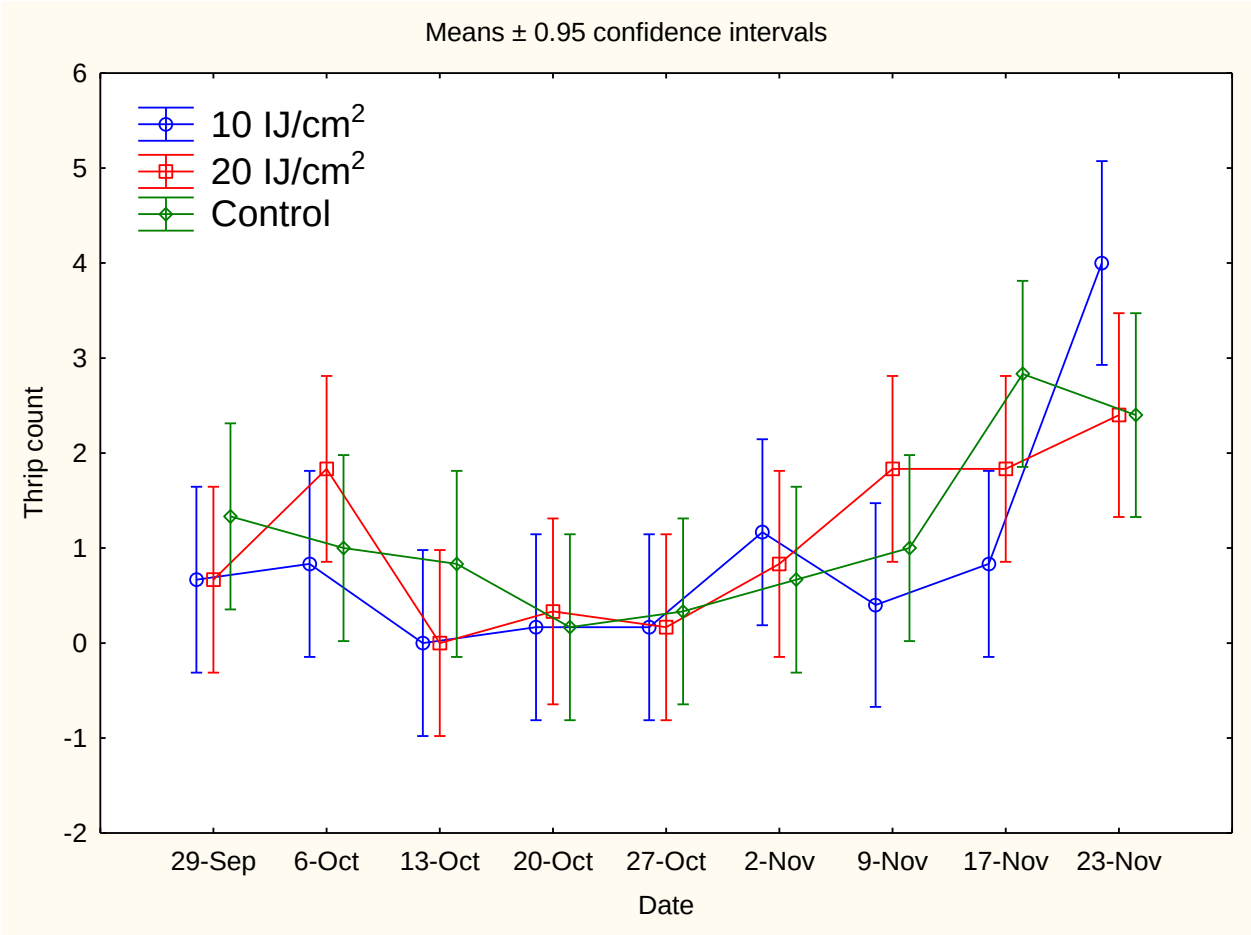

Figure 3.2.3.6. Mean ±SE of citrus thrips per trap per week for the three EPN treatments applied at the Crocodile Valley trial site (2011). (There were no significant differences between any of the treatments; α = 0.05).

FCM traps were hung for six weeks at the Boerboon trial site (26 September to 1 November 2011). As there was no survival of EPNs, these catches are irrelevant to the trial and have not been reported.

Initial survival and persistence of *S. feltiae* was better than for *H. bacteriophora* (Fig. 3.2.3.1). However, this rapidly declined. Simultaneously, EPNs were also detected in the untreated control, despite this not being the case before application. This very low level of EPNs detected from four weeks after application may not have been sufficient to provide any meaningful FCM control. The far higher level of FCM in the *S. feltiae* treatment might be explained that this treatment was applied in a separate orchard (orchard 8), which was separated from the orchard in which *H. bacteriophora* was applied which was used for the untreated control (orchard 11) by several hundred metres (Fig. 3.2.3.7). There may therefore have been a meaningful difference in the level of FCM in the two orchards, independent of the treatments applied. Moths were not monitored, as traps would have interfered with the monitoring of the FCM SIT programme being conducted on the farm.

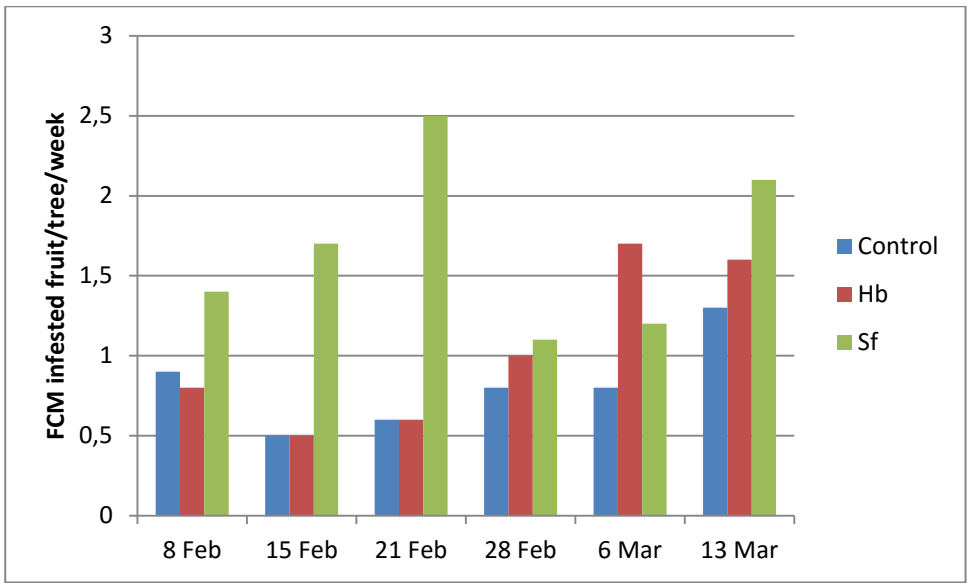

Figure 3.2.3.7. FCM infested fruit per tree per week in the three EPN treatment blocks at Oewer Farm. (Mean fruit infested for the full period was significantly higher for the Sf (*S. feltiae*) treatment than the other two, which did not differ from one another; LSD multiple range test, α = 0.05)

The most compelling results on FCM control were obtained at the Clanwilliam trial site. The lowest FCM trap catches and fruit infestation were recorded in the two treatments where EPN persistence was good i.e. the 10 IJ/cm^2 spray and the 20 IJ/cm^2 irrigation (Fig. 3.2.3.8). Moths were monitored weekly from 6 October 2011 to 15 March 2012. Fruit infestation was monitored weekly from 22 December 2011 to 15 March 2012. Relative to the untreated control, FCM infestation over this period was reduced by 63.64% and 54.55% by the 10 IJ/cm^2 treatment and the irrigation treatment. Relative to the 20 IJ/cm^2 treatment, which can be considered as a second untreated control, as no survival of EPNs was recorded beyond one week, infestation was reduced by 80.95% and 76.19%. Trap catches and fruit infestation were significantly higher in the microjet treatment (effectively a second control) than the other two EPN treatments (α = 0.05) (Fig. 3.2.3.8). Trap catches and fruit infestation between the microjet treatment and the untreated control were not significantly different (α = 0.05).

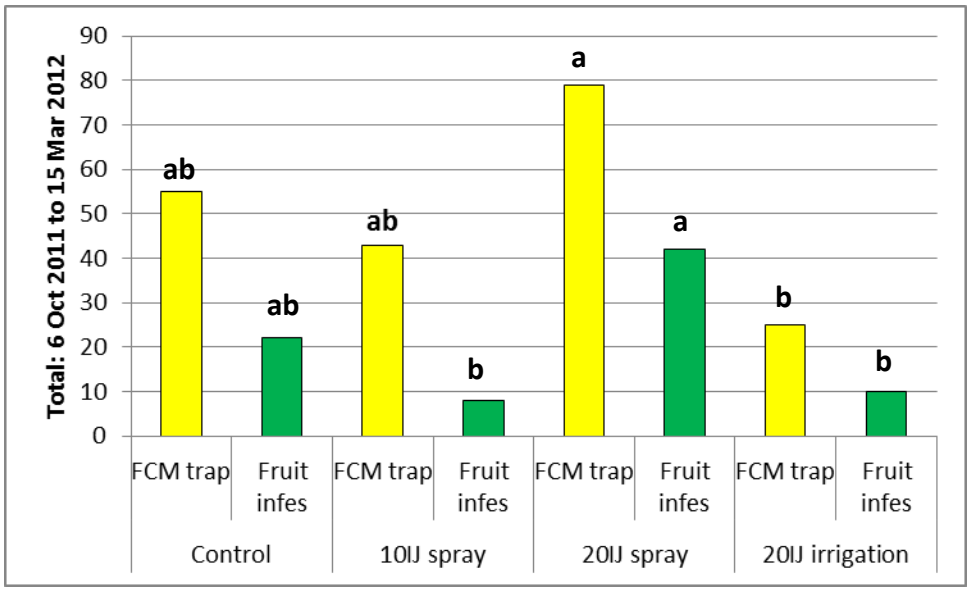

Figure 3.2.3.8. Total FCM caught per treatment (trap) from 6 October 2011 to 15 March 2012 and total fruit infested per 10 trees per treatment from 22 December 2011 to 15 March 2012 at Danie Smit's farm. (Bars of the same colour with the same letter are not significantly different; α = 0.05, Bonferroni multiple range test).

Moths were monitored at Crocodile Valley for a period of 14 weeks from 29 September 2011 to 19 January 2012 (Fig. 3.2.3.9). Monitoring was continued after this, but this formed part of a subsequent trial to determine the effect of the naturally occurring EPNs at the trial site. Mean numbers of moths caught per trap per week

for the three treatments were 6.1, 5.3 and 9.4 for 10 IJs/cm^2, 20 IJs/cm^2 and the untreated control respectively. Although there appeared to be a trend, there was no statistically significant difference (α = 0.05; Bonferroni LSD multiple range test), which is what was to be expected with the high background level of naturally occurring EPNs masking potential differences.

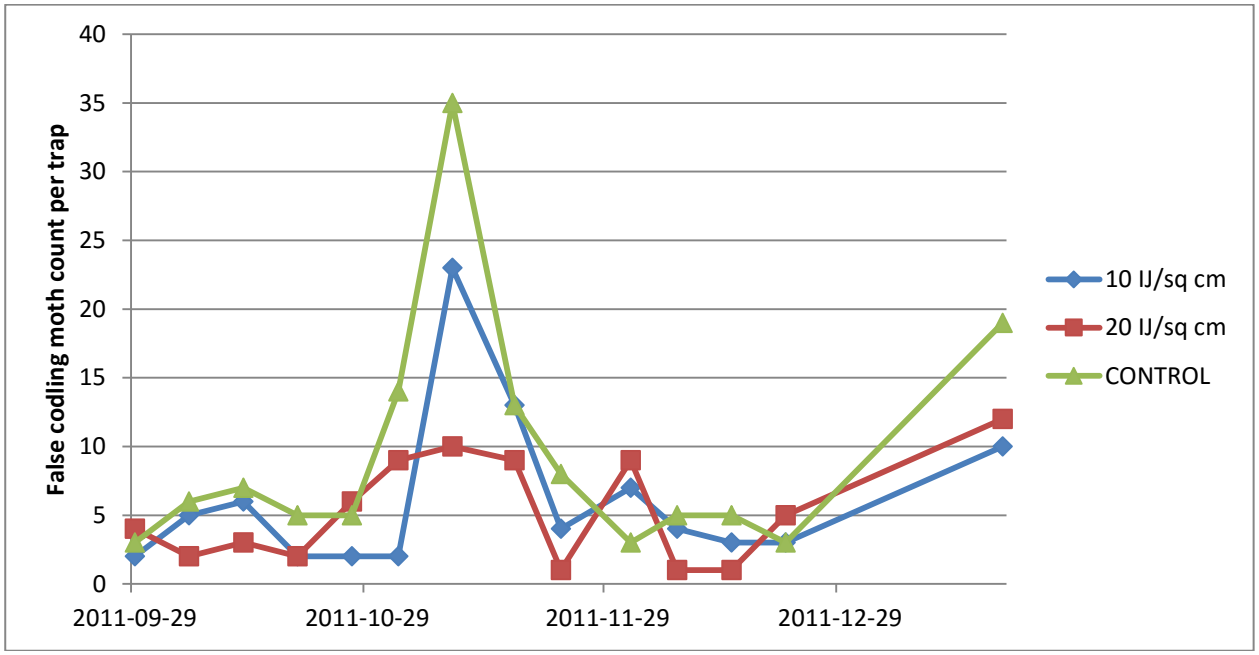

Figure 3.2.3.9. FCM caught per treatment (trap) per week from 29 September 2011 to 19 January 2012 at Crocodile Valley.

<u>Impact of naturally occurring EPNs</u>

Pre-application of cadusafos, EPN levels in the two orchards were similar (Fig. 3.2.3.10), albeit noticeably lower than recorded earlier in the season (Fig. 3.2.3.3). Cadusafos dramatically reduced the level of EPNs in the soil, but these seem to have fully recovered by eight weeks after treatment.

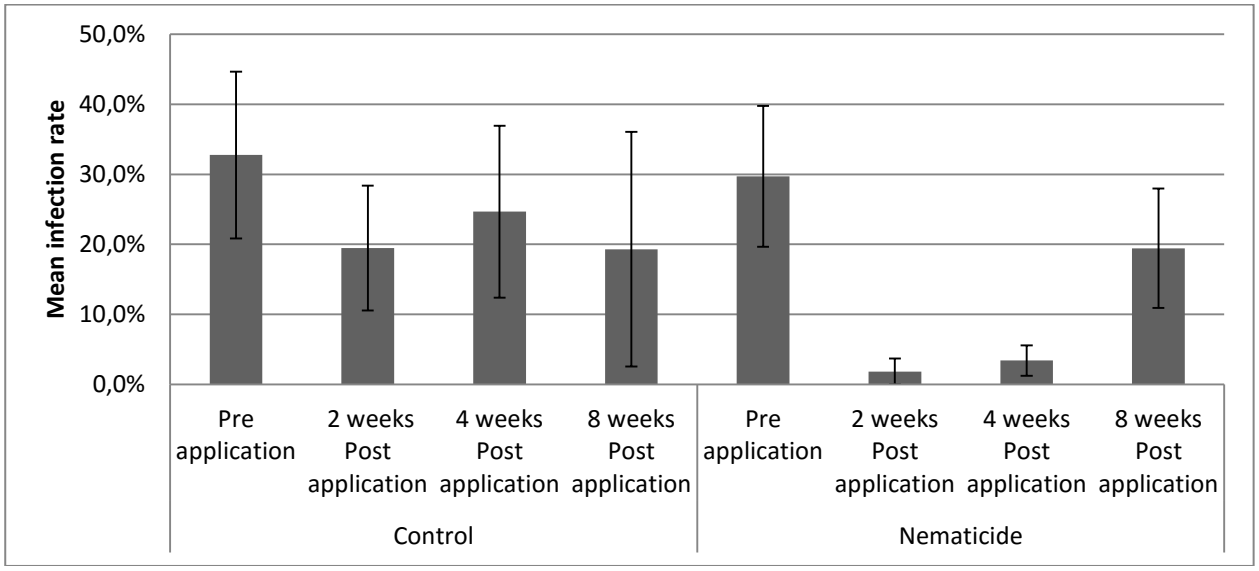

Figure 3.2.3.10. EPN induced mortality of caged planted sentinel FCM larvae in a cadusafos treated orchard and an untreated orchard at Crocodile Valley

Although no effect on FCM trap catches was recorded (Fig. 3.2.3.11), FCM infestation of fruit was notably higher where the nematicide had been applied (Fig. 3.2.3.12). Infestation was monitored on a weekly basis from 25 January to 25 April 2012. Mean fruit infested per tree per week for the untreated block was 0.09 ±

0.03 (mean ± SE), whereas for the nematicide treated block it was 0.22 ± 0.06. However, this difference was not statistically significant (α = 0.05, Student's t-test).

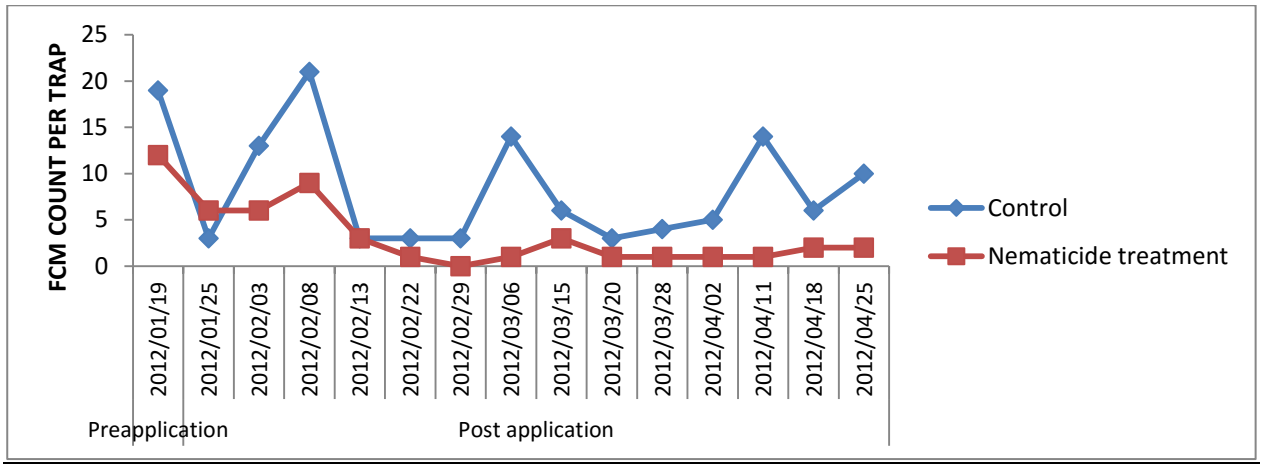

Figure 3.2.3.11. FCM caught per trap per week in a cadusafos treated orchard and an untreated orchard at Crocodile Valley (Date of nematicide application was 23/01/2012)

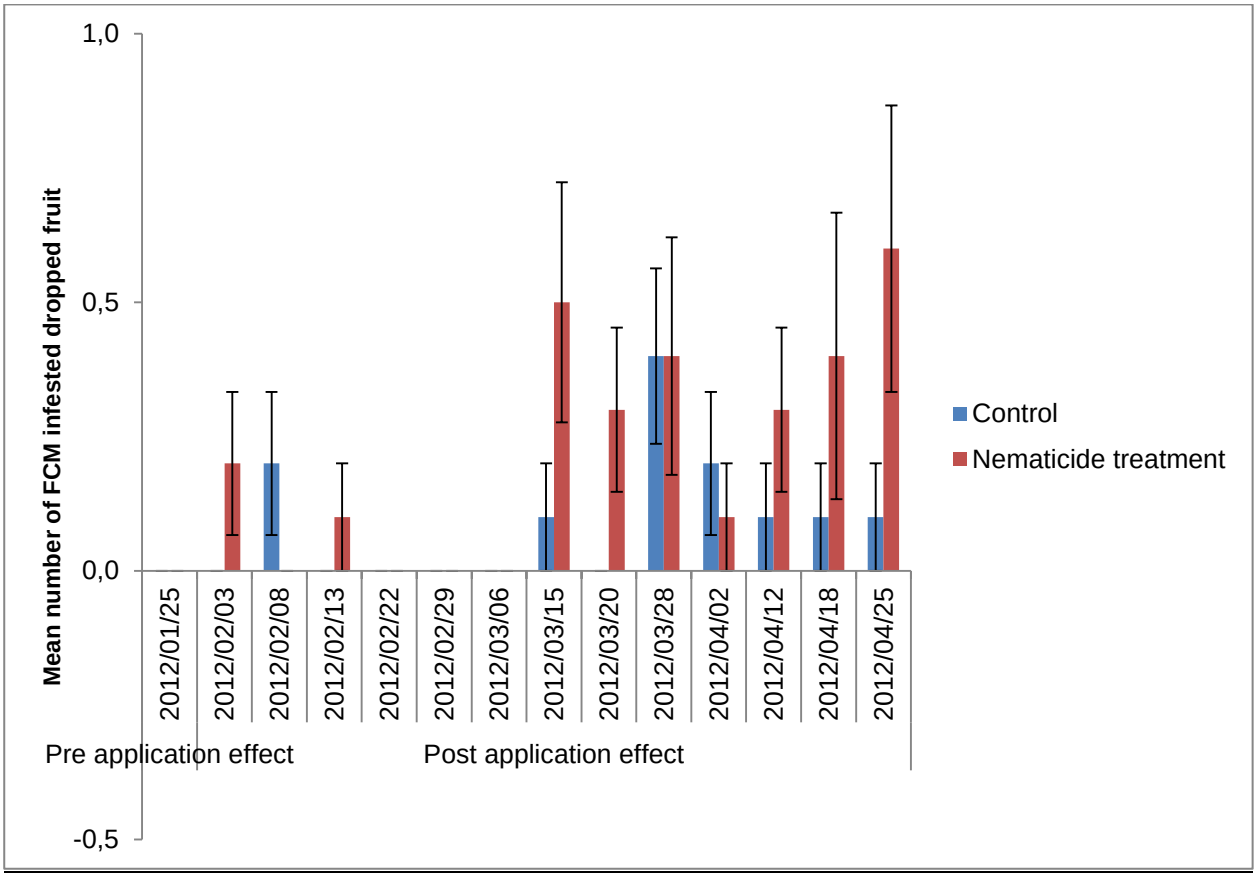

Figure 3.2.3.12. Mean number of FCM infested fruit per tree per week in a cadusafos treated and an untreated orchard at Crocodile Valley (Date of nematicide application was 23/01/2012)

Fruit fly traps were monitored weekly for five weeks from 20 March to 25 April 2012. Higher numbers of both Medfly (Fig. 3.2.3.13) and Natal fly (Fig. 3.2.3.14) were caught in Capilure-loaded Sensus traps in the nematicide-treated block than in the untreated control (however, no Medfly was caught in the last two weeks), indicating some controlling effect by the naturally occurring EPNs. A mean of 9.67 Medflies and 6.00 Natal flies were caught per trap per week in the nematicide-treated block, whereas only 0.67 and 2.67 of each were caught in the untreated control. These differences, although large, were not significantly different (α= 0.05, Student's t-test). However, this was due to the relatively small number of traps used.

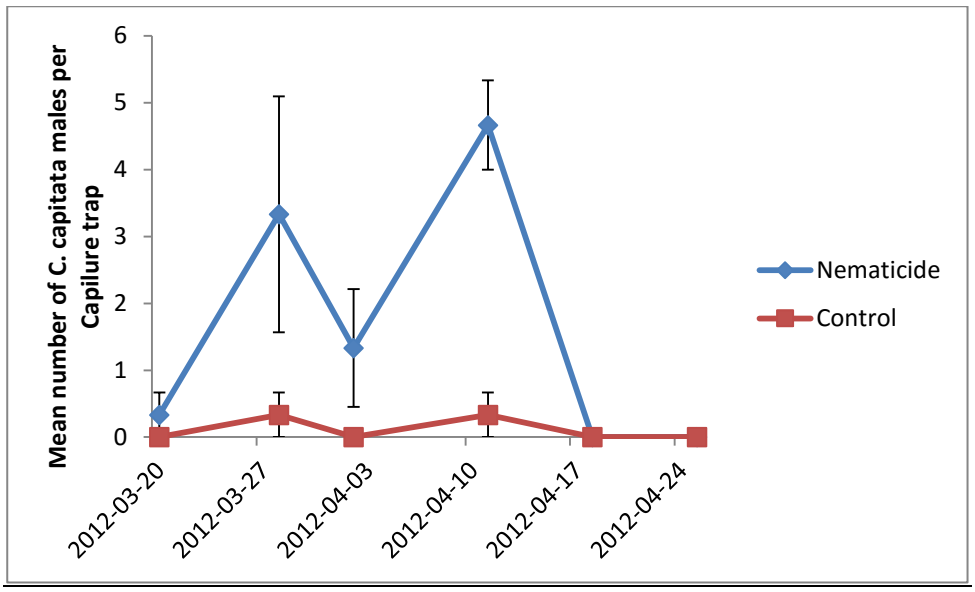

Figure 3.2.3.13. Mean number of Medfly males caught per Capilure-loaded Sensus trap (three traps per treatment) per week for five weeks. (Date of nematicide application was 23/01/2012)

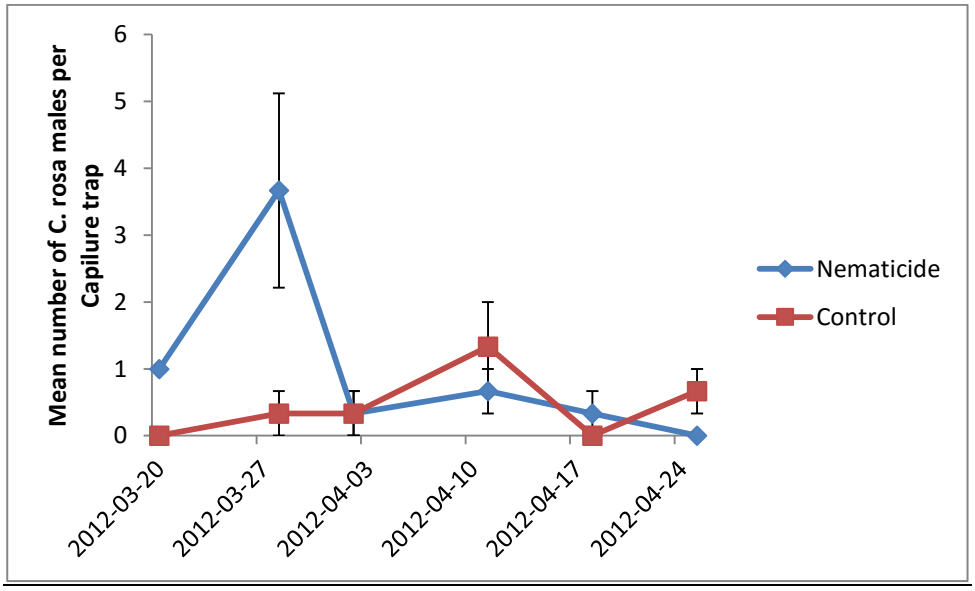

Figure 3.2.3.14. Mean number of Medfly males caught per Capilure-loaded Sensus trap (three traps per treatment) per week for five weeks. (Date of nematicide application was 23/01/2012)

Very low numbers of Marula males and females and Medfly and Natal fly females were caught. Catches in Questlure-loaded traps were very low.

Fruit fly numbers were not monitored at any of the other trial sites for various reasons.

2012/13

EPN survival and persistence

It was peculiar that no recovery of EPNs was made during the week after application of *H. bacteriophora* at Hexrivier in autumn (Fig. 3.2.3.15), particularly as EPNs were recovered 4 and 10 weeks after application, albeit at low levels. Soil temperature data must still be assessed; however, it is possible that temperatures were too low for *H. bacteriophora* to be active at this time. In order to increase the possibility of recovering EPNs, sentinel larvae cages were left in the soil for 10 days (as opposed to 7 days) in subsequent assessments during winter. Additionally, after removal from the soil, sentinel larvae were incubated for two days at temperatures above 20°C before dissection. Five months after the May application of *H. bacteriophora*, EPNs

were no longer recovered (Fig. 3.2.3.15). This was to be expected, as this species is not cold-adapted, being inactive below around 16°C.

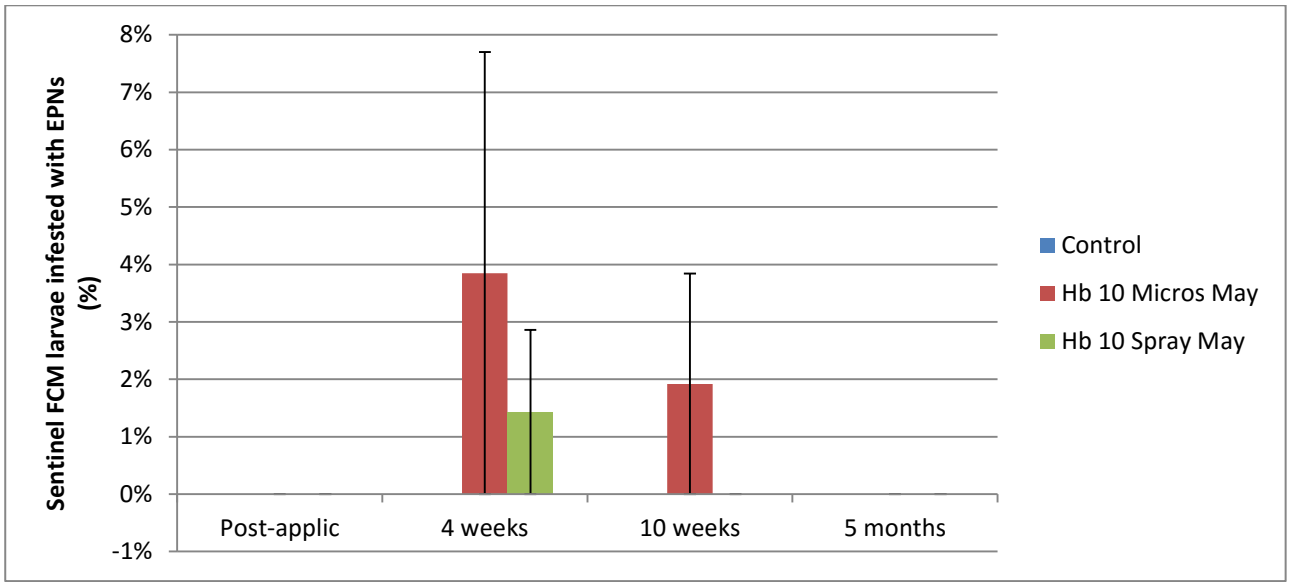

Figure 3.2.3.15. EPN induced mortality of caged planted sentinel FCM larvae at Hexrivier, Citrusdal, after autumn application of *H. bacteriophora*.

Recovery of EPNs after spring application of *H. bacteriophora* at Hexrivier was rather peculiar. Initially, EPNs were only recovered from the higher rate of application (20 IJ/cm^2) (Fig. 3.2.3.16). However, 11 weeks and 6 months after treatment, EPNs were recovered from all three blocks, including the untreated control. No explanation could be found for this. Additionally, EPNs were recovered at an increasingly high rate, despite pest levels in the orchard being extremely low.

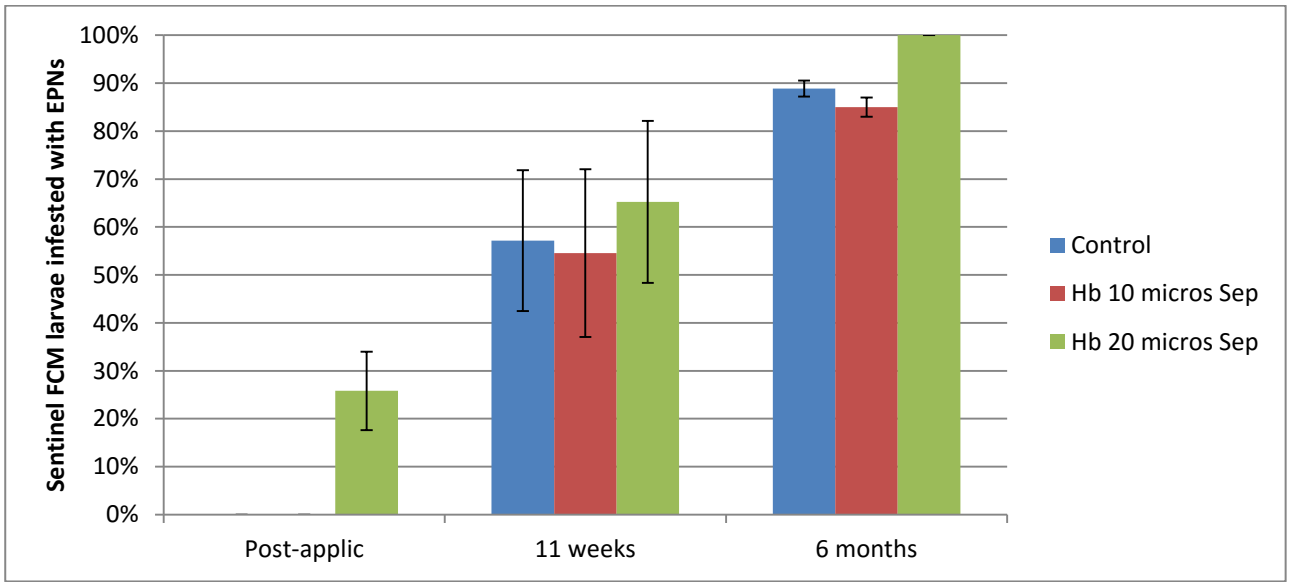

Figure 3.2.3.16. EPN induced mortality of caged planted sentinel FCM larvae at Hexrivier, Citrusdal, after spring application of *H. bacteriophora*.

At Klawervlei Farm, recovery of *S. feltiae* immediately after May application was very good (Fig. 3.2.3.17). Extremely low infection of sentinel larvae in the *H. bacteriophora*-treated and the untreated orchard, were recorded at 4 weeks after application, but thereafter, there was no sign of survival of *H. bacteriophora*. Some presence of *S. feltiae* was still recorded at 5 months after treatment (Fig. 3.2.3.17) and even at 7 months after

treatment (Fig. 3.2.3.18: 8 weeks after treatment (which is September treatments) is equivalent to 7 months after *S. feltiae* application in May).

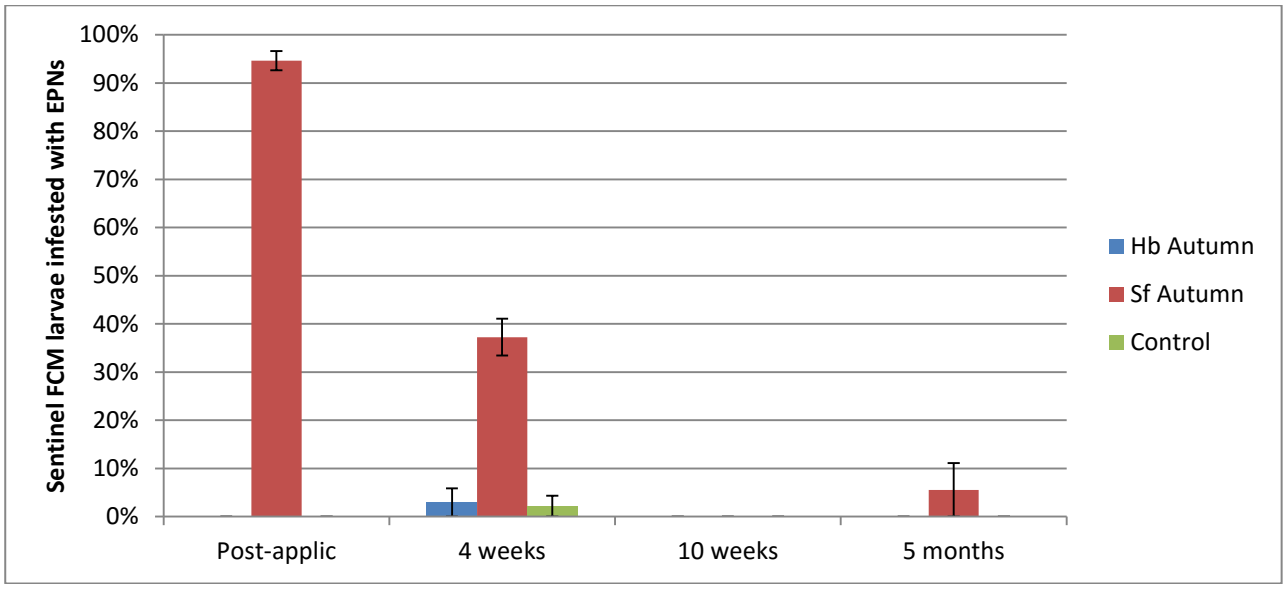

Figure 3.2.3.17. EPN induced mortality of caged planted sentinel FCM larvae at Klawervlei, Clanwilliam, after autumn application of EPNs.

Heterorhabditis bacteriophora showed poor survival after May application at Klawervlei Farm, whereas *S. feltiae* survived well up to 8 weeks after application (Fig. 3.2.3.18). At 16 weeks after application, no EPNs were recovered. It was noted on a couple of occasions when the site was visited, that orchard soil was very dry. During a trial conducted at the same site during the previous year, EPN survival and hence FCM control were excellent (Moore et al., 2012). The grower was possibly not as vigilant about regular irrigation during the second season or conditions may have been hotter and hence drier. As no researcher on this project was resident in the area, it was difficult to keep a regular check on the site. It should also be ascertained whether *S. feltiae* is more desiccation tolerant than *H. bacteriophora*.

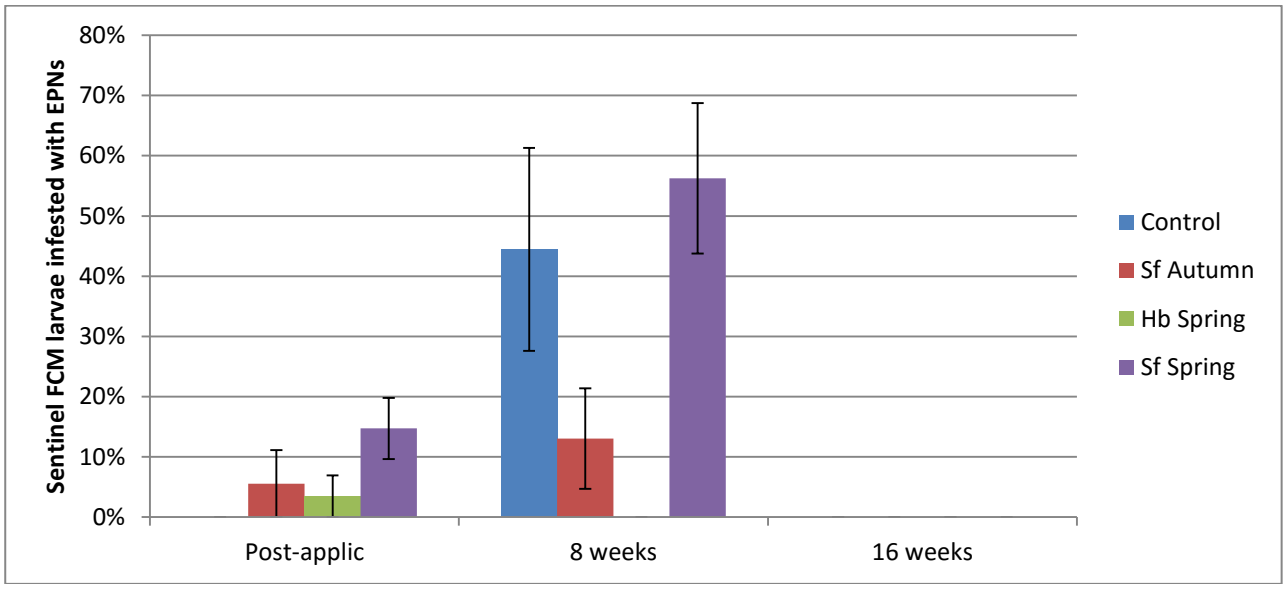

Figure 3.2.3.18. EPN induced mortality of caged planted sentinel FCM larvae at Klawervlei, Clanwilliam, after spring application of EPNs.

Recovery of *H. bacteriophora* after May application at Ronnievale Farm, although low, was achieved up to 15 weeks after treatment (Fig. 3.2.3.19). Survival of *H. bacteriophora* through winter was better (in the Eastern Cape) than was recorded in the two Western Cape trials (Klawervlei and Hexrivier) (Figs. 3.2.3.15 & 3.2.3.17). Winters are generally milder in the Sundays River Valley than in the Olifants River Valley of the Western Cape.

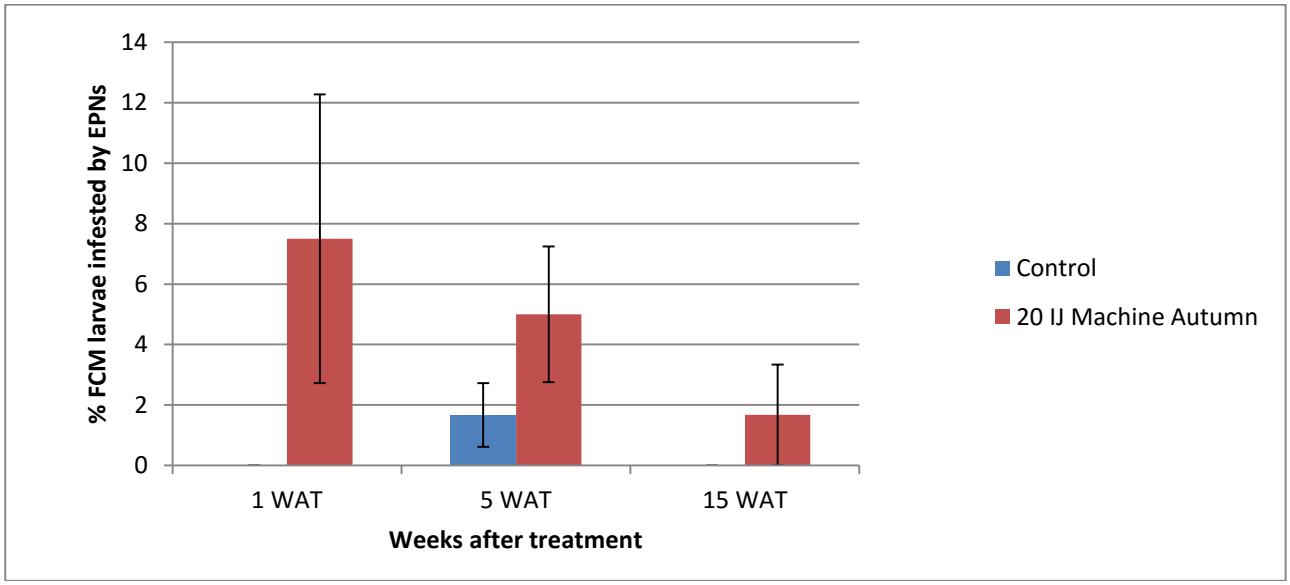

Figure 3.2.3.19. EPN induced mortality of caged planted sentinel FCM larvae at Ronnievale Farm, Sundays River Valley, after autumn application of *H. bacteriophora*.

Recovery of *H. bacteriophora* after September application at Ronnievale Farm (Fig. 3.2.3.20), was generally higher than after May application (Fig. 3.2.3.19). Recovery was slightly higher for the higher concentration (20 IJ/cm^2) than the lower concentration (10 IJ/cm^2).

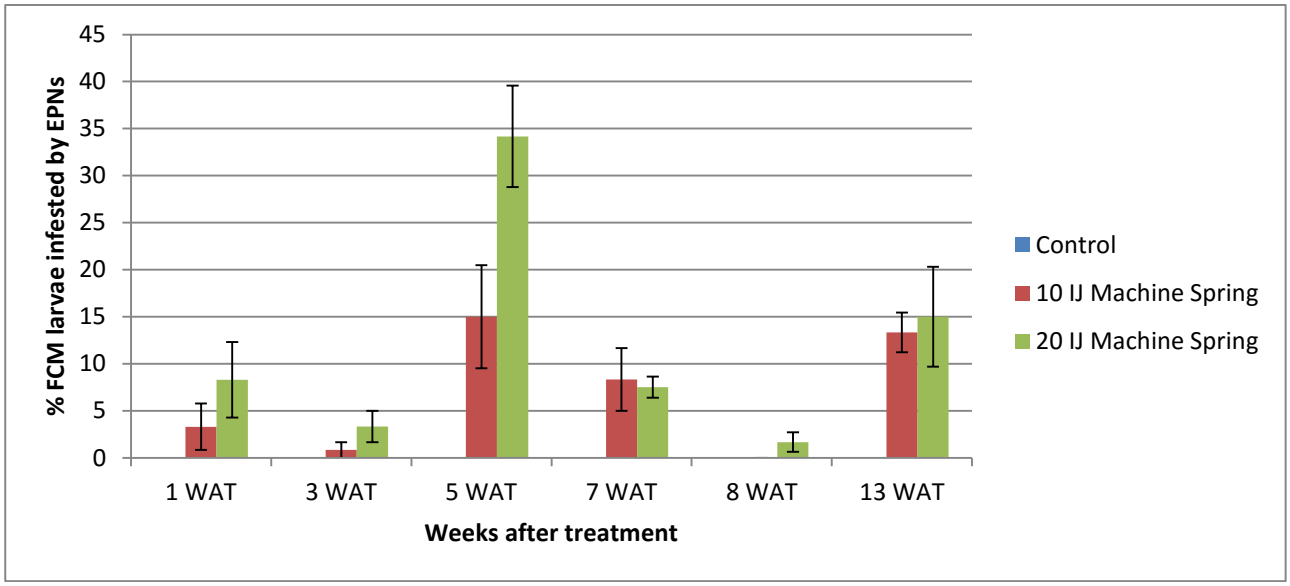

Figure 3.2.3.20. EPN induced mortality of caged planted sentinel FCM larvae at Ronnievale Farm, Sundays River Valley, after spring application of *H. bacteriophora*.

EPNs were similarly persistent at a similarly low level after autumn application at Huguenot Farm (Fig. 3.2.3.21).

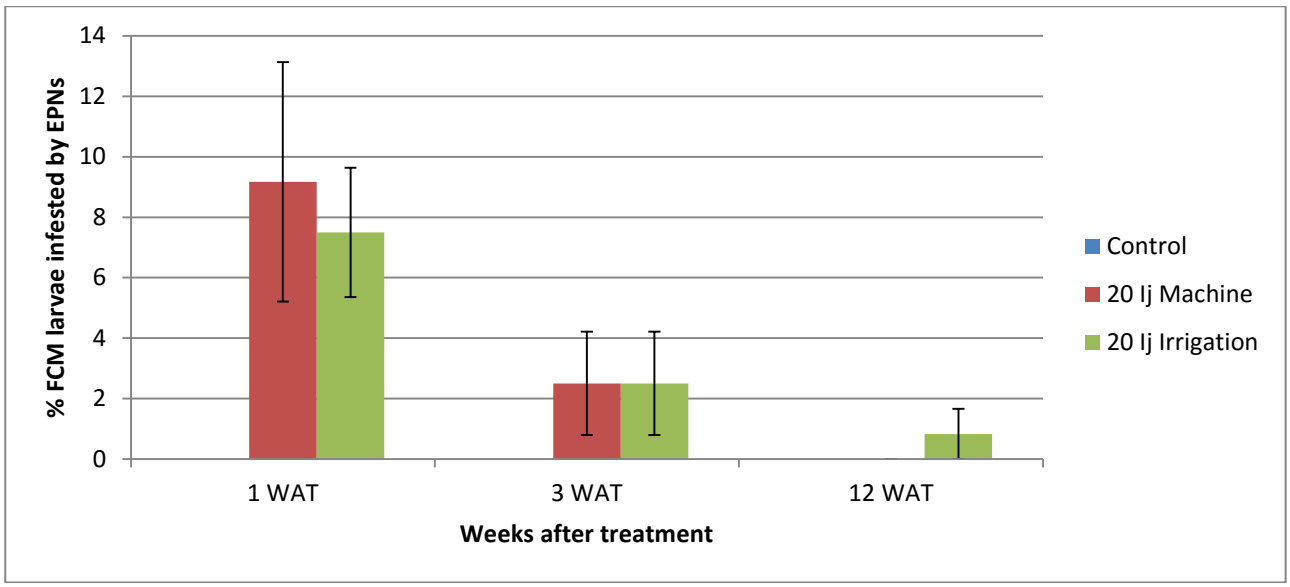

Figure 3.2.3.21. EPN induced mortality of caged planted sentinel FCM larvae at Huguenot Farm, Sundays River Valley, after autumn application of *H. bacteriophora*.

Although not initially apparent (probably due to cold winter temperatures), as was the case in the Mpumalanga field trial conducted during the previous season (Moore et al., 2012), there was once again a background level of naturally occurring EPNs at the trial site (Fig. 3.2.3.22). Despite this, during five out of the seven post-application monitoring periods, EPN recovery was higher for at least one of the treatments than for the untreated control.

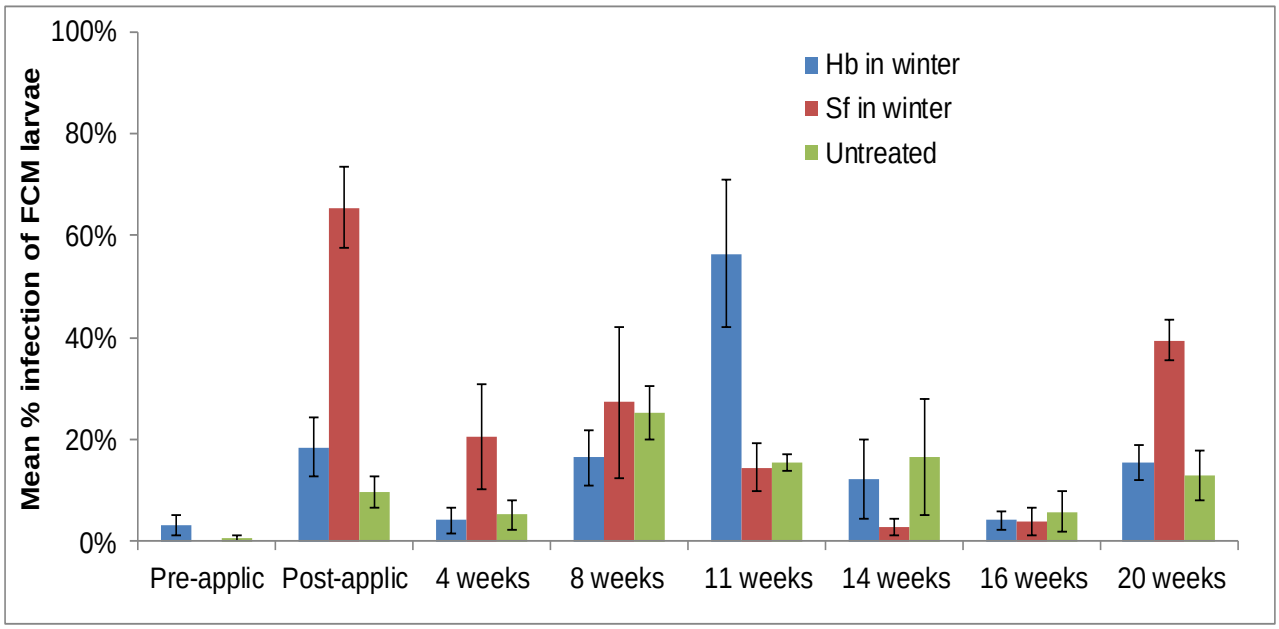

Figure 3.2.3.22. EPN induced mortality of caged planted sentinel FCM larvae at Montana, Nelspruit after winter application of EPNs.

This natural occurrence of EPNs was again evident in the control relative to the September *H. bacteriophora* application (Fig. 3.2.3.23). Surprisingly, EPN recovery was generally lower than after winter applications (Fig. 3.2.3.22).

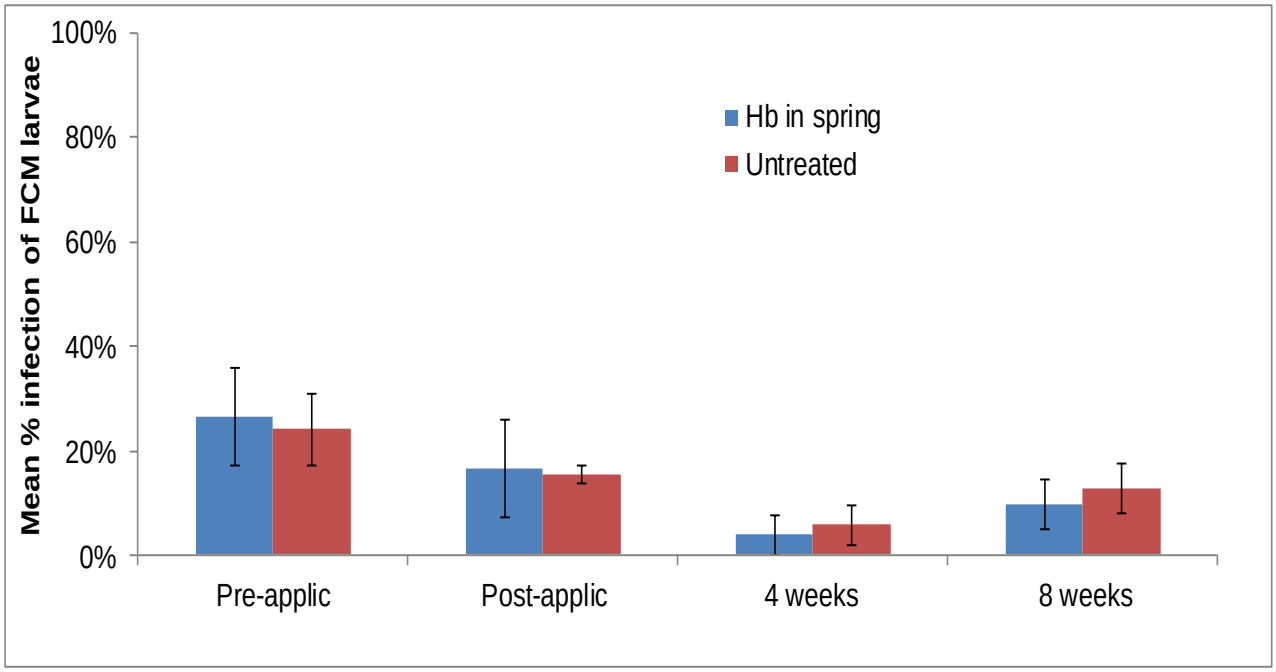

Figure 3.2.3.23. EPN induced mortality of caged planted sentinel FCM larvae at Montana, Nelspruit after spring application of EPNs.

FCM population suppression

Due to the very poor recovery of EPNs after the autumn applications at Hexrivier (Fig. 3.2.3.15), no monitoring of pest levels was conducted in these orchards. This was only done in the orchards where EPNs were applied in spring. Notably fewer moths were caught in the higher EPN treatment at Hexrivier than in the untreated orchard and the lower EPN treatment (Fig. 3.2.3.24), despite recovery of EPNs being only slightly higher in this orchard (20 IJ/cm^2) (Fig. 3.2.3.16).

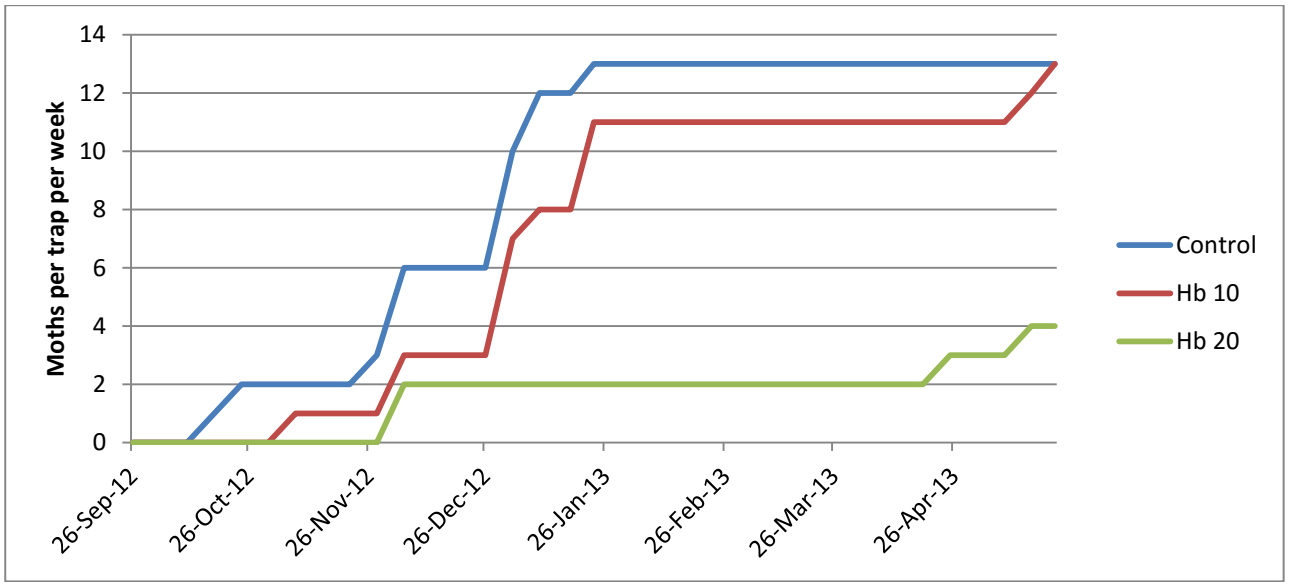

Figure 3.2.3.24. FCM caught per treatment (trap) per week at Hexrivier from 26 September 2012 to 22 May 2013.

At Klawervlei, both *S. feltiae* treatments (autumn and spring) appeared to be more effective than the *H. bacteriophora* treatment at suppressing the FCM population (Fig. 3.2.3.25).

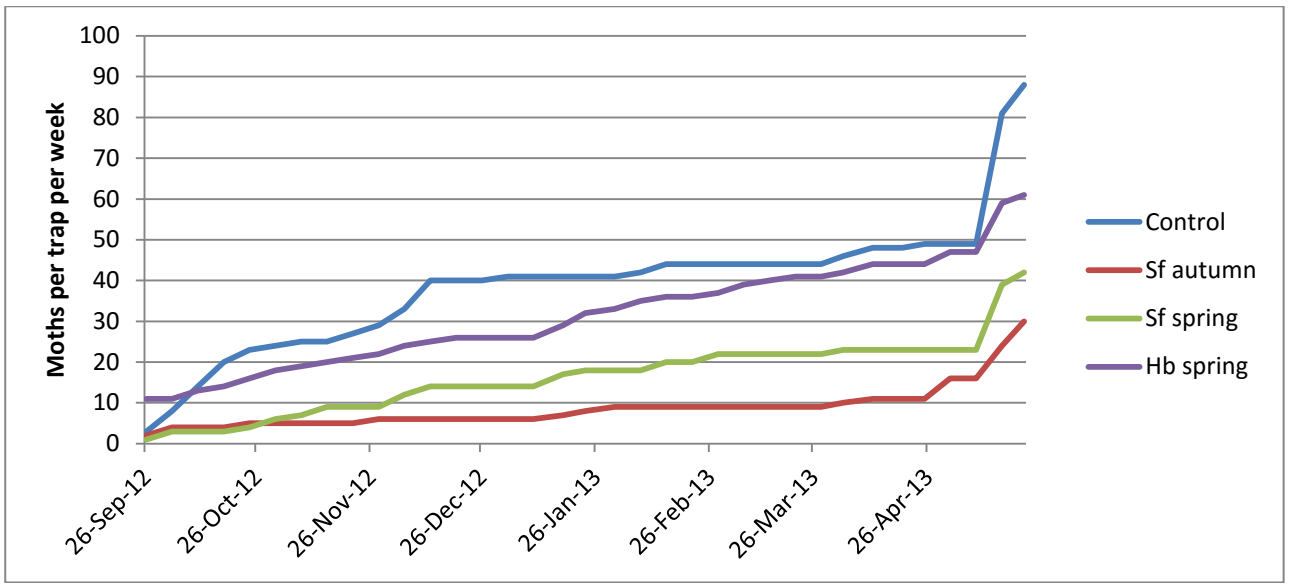

Figure 3.2.3.25. FCM caught per treatment (trap) per week at Klawervlei from 26 September 2012 to 22 May 2013.

At Ronnievale Farm, FCM trap catches were only lower where *H. bacteriophora* had been applied in spring (Fig. 3.2.3.26). Although a very low level of recovery of EPNs was recorded for a few weeks after the autumn application (Fig. 3.2.3.20), this was apparently not sufficient to suppress the FCM population relative to the untreated orchard.

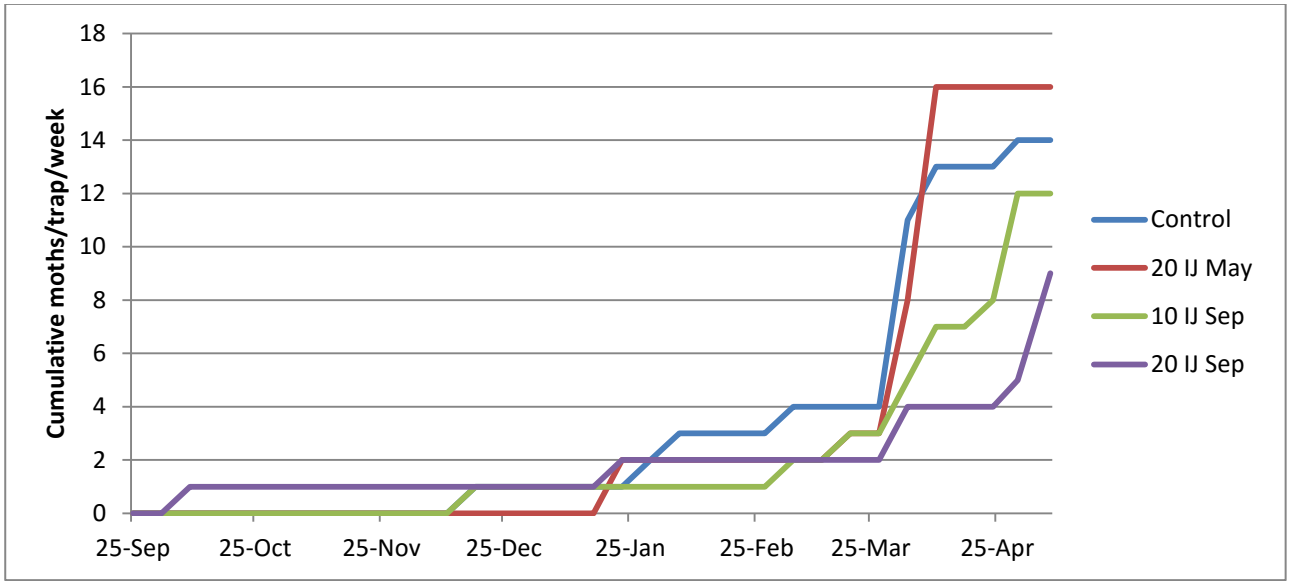

Figure 3.2.3.26. FCM caught per treatment (trap) per week at Ronnievale Farm from 25 September 2012 to 8 May 2013.

The low level of recovery of autumn applied EPNs at Huguenot Farm (Fig. 3.2.3.21) was apparently sufficient to suppress the FCM population relative to the untreated orchard (Fig. 3.2.3.27).

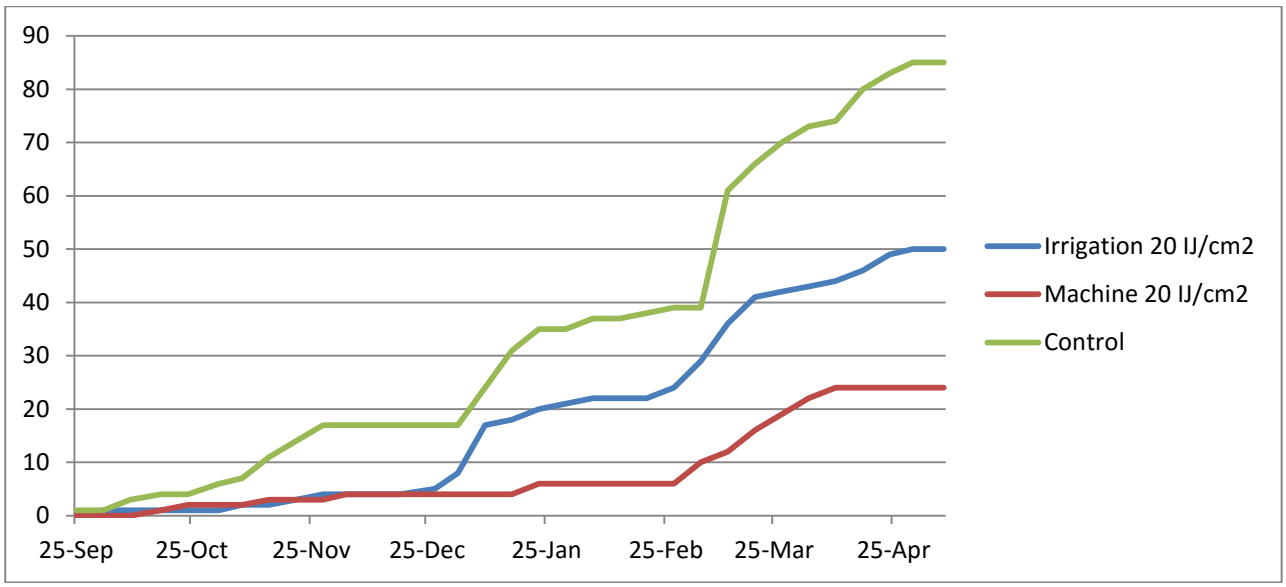

Figure 3.2.3.27. FCM caught per treatment (trap) per week at Huguenot Farm from 25 September 2012 to 8 May 2013.

Markedly higher FCM adult numbers were caught in the trap in the untreated block at Montana Farm (Fig. 3.2.3.28), confirming the trend recorded at almost every EPN trial site both during the 2012/13 season and the 2011/12 season.

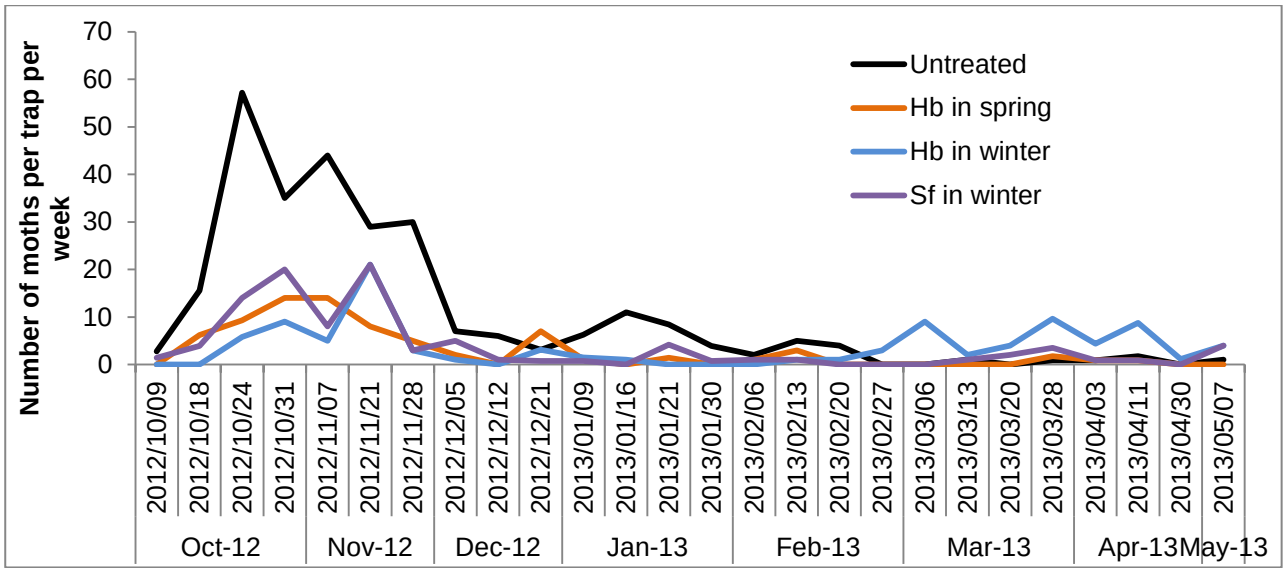

Figure 3.2.3.28. FCM caught per treatment (trap) per week at Montana Farm from 9 October 2012 to 7 May 2013.

<u>FCM damage reduction</u>

FCM infestation at Hexrivier was extremely low. Despite this, FCM infestation of fruit was lowest for the higher of the two *H. bacteriophora* treatments (Fig. 3.2.3.29).

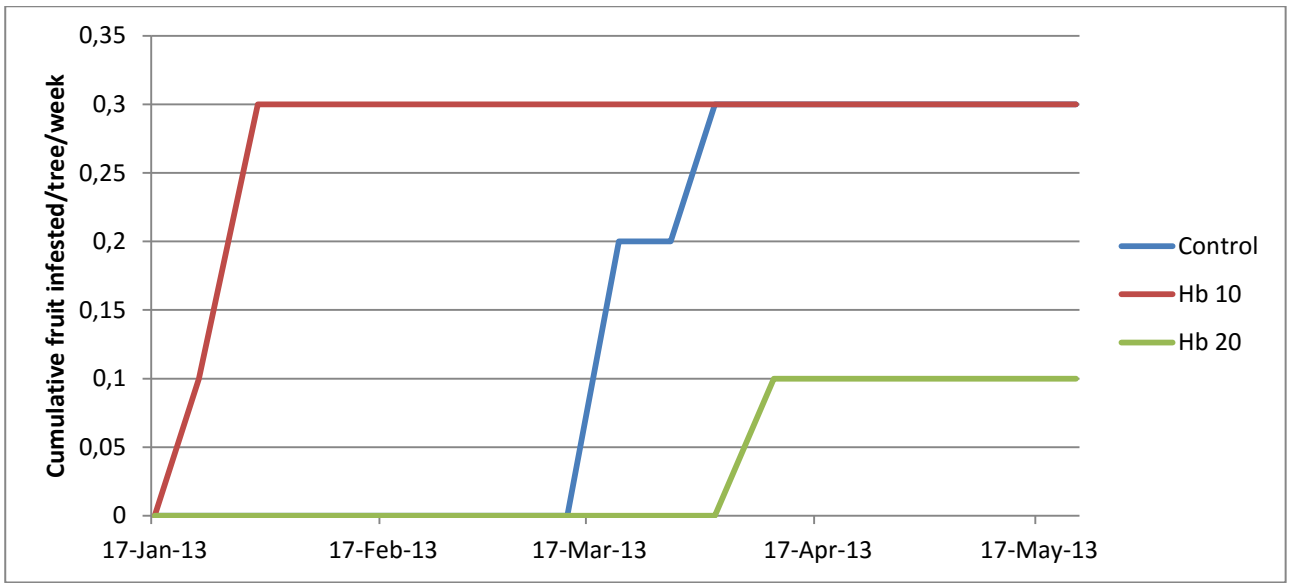

Figure 3.2.3.29. Cumulative FCM infested fruit per tree per week at Hexrivier from 17 January to 22 May 2013.

Application of EPNs at Klawervlei appeared to have no effect in reducing FCM (Fig. 3.2.3.30), contrary to the results from the EPN trial conducted on the same farm during the previous season, where a significant reduction in infestation was recorded (Moore et al., 2012). For *H. bacteriophora*, this may be reflective of the inadequate survival of EPNs in the soil, probably due to lack of good soil moisture (Figs. 3.2.3.17 & 3.2.3.18). However, particularly the spring application of *S. feltiae* persisted well, with good recovery after 8 weeks (Fig. 3.2.3.18). This apparent lack of a good result may therefore simply be due to unrelated differences in pest pressure between orchards, a constant risk with these sorts of trials where each treatment must be applied over a large area (at least 1 ha).

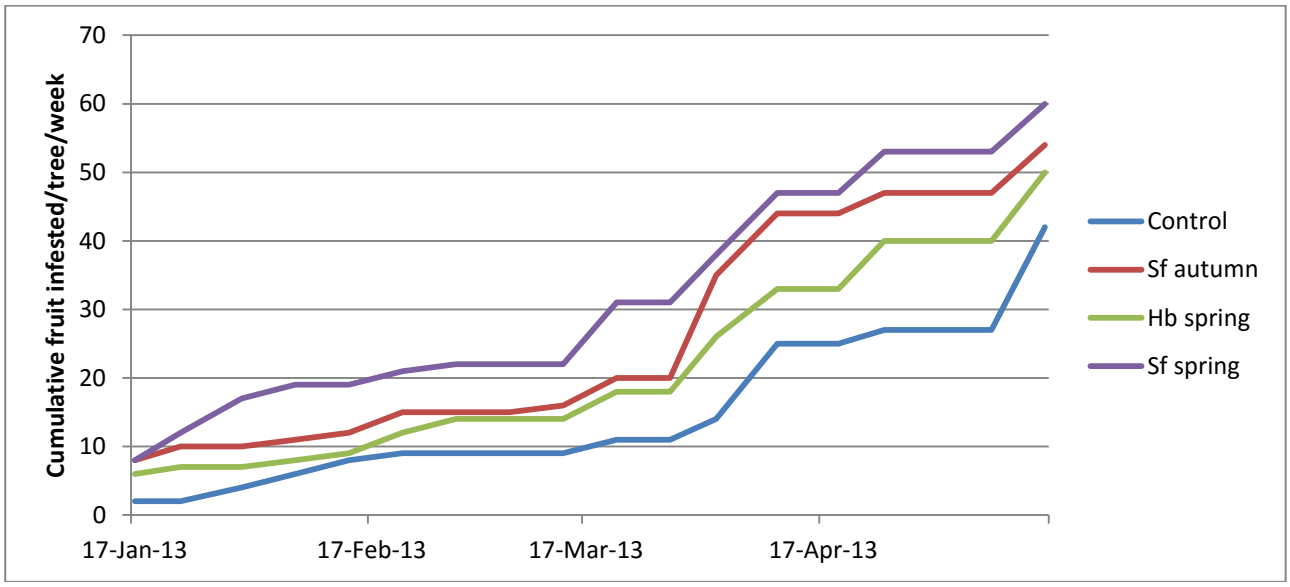

Figure 3.2.3.30. Cumulative FCM infested fruit per tree per week at Klawervlei from 17 January to 22 May 2013.

Reduction in infestation at Ronnievale Farm was impressive, with the autumn application of H. bacteriophora (at 20 IJ/cm^2) and the two spring applications of the same species (10 and 20 IJ/cm^2) resulting in 35%, 43% and 53% reduction in infestation respectively (Fig. 3.2.3.31).

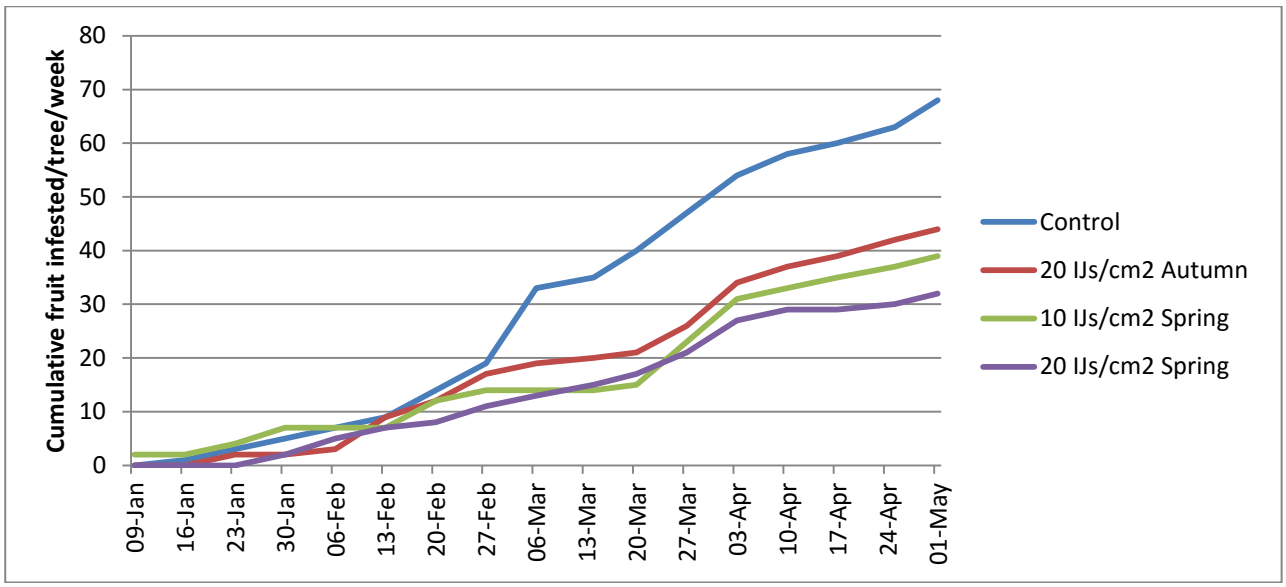

Figure 3.2.3.31. Cumulative FCM infested fruit per tree per week at Ronnievale from 9 January to 1 May 2013.

Although EPNs were recovered at Huguenot Farm up to 12 weeks after autumn application, this was only at 9% recovery after 1 week and substantially lower than that thereafter (Fig. 3.2.3.21). This may have been inadequate to cause a reduction in pest levels (Fig. 3.2.3.32), despite appearing to have some effect on trap catches (Fig. 3.2.3.27).

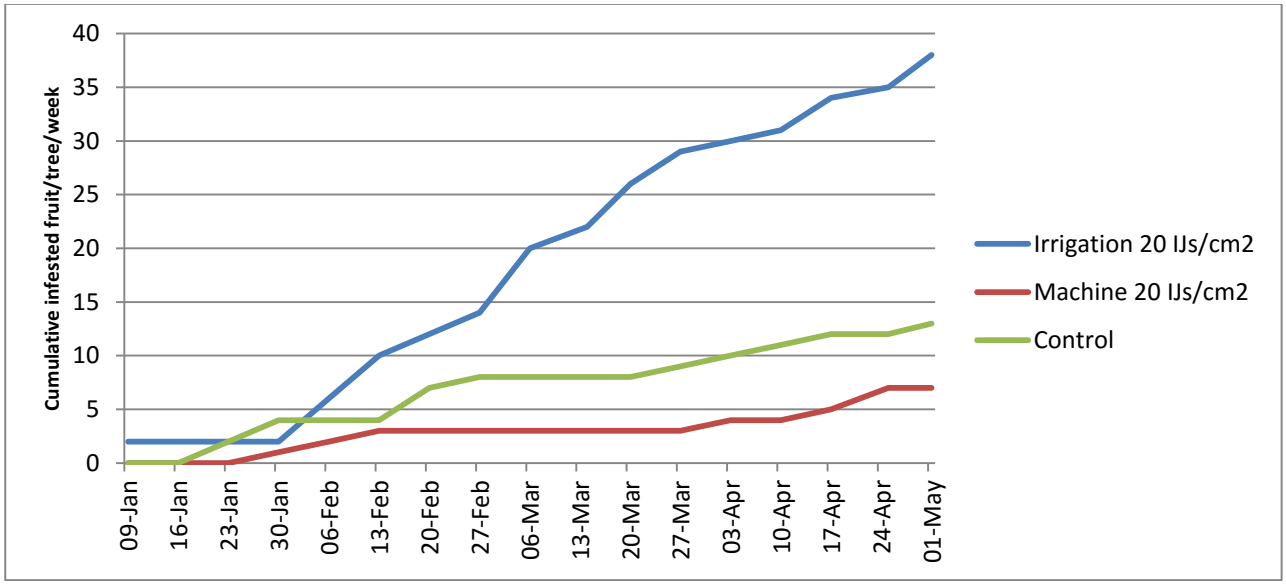

Figure 3.2.3.32. Cumulative FCM infested fruit per tree per week at Huguenot from 9 January to 1 May 2013.

At Montana Farm, FCM infestation of fruit in the untreated control orchard was the highest, along with that in the orchard where *H. bacteriophora* was applied in winter (Fig. 3.2.3.33). This is not surprising, as *H. bacteriophora* is not a cold-adapted EPN species. An 81% reduction in infestation was associated with the *S. feltiae* application and the spring application of *H. bacteriophora* resulted in a 43% decline in infestation relative to the control (Fig. 3.2.3.33).

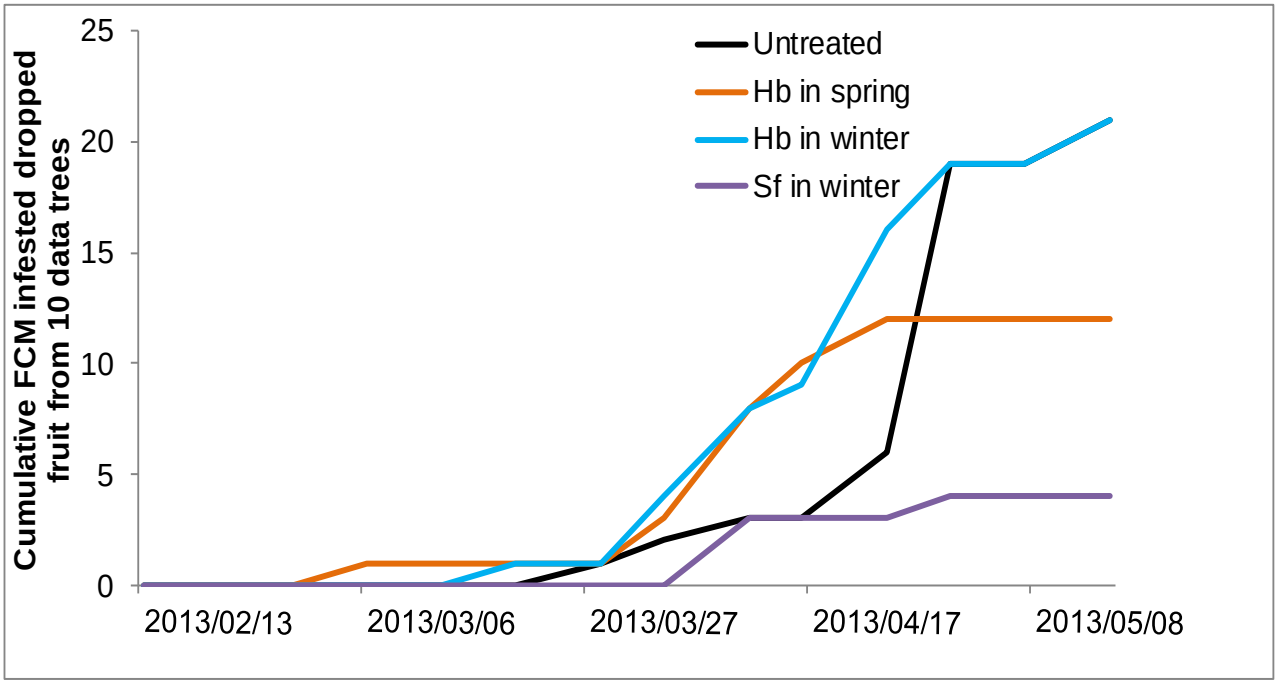

Figure 3.2.3.33. Cumulative FCM infested dropped fruit from 10 data trees at Montana, Nelspruit from 13 February to 15 May 2013.

<u>Fruit fly control</u>

Fruit fly traps were hung for four weeks leading up to harvest at Hexrivier (8 May to 5 June 2013). As fruit fly baiting was conducted diligently by the grower in all three trial orchards, very few flies were caught (fewer than 0.5 flies per trap per week) and therefore no conclusions could be drawn.

Ceratitis capitata was the dominant fruit fly species at Montana; very few female flies were captured in Questlure baited traps. Therefore, only data for male *C. capitata* is presented (Fig. 3.2.3.34). Flies were monitored for five weeks from 16 April to 15 May 2013. Catches were dramatically higher in the untreated control than in any of the EPN-treated orchards. Although extremely encouraging, this positive result was surprising, as fruit fly is known to be far less susceptible to EPNs than FCM, generally requiring much higher levels of inoculum in order for there to be a controlling effect (Manrakhan et al., 2012). This result may therefore have also been partly due to the higher number of dropped fruit in the untreated control which could have been a breeding site for fruit fly.

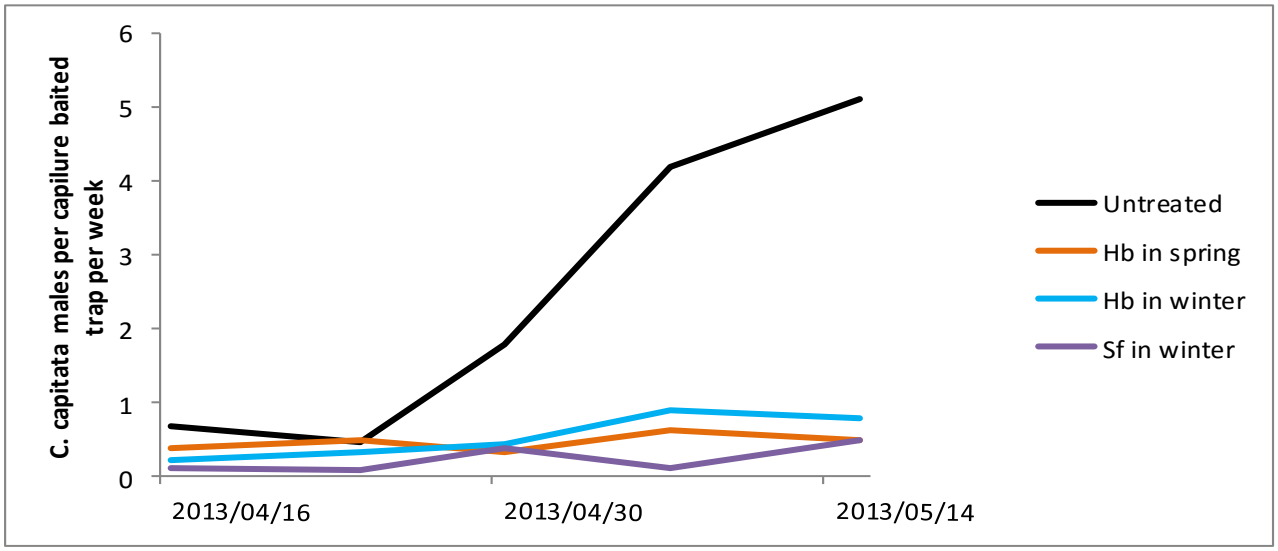

Figure 3.2.3.34 *Ceratitis capitata* males per trap per week at Montana, Nelspruit from 16 April to 15 May 2013 .

2013/14

EPN survival and persistence

Results of EPN infection of sentinel larvae at Waverley Farm from the first (September) assessment were inconclusive, at there was inexplicably a high level of natural mortality and some escape of larvae. These results have therefore been omitted (Figure 3.2.3.35). Thereafter, EPN infection of sentinel larvae was similar for both of the monthly treatments, which were conspicuously higher than for the once off September treatment. However, infection was never higher than 17%.

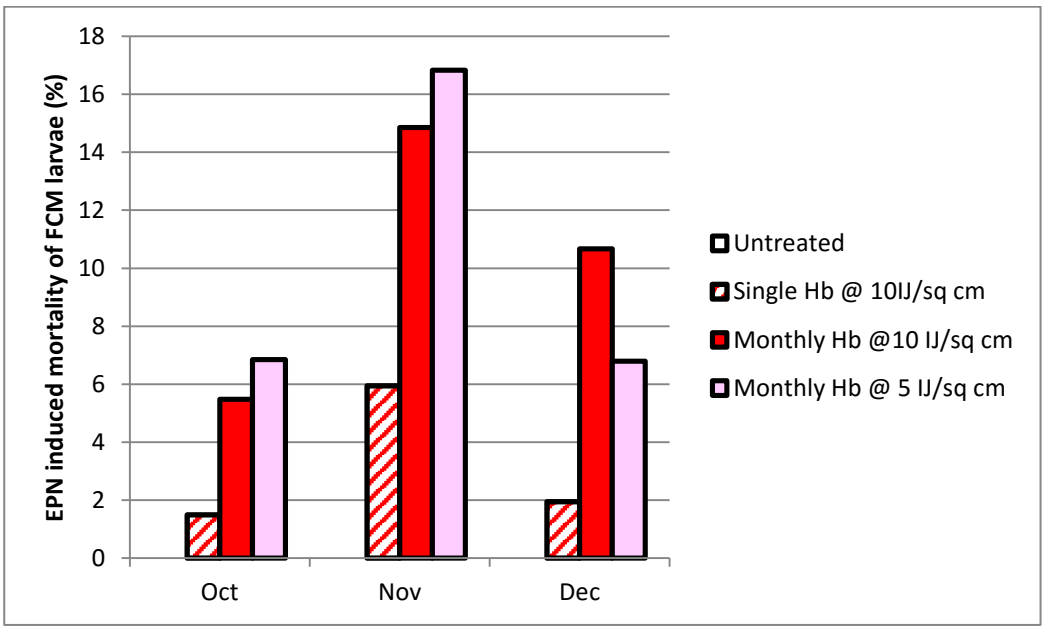

Figure 3.2.3.35. EPN induced mortality of 5th instar FCM larvae baited onto soil from Waverley Farm, Sundays River Valley, Eastern Cape, after EPN application in October, November and December 2013.

As has now become the norm for trials in Mpumalanga, there was a background level of naturally occurring EPNs in the trial site (Moore et al., 2012; Moore et al., 2013; Manrakhan et al., 2014) (Fig. 3.2.3.36). However, the background level was very low and did therefore not influence the results of the trial. infection of sentinel larvae was high for both of the monthly treatments, but slightly higher for the higher dose. infection for these two treatments was conspicuously higher than for the once off September treatment. infection peaked at just under 80% of sentinel larvae, which was far higher than for the Eastern Cape trial. This may at least in part be due to the sandier soils at the Mpumalanga trial site.

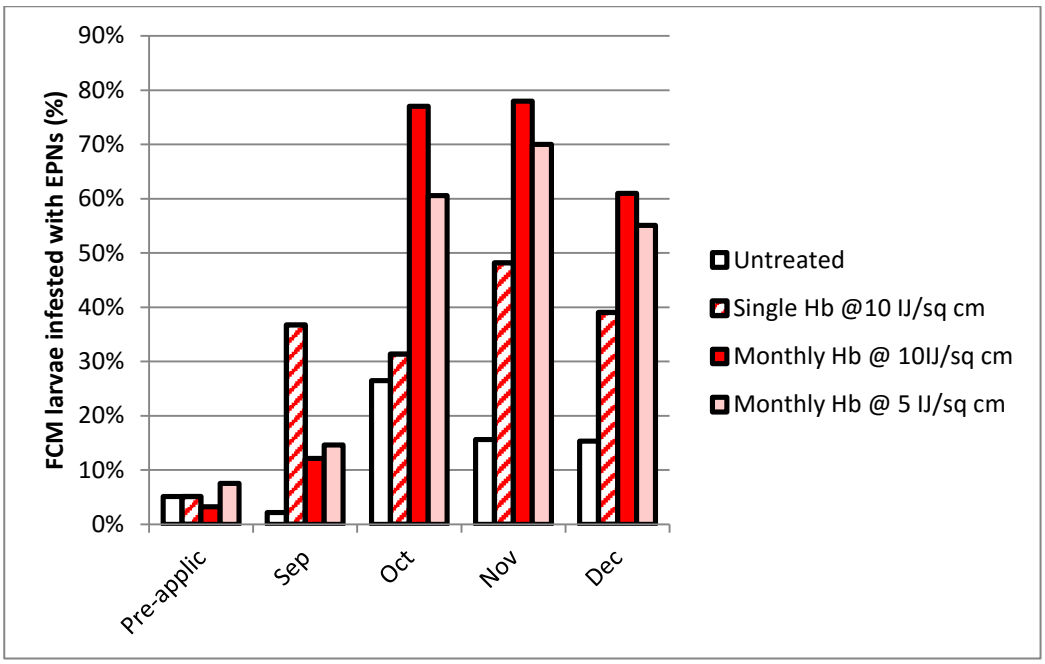

Figure 3.2.3.36. EPN induced mortality of 5th instar FCM larvae baited onto soil from Montana Farm, Nelspruit, Mpumalanga, before and after application of EPNs on various dates.

At the Western Cape trial site there was also a low background level of naturally occurring EPNs (Fig. 3.2.3.37). After the first application EPN infection of sentinel larvae was logically similar for the two 10 IJ/cm^2 treatments. However, thereafter, the difference in infestation became pronounced. What was inexplicable was that infestation for the repeated application also declined.

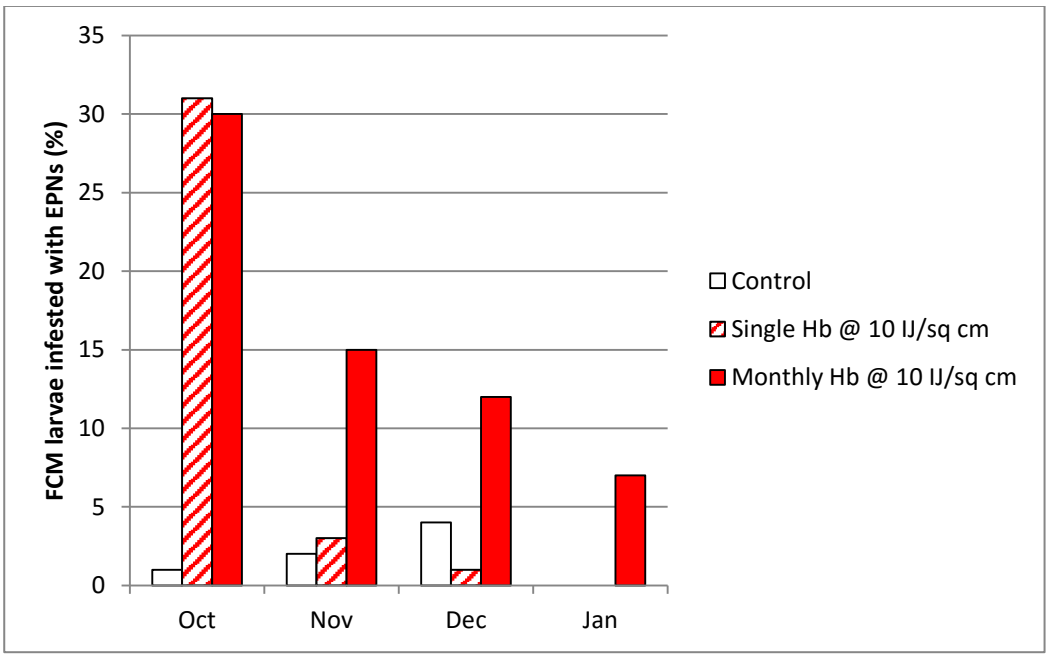

Figure 3.2.3.37. EPN infection of 5th instar FCM larvae baited onto soil from Ou Werf Farm, Citrusdal, Western Cape, before and after application of EPNs on various dates.

FCM population suppression

At Waverley Farm there was little difference in the numbers of moths caught per each treatment block (Fig. 3.2.3.38), indicating that this method for determining population suppression is variably reliable, as moths can enter the block from outside. However, unsurprisingly, slightly fewer moths were caught for the monthly 10 IJ/cm^2 treatment.

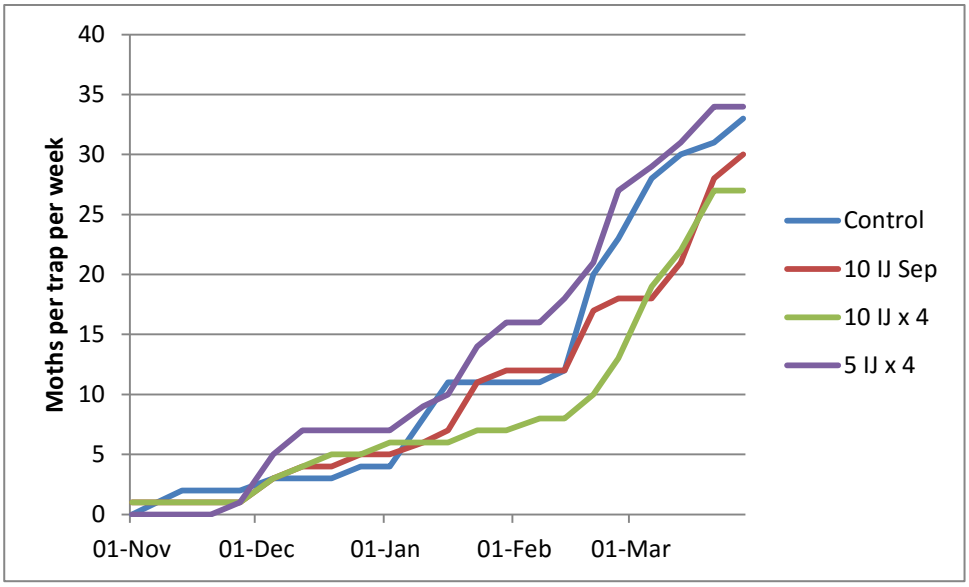

Figure 3.2.3.38. FCM caught per treatment (trap) per week at Waverley from 1 November 2013 to 28 March 2014, represented cumulatively.

Results of moth catches at the Montana trial site were a bit confusing, as by far the lowest catches were recorded for the 5 IJ/cm^2 treatment (Fig. 3.2.3.39). At best, one would have expected this to be similar to the monthly 10 IJ/cm^2 treatment, which was in fact slightly higher than the untreated control and almost identical to the once off 10 IJ/cm^2 treatment. Once again, either the shortcomings of using moth catches to indicate treatment efficacy, or the natural variability associated with using a large block for each treatment is being highlighted.

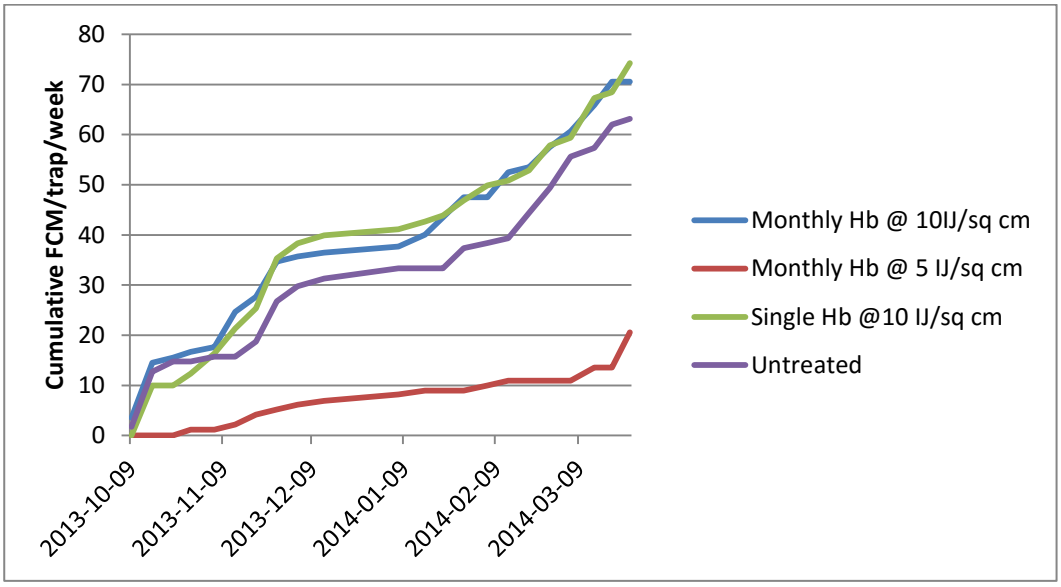

Figure 3.2.3.39. FCM caught per treatment (trap) per week at Montana Farm from 9 October 2013 to 26 March 2014, represented cumulatively.

Not a single moth was caught in any of the traps at Ou Werf Farm in the Western Cape during the trial period.

FCM damage reduction

By far the highest level of FCM infestation at the Waverley trial site was recorded for the 5 IJ/cm^2 treatment (3.2.3.40). This was peculiar and probably indicates that this particular block experienced extraordinary FCM pressure and that the low concentration of EPNs applied was insufficient to suppress this. However, results with the other treatments made sense, with the monthly 10 IJ/cm^2 treatment experiencing the lowest level of FCM infestation.

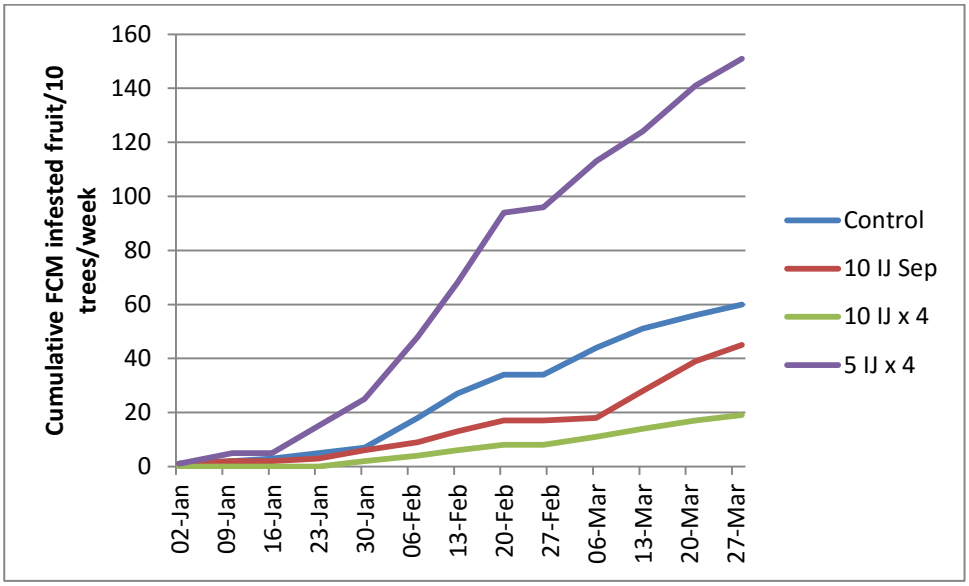

Figure 3.2.3.40. Cumulative FCM infested fruit per 10 trees per week at Waverley from 2 January to 28 March 2014.

No FCM infested fruit were recorded in any of the treatment blocks on Ou Werf Farm in the Western Cape up to the end of March 2014. Evaluation of fruit infestation at the Montana Farm trial site in Mpumalanga was only initiated after March 2014, as FCM levels were negligible up to this time.

Hot-spot trials

EPN recovery from FCM larvae exposed to soil samples taken immediately after application was 8% for the 10 IJ/cm^3 and 11% for the 20 IJ/cm^3 treatment. FCM trap catches were monitored from 28 March to 15 May. There was very little difference between treatments, with 16, 13 and 12 moths caught in the untreated control, 10 IJ/cm^3 and 20 IJ/cm^3 treatments respectively. FCM infestation was unfortunately very low, with 4, 2 and 2 fruit infested for the untreated control, 10 IJ/cm^3 and 20 IJ/cm^3 treatments respectively.

2014/15

At Stenhope Farm, EPN recovery was low for the two treatments (Fig 3.2.3.41). A second set of soil samples were taken on 18 June, 13 weeks after application. No EPNs were recovered from FCM exposed to these soil samples.

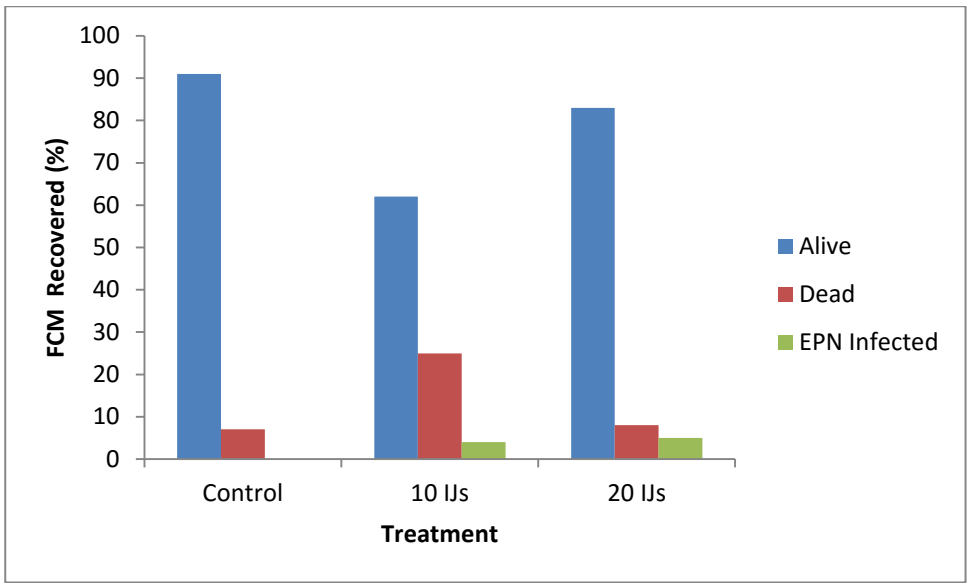

Fig 3.2.3.41. Mortality and EPN infection of FCM for various treatments in soil samples taken a day after application on 12 March 2015.

FCM infestation, monitored from 9 April to 26 May, can be seen in Figs. 3.2.3.42 and 3.2.3.43. The 10 IJ/cm^3 treatment resulted in a 47% reduction in infestation over the 8 week period of monitoring. However, the results were heavily influenced by very high infestation for one week (week 5) in the untreated control. Surprisingly there was no reduction in infestation as a result of the 20 IJ/cm^3 treatment.

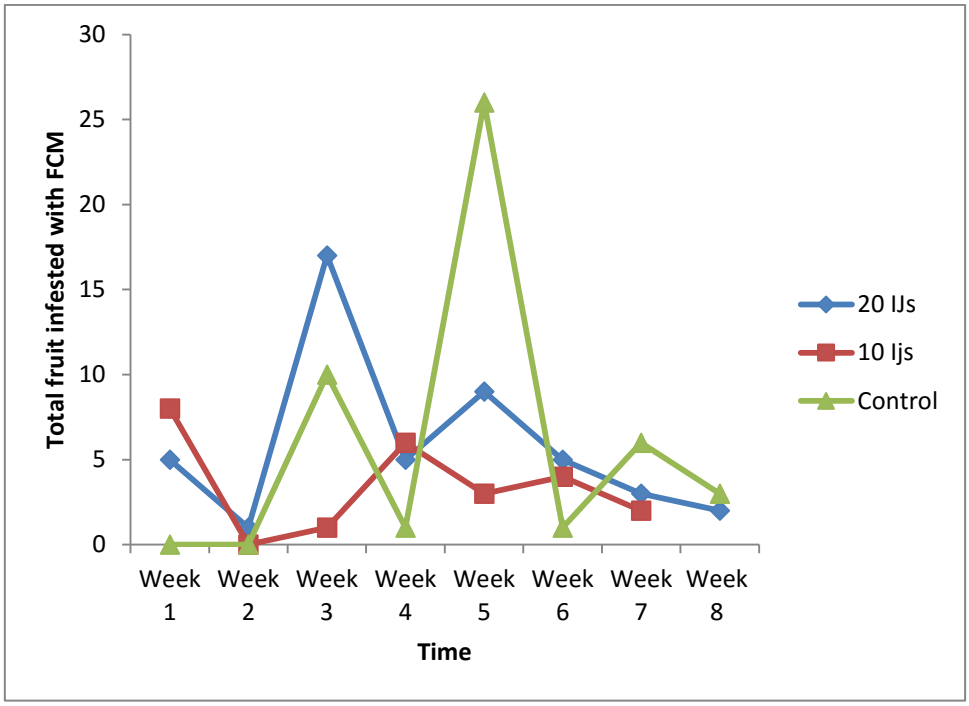

Fig 3.2.3.42. Weekly FCM infestation for untreated control and 2 EPN treatments, monitored from 9 April to 26 May 2015.

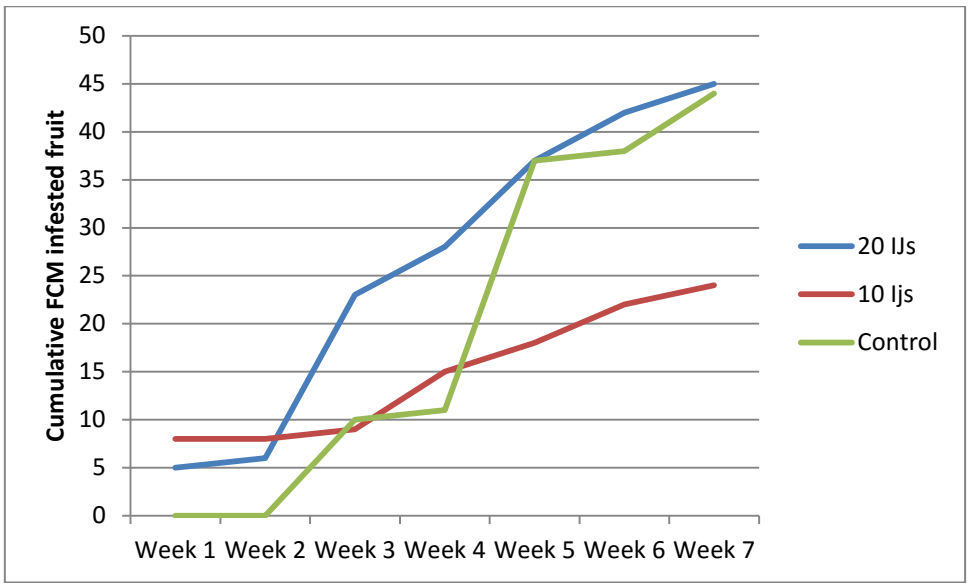

Fig 3.2.3.43. Cumulative FCM infestation for untreated control and 2 EPN treatments, monitored from 9 April to 26 May 2015.

Conclusions

Field trials provided substantial evidence that commercial application of EPNs to the soil in citrus orchards can meaningfully contribute to FCM control. A couple of trials also showed marked reduction in fruit fly levels. However, as with virtually all FCM control measures, EPNs are unlikely to be a stand-alone option, but can be an important component within an integrated approach, at a time (autumn to spring) and in a zone (soil) where no other control measures are currently directed. Additionally, it was clear that there are potentially a number of restraints to the efficacy of EPNs. Probably the most important of these is lack of moisture in the orchard, disqualifying drip irrigated orchards from the use of EPNs unless good and persistent rain can be guaranteed.

It was also apparent that monthly applications of EPNs may provide improved FCM control relative to a once-off spring application. However, it appears that at least in certain cases, it will not be possible to drop the concentration to below 10 IJ/cm^2.

Unfortunately, results with late season corrective trials were not as impressive as hoped, or as impressive as early season preventative applications, particularly monthly applications of EPNs for the first few months of the season.

Finally, where meaningful levels of EPNs occur naturally in the soil, these should be conserved, as they can contribute towards control of FCM populations and possibly fruit flies too.

Future research

As EPNs have been registered and are commercially available to growers, no further research is planned on this topic. However, if further research was to take place, the following aspects could be investigated:

- A more in depth study on the role of soil type.
- A more in depth study on the role of soil moisture, to the point of frequency of irrigation required, including relative to soil type.
- An survey for more indigenous EPN species, particularly those which might be more cold tolerant/active and thus usable in winter.
- Compatibility of EPNs with EPFs and potential synergism (a study on this was initiated a couple of years ago, but was terminated due to withdrawal of the student; the project might be picked up again in 2017).

Technology Transfer

The following technology transfer has been conducted from this project:

- A poster presentation was made at the biennial Citrus Symposium in the Drakensberg in August 2012.

- Feedback was provided to all regional grower study groups during September 2012.
- A paper was presented at an EPN workshop organised by River Bioscience in Stellenbosch during May 2013.
- An oral paper was presented at the IOBC meeting of the WPRS on Insect Pathology and Entomopathogenic Nematodes in Croatia in June 2013.
- Feedback was provided to all regional grower study groups during September 2013.
- An oral presentation was made at the Entomological Society of South Africa Conference in Grahamstown during July 2014.
- An oral presentation was made at the International Nematology Congress in Cape Town in May 2014.
- An oral presentation was made at the biennial Citrus Symposium in the Drakensberg in August 2014.
- A paper was published in Biocontrol, Science and Technology on the impact of naturally occurring EPNs, with Aruna Manrakhan as first author.

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3.2.4 FINAL REPORT: A feasibility study on the use of sniffer dogs for detecting FCM infested fruit post-harvest

Project 1071 (April 2013 – Mar 2016) by Sean Moore, Tim Grout, Wayne Kirkman (CRI), Stan Gillham (The Gillham Trust) and Pierre Olivier (SAPS)

Summary

This project proposes the use of dogs for detecting FCM-infested fruit post packhouse processing. The Gillham Trust is cooperating with us in conducting imprinting and training exercises with a 3-year old German Shepherd, named Max. After only one month of training, the dog already demonstrated an impressive ability to consistently and accurately detect oranges that had been infested with FCM only 3 or 4 days previously. Imprinting of the dog was continued to overcome barriers such as waxing of fruit, wrapping of fruit and burying of the infested fruit in a carton of healthy fruit. A series of trials were conducted where the dog was offered 10 cartons of oranges, of which usually only one contained an infested fruit. From a total of 140 runs over 6 replications, the dog achieved 98.9% true positive detections, of which 81.2% were on the first passing. It was clear that the dog's confidence and accuracy in detection improved with experience over time. The extraneous stimuli of a packhouse environment did not distract the dog.

Opsomming

Hierdie projek stel die gebruik van honde voor om VKM besmette vrugte na die pakhuis verwerkingsproses op te spoor. The Gillham Trust werk saam met ons op inprent en opleidings oefeninge met ŉ 3-jaar oue Duitse Herder, geenaamd Max. Na net een maand se opleiding het die hond alreeds ŉ indrukwekkende vermoë getoon om konstant en akuraat lemoene op te spoor wat net 3 tot 4 dae tevore met VKM besmet is. Opleiding van die hond het voortgeduur om struikelblokke te oorkom, soos waks op die vrugte, toegedraaide vrugte en die verberging van die besmette vrug in ŉ karton vol gesonde vrugte. ŉ Reeks proewe is uitgevoer waar 10 kartonne lemoene vir die hond aangebied is, waar daargewoonlik net een besmette vrug in een van hulle was. Uit ŉ totaal van 140 toetse oor 6 herhalings het die hond 98.9% ware positiewe opsporings bereik, waarvan 81.2% op die eerste verbystap was. Dit was duidelik dat die hond se selfvertroue en akuraatheid met opsporing oor tyd verbeter het met ondervinding. Die versteurende stimulasies van die pakhuis omgewing het nie die hond afgelei nie.

Introduction

Sniffer dogs have been tested and are now being used commercially in Florida for detecting the occurrence of citrus canker in orchards and on fruit in cartons (Jackson, 2012). They are also being used to detect vine mealybug in vineyards (Jackson, 2012), amongst a very wide range of other uses outside of agriculture, such as bed bugs (Cooper et al., 2014), mosquitoes (Sohn, 2014) and pine weevils (Suma et al., 2014). According to Tim Gottwald (USDA, Florida), dogs are extremely reliable (around 98-99%) and are rapid. Considering the impending FCM crisis in Europe, it would be irresponsible if we did not expeditiously investigate the potential for using dogs for detecting FCM-infested fruit. The ground work for such a venture has been established, through the study being conducted with NMMU on identification of volatiles associated with FCM-infested fruit (van der Walt, 2012). The idea is that dogs should be trained to sniff specific volatiles (and levels) associated with FCM-infested fruit and that these dogs should then be used in packing facilities, either on an individual fruit basis in a specially constructed line post-grading, or on a per carton basis pre-palletising. A collaborative relationship has been established with The Gillham Trust, which appointed a dog trainer to imprint a dog or dogs on FCM infested fruit relative to healthy fruit. This culminated in a series of replicated trials under different conditions, ultimately simulating commercially citrus packing conditions.

Objectives

1. To get an overview of the abilities and limitations of sniffer dogs and the implications of using them for detecting FCM-infested fruit, by visiting existing units which use dogs for detection.
2. To determine whether sniffer dogs can be trained to detect the difference between healthy and FCM-infested fruit and whether this applies at a very early stage of infestation.
3. To determine whether sniffer dogs can detect volatiles of FCM-infested fruit infused into odour filters.
4. Conduct a replicated trial with large numbers of fruit to determine the level of accuracy of sniffer dogs in finding FCM-infested fruit among healthy fruit.

Materials and methods

2012/13

This was not originally proposed as a scientific trial but as a feasibility study which would be conducted through visiting existing units that study, train or implement the use of dogs for detecting specific targets. Consequently, the Sedupe k9 (DAFF) Dog Unit at OR Tambo Airport and The Gillham Trust were visited in August 2013.

Thereafter, a study was initiated with the Gillham Trust. Healthy and FCM infested fruit were supplied to a dog handler appointed by the Gillham Trust in order to begin imprinting dogs with odours associated with infested fruit. Unfortunately, the initially appointed dog handler did not make good progress and fairly late during the 2013/14 season it was necessary to replace him with another dog trainer – Pierre Olivier. Pierre was supplied regularly with infested and healthy fruit, which he used for imprinting two dogs.

2013/14

A continuous supply of FCM infested fruit and healthy fruit was made available to Pierre Olivier for imprinting and training the dogs. In July 2014, after one month's training, a visit was paid to Pierre Olivier and his dog, Max, a 3 year old German Shepherd (the other dog had since been considered unsuitable). Eight 2 L glass bowls were placed in a circle on the floor and 3-4 healthy fruit were placed into each one. Thereafter, one fruit which had been infested with an FCM larva 4 days previously, was placed into one of the bowls. The dog was

then released to circle the bowls and sniff which one contained the infested fruit. This was repeated 40 times, changing the position of the bowl with the infested fruit on each occasion.

In September, after two months of training, a series of trials were conducted with fruit in cartons. This was done at CRI in Nelspruit. Eight cartons were filled with healthy unwaxed fruit. Into one of the cartons was placed a single FCM infested fruit, which had been infested four days previously. The eight cartons were placed in a row, with approximately 50 cm separating the cartons from one another. The ability of the dog to systematically sniff which carton contained the infested fruit was determined during a series of trials conducted over one and a half days. After each run, the position of the box with the infested fruit was changed. After approximately 10 runs, the carton with the infested fruit was replaced with a fresh carton with fresh healthy and infested (one) fruit. In total, about 100 runs were conducted.

2014/15

Imprinting

In May 2015, as soon as Navel oranges were again ripe for harvest, fruit were again infested with FCM larvae and healthy and infested fruit were sent to Pierre Olivier to resume training Max. A full set of replicated trials, conducted in such a manner that the results will be publishable in a scientific journal, is scheduled for September 2015.

Detection trials

For all trials, standard (not Supervent) 15 kg cardboard export cartons were used. Trials were conducted on the floor in a large garage (16 m x 19 m), which had been cleaned by sweeping and mopping before the trials were initiated. Cartons were always placed in a straight row, with approximately 40 cm between cartons and lids were always placed onto cartons for the trials. Positioning of cartons containing infested fruit was determined using a random number table. The position of the infested carton was thus changed for each replicate. The dog handler turned his back when this was done so that he could not see where the carton/s with the infested fruit was positioned. The carton was cryptically marked by the trial master using a marker pen. This was not observable to the dog handler. The dog was then collared and led by a leash by the dog handler, ensuring that each carton was sniffed in passing. The dog was scored on each replicate, out of a total of 20 or 30. This included whether he detected the infested carton or not, and on which passing he detected the infested carton (first, second etc.).

Fruit and infestation

Approximately 760 fruit from a Midknight Valencia orchard on Crocodile Valley Estates (25°28'39"S and 31°03'59"E) were obtained on 27 August 2015. The fruit were Count 56, meaning that approximately 56 fruit could be packed into a standard 15 kg export carton. Approximately 700 of the fruit had been through the packhouse and were thus treated with fungicide (imazalil and/or thiabendazole), waxed and packed in cardboard cartons as if for export. The remaining 60 fruit had not been through the packhouse.

On 28 August, each of these 60 fruit was inoculated with 4 neonate *T. leucotreta* larvae (less than 24 h old), by placing each larvae onto a fruit using a fine (size 000) paint brush. This was done in a laminar flow cabinet and fruit were left in the cabinet for at least 1 h in order to allow larvae to safely penetrate into the rind of fruit. On 31 August, fruit were inspected for certain signs of larval penetration i.e. frass protrusion from a minute penetration mark. Half of the clearly infested fruit were waxed in the packhouse and the remaining fruit were left unwaxed. On 1 September, 4 days after inoculation of fruit took place, fruit were again inspected for certain signs of larval penetration. These fruit were marked with small round green stickers.

Detection of unwaxed infested fruit

Ten cartons were placed in a row on the floor. Two waxed uninfested fruit were placed into each carton. In addition, one infested unwaxed fruit was placed into one of the cartons. Within a few seconds of the infested fruit being placed into the carton and the lid being closed, the trial began. After each five replicates, the carton containing the infested fruit was replaced i.e. a fresh carton with other healthy and infested fruit.

Detection of waxed infested fruit

This trial was conducted identically to the previous one, except that waxed infested fruit were used.

Detection of waxed infested fruit in cartons of fruit

This trial was conducted in a similar manner to the previous two, except that a total of 30 healthy waxed fruit were placed into each carton and two out of the 10 cartons contained an infested fruit.

Detection of waxed wrapped infested fruit

Two fruit were placed into each of 10 cartons, one of the fruit being wrapped. The wrapped fruit in one of the cartons was infested and waxed.

Detection of waxed wrapped buried infested fruit

One infested, waxed and wrapped fruit was placed at the bottom of a carton and covered with 40 uninfested waxed fruit. The remaining nine cartons also had 40 waxed fruit but no infested fruit. Several wrappers were also placed into all cartons.

Packhouse visit

After completion of the trials, a visit was made to the packhouse at Crocodile Valley Estates in order to test Max's performance in a busy operational packhouse. A 20-run trial was conducted with 10 cartons, all of which contained about 30 healthy fruit and one of them contained one infested fruit. Cartons were placed on the floor in the packhouse.

Thereafter, cartons were elevated onto a roller and Max's ability to detect infested fruit at this new height was tested.

Results and discussion

2012/13

Visit to Sedupe K9 (DAFF) Dog Unit

Sean Moore arranged with the head of the unit, Amanda Steyn, to visit the unit at OR Tambo Airport. However, he was hosted by Alex Mangole (head of training) and Ronald Shibambu (head of operations). The international arrivals baggage collection area was visited and the dogs were observed at work. Thereafter, a visit was made to the unit at the airport where the dogs are housed and trained. On arriving at the baggage collection area, there were no dogs operating on the floor. This was disappointing, as there were many international travellers collecting their baggage and it was clear that prohibited agricultural goods could easily have entered the country without detection. The hosts were equally unimpressed and rapidly triggered at least one handler and his dog into action. The dog was observed operating on the floor for a few minutes. He detected bread in a traveller's bag (this was not prohibited). Earlier that morning (not observed by us), a dog had detected figs and mint in baggage from Tel Aviv, which had been confiscated.

The unit was started in 2004 with a visit by a few potential dog handlers to the USDA dog unit in Florida, where they received 12 weeks training and returned with dogs. There are now 22 dogs at OR Tambo and fewer at Cape Town and Durban international airports. Training now takes place at OR Tambo. Most of the dogs are Beagles. After 10 weeks of training, they are employed at international arrivals. The remainder of the dogs are Labradors, which after 14 weeks of training, are used for sniffing cargo. Training of dogs can begin as early as 3 months old, but usually not as early as this. Dogs need to have the right temperament for the job i.e. they must be good with people, must be very fond of food and must be energetic.

In training, dogs are initially imprinted on five cues – citrus, apples, mango, pork and beef. Initially, these five cues are lumped together and then separated, once the dog is successfully imprinted. Gradually, additional plant and meat products are added, so that dogs learn to generalise. A demonstration of training at the dog unit was observed. When a dog makes a positive detection, it is rewarded with a small snack. In training, dogs are also taught to detect prohibited goods through all types of possible disguises, such as coffee, perfumes and wrappings.

The dogs are very well looked after and all appear happy and in good condition. Their weight is strictly monitored and diet modified if necessary. The also receive regular veterinary check-ups, have their teeth brushed daily and are on an exercise programme. It is very clear that they love their work.

The Gillham Trust

Sean Moore also visited The Gillham Trust in Pretoria. Stan Gillham told about his background as Brigadier and head of the SA Police Dog Unit and about the work of the The Gillham Trust. The Gillham Trust has operated in countries throughout the world, mostly training and implementing sniffer dog units to detect landmines and other explosives. They use what they call "high drive" dogs to do this work. Such dogs can apparently work non-stop for three quarters of a day. The best dogs are Springer Spaniels, Beagles and Collies.

Tim Grout provided the Gillham Trust with some FCM and fruit fly infested fruit to conduct initial imprinting trials with a couple of their dogs. Initially, fruit in an advanced state of decay were used, apparently eliciting a good response from the dogs. However, progress thereafter was unsatisfactory and the initially appointed dog handler had to be replaced by a second handler, Pierre Olivier. Thereafter, progress was positive and rapid.

Unfortunately, one of the dogs proved to fatigue rather quickly, and so further imprinting was continued with only one dog.

2013/14

Unfortunately, one of the dogs proved unsuitable, as he was over-excitable. However, the remaining dog, Max, made excellent progress in a short space of time. As a previous handler was originally appointed and as this handler proved to be unsuitable and had to be replaced, there were only two months before the end of the season, during which time Max could be trained before the series of trials was conducted.

In the preliminary trial conducted in glass bowls, Max correctly and rapidly (approximately 2 s on each occasion) detected the bowl with the infested fruit on each of 40 occasions.

Unfortunately, the trials conducted in cartons in September 2014 were not as successful as hoped, as the last fruit which Max was imprinted with were in a very advanced state of infestation and for a couple of weeks before the trials, no infested fruit was available. Consequently, most of the one and a half days of trials was spent re-imprinting Max. However, at the end of this period, Max did achieve a successful series of detections (10 out of 10).

2014/15

Max's detection of unwaxed infested fruit was 100% (80% on the first pass) (Table 1.2.x.1). However, this declined to 95% (70% on the first pass) in the first waxed infested fruit trial. Although this appeared to only be slightly improved in the third trial in which there were two cartons with waxed infested fruit (96.7% detection with 73.3% on the first pass), the improvement was in fact better than this. Initially only 20 runs (40 detections) of trial 3 were conducted, for which Max's detection was 95% (75% on the first pass). After a break, a further 10 runs (20 detections) were conducted for which detection was 100% (70% on the first pass). It therefore appears that changing from unwaxed to waxed fruit required quite a period of re-imprinting.

Table 3.2.4.1. Performance of the sniffer dog in detecting cartons containing single FCM infested fruit in a series of trials

Trial		**Runs**	**Correctly detected**	**Passing on which detected**			**Incorrect selection**
				1st	**2nd**	**3rd or more**	
1	Unwaxed fruit	20	20	16	2	2	0
2	Waxed fruit	20	19[2]	14	4	1	1
3	Waxed fruit in 2 cartons	30 (60)[1]	58	44	10	3	2
4	Waxed and wrapped fruit	20	20	20	0	0	0
5	Fruit waxed, wrapped and buried	20	20	19	1	0	0

[1]30 runs but 60 detections i.e. 2 boxes with an infested fruit in each run.

[2]After four unsuccessful attempts, the infested fruit was changed after which there was an immediate correct detection.

In total, Max was given 140 detection opportunities, making 137 correct decisions (97.86%), of which 113 were correct on the first pass (80.71%) (Table 3.2.4.1). In the final trial conducted, once Max had been thoroughly imprinted with the odour of wrapped, waxed infested fruit, he detected 100% of infested fruit. All, but the very first run were detected on the first passing (95%). Calculated as mean performance values of the six replicates, the performance was even better (Table 3.2.4.2).

Table 3.2.4.2. Mean (± SE) performance of the sniffer dog in detecting cartons containing single FCM infested fruit in six trials, each considered as a separate replicate

True/positive responses (mean % ± SE)	True/positive responses on the first attempt (mean % ± SE)	False/positive responses (mean % ± SE)
98.88 ± 1.05	81.25 ± 5.39	1.67 ± 1.05

All supposedly infested fruit used in the trials were dissected immediately after completion of the trials. Larvae were found in all but one of the fruit. Larvae were approximately 2 mm long and between first and second instar.

Packhouse visit

Max detected 100% of infested cartons correctly. All but the last two runs were detected on the first passing. This may have been an indication that Max was starting to tire after a long day of hard work.

Max struggled to detect the correct carton, when cartons were elevated onto a roller. The handler spent approximately 40 minutes conditioning Max to sniff upwards as opposed to downwards, as in all of his previous conditioning. It was clear that Max was capable of detecting the infested box on the roller, but that he needed for time to be re-imprinted with this change in protocol and to rebuild confidence in his ability.

Conclusion

A 3-year old German Shepherd demonstrated an impressive ability to consistently and accurately detect recently FCM infested oranges, even when they were waxed after infestation, wrapped and buried in a carton of healthy fruit. The performance of the dog appeared to improve with continued exposure to and imprinting on the exact scenario used. The stimuli filled environment of an operational citrus packhouse did not seem to distract the dog nor compromise its performance. It is thus clear that sniffer dogs have potential for employment in a citrus packhouse set up, in order to improve the current ability to detect FCM infested fruit. This could be employed for inspection of all packed fruit coming through the packhouse or for representative samples from consignments of fruit, whichever is the most practical in any particular situation. It may be necessary for some sort of dispensation from current regulation in order to allow sniffer dogs to operate in a packhouse.

Technology Transfer

Reports have been made at several grower meetings of this project and the promise that the technology holds. A presentation and demonstration is planned for the biennial Citrus Symposium in the Drakensberg in August 2016.

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3.2.5 **FINAL REPORT: Classical biocontrol introduction of *Agathis bishopi* into the Western Cape**

Project 1077 (2013/14 - 2015/16) by Martin Gilbert (CRI)

Summary

Larval parasitoids of false codling moth have never previously been found in the Western Cape. In 2013 sampling of sanitation fruit was begun to see whether previous observations regarding absence of larval parasitism were still valid. If absent, then *Agathis bishopi*, from a culture at Rhodes University, would be released in the Citrusdal area in an attempt to aid biological control of FCM. In 2013/14 no *A. bishopi* was found in the Citrusdal area. In 2014/15 and 2015/16 sanitation fruit was again sampled on a weekly basis, from two farms within the same production area that were considered hotspots for FCM. The presence of *A. bishopi* was not detected in any samples. The *A. bishopi* culture at Rhodes University experienced prolonged fungal contamination during 2014/15 and adults were not available for release in the Western Cape. Unfortunately, the colony is no longer in existence. It is now more logical to attempt to breed *A. bishopi* in Stellenbosch. Previous releases were done on a small scale in the Citrusdal area without success. It may be possible to establish *A. bishopi* in other fruit production areas of the Western Cape. In 2016 FCM has been of growing importance as a pest in grapes in the Western Cape. Grapes and citrus are often grown in close proximity in this region. The increasing adoption of grapes as a host (close to citrus) increases the risk to the latter crop. Improvement of biocontrol of FCM remains therefore an important goal. A new proposal and budget for the breeding of *A. bishopi* at Stellenbosch will be drawn up.

Opsomming

Parasitoeïde van die larwes van valskodlingmot (VKM) is nie vantevore in die Wes Kaap gevind nie. In 2013 is daar begin om monsters te neem van sanitasievrugte om te bepaal of vorige waarnemings ten opsigte van hierdie afwesigheid van sulke parasitoeïde, nog geldig is. Indien nog afwesig, sal *Agathis bishopi* van Rhodes Universiteit in die Citusdal area vrygelaat word in ʼn poging om die biologise beheer van VKM te verbeter. In 2013/14 is geen *Agathis bishopi* in die Citrusdal area gevind nie. In 2014/15 is die ondersoek op twee plase wat bekend is vir die hoë voorkoms van VKM voortgesit. Daar is egter steeds geen *A.bishopi* in enige van die monsters gevind nie. Die teling van *A.bishopi* by Rhodes Universiteit gedurende 2014/15 het baie probleme ondervind met swam kontaminasie wat tot gevolg gehad het dat insekte nie vir loslating in die Wes Kaap beskikbaar was nie. Ongelukkig bestaan die kolonie nie meer nie. Dit maak dus nou meer sin om *A. bishopi* op Stellenbosch te probeer teel. Alhoewel vorige loslatings van *A. bishopi* op Citrusdal onsuksesvol was, mag dit dalk nog moontlik wees om dit in ander dele van die Wes Kaap te vestig. Die fokus op VKM as ʼn plaag van ander vrugtesoorte insluitent druiwe in die Wes Kaap neem skerp toe. Die verbetering van biologiese beheer van VKM bly ʼn belangrike mikpunt. Dus sal ʼn nuwe navorsings voorstel en begrooting vir die teling van *A. bishopi* in Stellenbosch opgestel word.

Introduction

Agathis bishopi is a larval parasitoid of false codling moth (FCM) which appears to be limited to the Eastern Cape (Prinsloo 1984) and contributes, sometimes significantly, to natural mortality of the pest on commercial citrus in this area (Moore *et al.* 2009, Zimba *et al* 2015).

To date no evidence of larval parasitism of FCM in the Western Cape citrus-growing regions has been found (Sishuba 2003, Gendall 2007). In spite of this, there existed the need for a more detailed survey in order to establish the status of any *A. bishopi*, or other FCM larval parasitoids, that may hitherto have escaped detection.

Objectives

- To confirm the presence or absence of naturally occurring *A.bishopi* in Western Cape citrus-producing areas.
- If found to be naturally absent, the introduction of *A.bishopi* adults from a culture at Rhodes University into Western Cape citrus-producing areas.

Materials and Methods

Sanitation fruit were sampled from 20 data trees per week at a farm with a high incidence of FCM in the Citrusdal area during January to early May 2014 - 16. 20-40 collected fruit per week were returned to the lab and placed into emergence boxes with sand covering the floor of the box. Any emerging parasitoids resembling *A. bishopi* would have been sent for identification.

Results and Discussion

Despite sampling occurring over a long period of time, no *A. bishopi* were recovered from FCM-infested fruit and no other larval parasitoids were found. The difficulty of breeding significant numbers of *A. bishopi* became apparent from the inability to maintain a long-term culture in the Eastern Cape. Adults were therefore not available for a release programme in the Western Cape.

Conclusion

This survey confirms that *Agathis bishopi* does not occur naturally on citrus in the Citrusdal area of the Western Cape. It was also not possible to attempt to establish *A. bishopi* in this area at this time as was originally planned.

An attempt should be made to establish a breeding colony of *A. bishopi* in Stellenbosch as done in the Eastern Cape (Zimba *et al* 2016) and this will be proposed for a future project. The increasing importance of FCM as a pest on other fruit types, often grown in close proximity to citrus, in the Western Cape makes such a project of greater significance than before.

Technology Transfer

None.

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3.2.6 PROGRESS REPORT: Laboratory handling and quality control for SIT: an experimental assessment of FCM chilling and flight performance with respect to the improvement of moth production parameters, particularly pertaining to improved cold-tolerance

Project 1079 (2014/15 – 2015/16) by Nevill Boersma (Xsit), John Terblanche (SU) and Martin Gilbert (CRI)

Summary

Sterile false codling moth adults (FCM) are known to be less active at low temperatures than their wild counterparts. This has implications for the efficacy of a Sterile Insect Release (SIT) programme for the control of FCM, particularly during the cooler months when harvesting of citrus approaches. The project aim is to expose sterile FCM to different thermal conditions and to assess the effect of these on flight-, and mating ability. Changes could then be incorporated into the rearing process in order to improve the fitness of released sterile moths.

Critical Thermal limit. Varying the acclimation temperatures does not yield significant results. This was especially clear when considering adult males. Instead, the critical thermal minimum of *T. leucotreta* is most influenced by its sex. CTmin is also influenced by the treatment temperature. This was observed for both males and females, but the effect is greater in females than in the males.

Fecundity. Results of treatments where normal pairings were involved were compared to those without. Fecundity is reduced when normal insects are included. However normal male x treated female pairings showed significantly higher fecundity than the treated males x normal female pairings. In fact, normal male x treated female pairings remained within the same order of significance as treated male x treated female pairings, showing that females respond more strongly to prior thermal acclimation than males, but when both sexes have been exposed it is equivalent to the females' response alone despite the treatment. The experiments showed that the sex of the treated individual within the pairing has a significant effect, but that the contribution of treated males to the fecundity of treated male x treated female pairing is nearly negligible.
Longevity. From the longevity experiments, the difference between longevity for males and females is the most prominent characteristic. Clearly, females demonstrate a greater variance in their longevity due to environmental influences than males. Stated differently, males exhibit less plasticity than females, which was true also for fecundity and CTmin characteristics. The statistical analysis states that both acclimation and temperature treatments have a significant impact

Opsomming

Dit is bekend dat steriele valskodlingmotte (VKM) minder aktief is by laer temperature as wat wilde motte is. Dit hou implikasies in vir die effektiwiteit van ŉ steriele insek tegniek program (SIT) vir die beheer van VKM gedurende die koeler maande voor die begin van die sitrus plukseisoen. Die projek het ten doel om steriele VKM aan verskillende temperatuur kondisies bloot te stel en om die effek van hierdie vlieg- en paringsvermoë te assesseer.
Kritieke termiese minimum. Alhoewel temperatuur behandeling VKM se kritieke termiese minimum beïnvloed, is die invloed van geslag meer merkwaardig. Byvoorbeeld, alhoewel die kritieke termiese minimum van mannetjies ook varieer soos wat temperatuur behandeling varieer is dieselfde effek meer prominent in wyfies. Ten spyte hiervan dui resultate dat akklimasie temperatuur nie ŉ wesenlike rol speel in die kritieke termiese minimum van VKM nie, ongeag die geslag.
Fekunditeit. Statistiese analiese wys dat fekunditeit laer is wanneer slegs een individu van ŉ mot paar onderworpe word aan akklimasie en temperatuur behandelings. Verder wys resulate dat die geslag van die individu wat behandeling ontvang, ŉ merkwaardige rol speel in eierlegging en dus ten einde die effek van akklimasie en temperatuur beïnvloed. Resultate wys dat paring tussen ŉ wyfie wat behandeling ontvang het en normale mannetjie aansienlik meer eiers sal lewer as ŉ paring tussen ŉ normale wyfie en ŉ mannetjie wat behandeling ontvang het. Meer spesifiek, die eierlegging van hierdie paar (behandelde wyfie met normale mannetjie) is vergelykbaar met dié van ŉ paar waar beide individuee onderworpe was aan behandeling. Hierdie voorbeeld dui dat die byvoeging van ŉ behandelde mannetjie weglaatbaar is spesifiek omdat akklimasie en temperatuur behandelings ŉ groter invloed het op wyfies.
Langlewendheid. Alhoewel statistiese ontleding klem lê op die impak van akklimasie en temperatuur behandelings op langlewendheid, word die invloed van geslag meer prominent ten toon gestel op grafieke. Variasie in langlewendheid, wat dui op mate van plastisiteit te midde van veranderende omgewingstoestande, is meer prominent in wyfies.

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

Project 1111 (Oct 2014 – Dec 2015) by Sean Moore and Sean Thackeray

Summary

A total of 29 citrus growers and citrus cooperatives/companies in seven general regions throughout South Africa and Zimbabwe were contacted to collect and supply monitoring data for FCM for the duration of the 2015 season until harvest. Additionally, Xsit agreed to supply their data from Citrusdal, the Sundays River Valley and the Gamtoos River Valley. The data will be used to determine the current ability in each region to comply with the proposed standards of the drafted FCM systems approach and to determine which areas can be regarded as areas of low pest prevalence. The latter will indicate the potential of the area for achievement of pest freedom. It has subsequently been decided to collect such data for a second subsequent season in order that the data might be publishable. The preparation of a final report on this project has thus been postponed for a year.

Opsomming

In totaal is 29 sitrus produsente en sitrus koöperatiewe/maatskapye in sewe algemene streke deur Suid-Afrika en Zimbabwe genader om VKM moniterings data deur die loop van die 2015 seisoen tot oestyd te versamel en om dit aan ons te voorsien. Boonop het Xsit ingestem om hulle data van Citrusdal, die Sondagsrivier Vallei en Gamtoosrivier Vallei te voorsien. Die data sal gebruik word om te bepaal was is die huidige vermoë in elke

streek om die voorgestlede standaarde in die VKM stelselsbenadering na te kom en om te bepaal watter areas as streke van lae plaag voorkoms beskou kan word. Laasgenoemde sal die moontlikheid aandui om plaag-vryheid in ʼn streek te bereik. Dit is onlangs besluit om sulke data vir ʼn tweede agtereenvolgende seisoen te versamel omrede die data dan moontlike publiseerbaar sal wees. Die voorbereiding van ʼn finale verslag op hierdie projek is dus vir ʼn jaar uitgestel.

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

Project 1024 (Apr 2015 – Mar 2016) by S.D. Moore (CRI), Martin P Hill (RU), J.F. Dames (RU) and C.A. Coombes (RU)

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 Bb1 under micro-sprinkler irrigation, and 60% for isolate Ma2 under drip irrigation. In addition, Bb1 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. The thesis, which is available on request, is also being published as three journal papers. However, the study is continuing and a final report will be submitted thereafter.

Opsomming

Die effektiwiteit en nawerking van drie EPS isolate vir die beheer van VKM,is onder ʼn verskeidenheid veldtoestande ondersoek gedurende die verloop van vyf veldproewe uitgevoer tussen die 2013/2014 en 2014/2015 sitrus groeiseisoen. Resultate is tot dusver uitstekend, met ʼn 80% afname in VKM besmetting aangeteken vir die isolaat Bb1 onder mikrobesproeiing en 60% vir die isolaat Ma2 onder drupbesproeiing. Daarbenewens was Bb1 ʼn doeltreffende beheermaatreël wanneer dit ongeveer twee maande voor oes toegedien is, met ʼn afname in besmette vrugte van tussen 50 en 80%. In alle veldproewe is ʼn afname in vrugval binne alle behandelings blokke ook gevind. Alle isolate het ʼn nawerking van vyf maande na toediening getoon, met ʼn verhoging in swam titer (konsentrasie) by sekere persele aangetoon. Resultate dui ʼn beter nawerking van swamme in natter as droër gronde aan, as gevolg van die besproeiings sisteem wat gebruik is. Benewens evaluaring van die effektiwiteit en nawerking in die veld, is die verenigbaarheid van hierdie isolate ook met agt geregistreerde swamdoders geëvalueer, wat aangedui het dat net macozeb ʼn effense probleem vir EPSe mag inhou. Voorlopige evaluasie van formulasies is ook uitgevoer. Candice Coombes het haar tesis voltoei en is met ʼn PhD toegeken. Die tesis, wat op versoek beskikbaar is, word ook tans as drie joernaal manuskripte gepubliseer. Nogtans word die studie nog egter voortgesit en ʼn finale verslag sal daarna ingedien word.

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

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

Summary

Due to a Pest Risk Assessment (PRA) having been recently conducted by the European Plant Protection Organisation (EPPO) on FCM and the impending debilitating outcome of this PRA for export of southern African citrus to Europe, a study on the effect of abbreviated cold treatments on FCM survival was initiated in 2012. Most work conducted between 2012 and 2014 was aimed at demonstrating the efficacy of incomplete cold treatments for use as a step in a systems approach. Subsequently, studies were conducted to determine whether probit 9 efficacy of cold treatments against fourth and fifth instar FCM, the most cold-tolerant larval life stages, could be achieved with improved postharvest cold treatments i.e. warmer temperatures and/or shorter duration. Three time-temperature combinations were evaluated: 20 d, 18 d and 16 d at average temperatures in the range of -0.14 °C to -0.43 °C. All treatments were shown to cause mortality at or in excess of the probit 9 level (99.9968% efficacy at the 95% confidence level). These results validate the efficacy of the following postharvest cold treatments for FCM for phytosanitary treatment purposes: 16 d at -0.2 °C, 18 d at -0.4 °C and 20 d at -0.4 °C, which are recommended for conversion into the following treatment protocols: 16

d at or below -0.1 °C, 18 d at or below -0.3 °C and 20 d at or below -0.3 °C. The shorter duration and in some cases higher temperatures will be less costly and logistically easier to apply with reduced adverse effects in the form of fruit chilling injury than the cold treatments currently in use. Finally, a trial is currently underway to establish a probit 9 efficacy treatment at 1°C. Thus far it has been determined that 19 d is the probably duration of exposure required. Three replicates, making up a total of almost 100 000 larvae, will be conducted under these conditions.

Opsomming

As gevolg van ŉ Plaag Risiko Analise (PRA) wat pas deur die Europese Plantbeskermings Organisasie (EPPO) op VKM uitgevoer is en die dreigende uitkoms van die PRA vir die uitvoer van sitrus van suidelike Afrika Europa toe, is ŉ studie op die effek van verkorte koue behandelings op VKM oorlewing in 2012 gëinisieer. Meeste werk wat tussen 2012 en 2014 uitgevoer is het die werking van onvoledige koue behandelings as ŉ stap in ŉ stelselsbenadering gedemonstreer. Daarna is studies uitgevoer om te bepaal of probit 9 doeltreffendheid van koue behandelings teen vierde en vyfde instar VKM, die mees koue tolerante lewensstadiums, met verbeterde na-oes koue behandelings bereik kon word dws warmer temperature en/of korter blootstelling. Drie tyd-temperatuur kombinasies is geëvalueer: 20 d, 18 d en 16 d teen gemiddelde temperature van -0.14 °C tot -0.43 °C. Alle temperature het hoër mortaliteit as die probit 9 vlak (99.9968% effektiwiteit teen ŉ 95% vlak van vertroue) veroorsaak. Hierdie resultate verifieer die doeltreffendheid van die volgende koue behandelings vir VKM vir fitosanitêre doeleindes: 16 d teen -0.2 °C, 18 d teen -0.4 °C en 20 d teen -0.4 °C. Dit word aanbeveel dat hulle tot die volgende behandelings protokolle omgesit word: 16 d teen of onder -0.1 °C, 18 d teen of onder -0.3 °C en 20 d teen of onder -0.3 °C. Die korter duur en in sekere gevalle hoër temperature sal nie so duur wees nie en logisties makliker wees om toe te pas met minder nadelige effekte as huidige koue behandelings, veral wat koueskade betref. Laastens, is ŉ proef tans aan die gang om ŉ probit 9 effektiwiteits behandeling teen 1°C te ontwikkel. Dit is tot dusver bepaal dat 19 d die mees waarskynlike blootstellings tydsduur sal wees. Drie replikate met omtrent 100 000 larwes, sal onder hierdie omstandighede uitgevoer word.

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

Project 1060 (2013/4, 2015/6-2016/7) by Tim G Grout and Peter R Stephen (CRI)

Summary

A feasibility study in 2013/4 indicated that it would be worthwhile constructing a machine that could treat citrus fruit with hot air at high relative humidity. Phillip Lewis built such a machine from an old chest freezer and it can be used to heat fruit up to 50°C at relative humidities above 70%, which was not possible with an environmental chamber. The small heat chamber was used to conduct trials on the susceptibility of false codling moth (FCM) eggs on fruit and first, second and third instar FCM in fruit, to a temperature of 44°C and relative humidity of 60 to 80%. The eggs and larvae were treated for 6 h and then allowed to cool down in ambient air before assessment. Third instars were the most heat tolerant of the life stages tested. Three trials using third instars were then conducted at 44, 46 and 48°C where fruit were exposed to these temperatures for 6 h. Intermediate mortalities were obtained at 44°C but 100% mortality at 46 and 48°C. The treatment at 46°C involved 681 larvae in 40 fruit and requires further investigation, but research was suspended so that PRS could run urgent cold treatment trials on FCM (project 1039). Once the cold treatment research has been completed this hot air research will continue and may need to be extended for another year.

Opsomming

ŉ Uitvoerbaarheidstudie in 2013/4 het aangedui dat dit die moeite werd sal wees om ŉ masjien te bou wat sitrusvrugte met warm lug teen ŉ relatiewe hoë humiditeit kon behandel. Phillip Lewis het so ŉ masjien uit ŉ ou vrieskas gebou en dit kan gebruik word om vrugte tot 50°C te verhit teen ŉ relatiewe humiditeit hoër as 70%, wat nie met ŉ omgewingskamer moontlik was nie. Hierdie klein verhittingskamer is gebruik om proewe te doen op die vatbaarheid van valskodlingmot (VKM) eiers op vrugte en eerste, tweede en derde instar VKM in vrugte te bepaal, tot ŉ temperatuur van 44°C en relatiewe humiditeit van 60 tot 80%. Die eiers en larwes is vir 6 h behandel en toe in omgewingslug afgekoel voor hulle ontleed is. Van die lewensstadiums wat getoets is is derde instars die mees hitte verdraagsaam. Drie proewe met derde instars is toe teen 44, 46 en 48°C uitgevoer, waar vrugte vir 6 aan hierdie temperature blootgestel is. Matige mortaliteit is op 44° C verkry, maar op 46 en 48°C is 100% mortaliteit verkry. Die behandeling by 46°C het 681 larwes in 40 vrugte gebruik en vereis ŉ verdere ondersoek, maar navorsing is geskors sodat PRS dringende kouebehandelings proewe op VKM (projek 1039) kon uitvoer. Sodra die koue behandeling navorsing afgehandel is, sal hierdie warm lug navorsing voortgaan en mag dus dalk nog ŉ jaar gebruik.

3.2.11 **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 accidently, that 7-vinyldecyl acetate 1 (7-VDA) was capable of preventing adult false codling moth (FCM) males from locating virgin females. Consequently, we decided to examine this further with a view to developing a novel mating disruption, or rather a mating inhibition, technology. A novel polyethylene dispenser was developed, which allowed a consistent release rate of 7-VDA, comparable to or better than that of the female FCM pheromone from a commercial dispenser. Laboratory mating inhibition trials were conducted with virgin pairs of moths in plastic containers, with dispensers loaded 24 h before moths were introduced. Both FCM pheromone and 7-VDA showed the ability to reduce fecundity, an indication of reduced mating. Results were variable, but on average FCM pheromone appeared to be more effective. However, a subsequently tested combination of 7-VDA (5-10%) and FCM pheromone (90-95%) proved to be the most effective treatment, reducing fecundity by 69%. Trials were repeated using a modified protocol, designed to reduce variability, where females were removed from the plastic containers after 48 h and placed into petri dishes to record fecundity and fertility. Although the efficacy of the 7-VDA-FCM pheromone combination to reduce mating was confirmed, again achieving up to 69% reduction in fecundity, results continued to be variable. As a result of this variability and variability in previously conducted field cage studies, future studies will focus on full scale field trials in order to reliably determine the true potential of the technology. Consequently, adequate volumes of 7-VDA are currently being synthesised.

Opsomming

Jare gelede is dit ontdek, amper toevalig, dat 7-vinieldesielasetaat 1 (7-VDA) die vermoë het om volwasse valskodlinmot (VKM) mannetjies te verhoed om ongepaarde wyfie motte te vind. Daarom het ons besluit om hierdie verder te ondersoek met die moontlikheid van ʼn oorspronklike paringsontwrigting – of liewer paringsverhoeding – tegnologie te ontwikkel. ʼn Nuwe polietileen vrysteller is ontwikkel, wat ʼn konstante vrystellings tempo van 7-VDA toegelaat het en wat vergelykbaar of selfs beter was as die VKM wyfie feromoon in ʼn kommersiële vrysteller. Laboratorium parings inhibisie proewe is met ongepaarde mot pare in plastiek houers uitgevoer. Vrystellers is in die houers gelaai 24 ure voor die motte ingesit is. Albei VKM feromoon en 7-VDA het die vermoë getoon om fekunditeit te verminder, ʼn aanduiding van verminderde paring. Resultate is wisselvalig maar oor die algemeen het die VKM feromoon meer doeltreffend voorgekom. In proewe daarna was ʼn kombinasie van 7-VDA (5-10%) en VKM feromoon (90-95%) die mees doeltreffend en het fekunditeit met 69% verminder. Proewe is met ʼn verbeterde protokol herhaal, wat ontwikkel is om variasie te verminder. Na 48 ure is wyfies van die plastiek houers verwyder en in petribakke gesit om hulle fekunditeit en fertiliteit te meet. Al is die werking van die 7-VDA-VKM feromoon kombinasie bevestig, met alweer ʼn afname in fekunditeit van tot 69%, was resultate weereens wisselvallig. As gevolg van hierdie variasie en die variasie in voorheen uitgevoerde veld-hokproewe, sal navorsing in die toekoms op volskaal veldproewe fokus om die ware potensiaal van dié belowende tegnologie te bepaal. Gevolglik word, voldoende volumes 7-VDA huidig gesintetiseer.

3.2.12 **PROGRESS REPORT: Novel approaches to mating disruption of FCM**
Project 1080 (2013/14 – 2017/18) by Martin Gilbert and Claire Love (CRI)

Summary

The aim of this project is to investigate FCM control by the increased use of mating disruption (MD) products beyond levels that are presently registered. Another aspect of this is the use of MD products in combination with Sterile Insect Release (SIT). Both of these aspects, if proved to have merit, would make the possibility of local FCM pheromone synthesis more viable by increasing the potential demand for the pheromone. During 2015 / 16 work continued at Bo-Bergvlei in the Citrusdal area as this is an FCM hotspot. The experimental block was under the Xsit SIT programme. At this farm one half (2 ha) of a 4 ha block of Washington Navels received two applications of Isomate as per registration. This was followed up by three applications of Checkmate at 150 ml per ha. One hectare received a single Isomate at the end of January. A further hectare received only SIT treatments. Aerial releases of sterile FCM were carried out as usual over the whole block by Xsit as part of their commercial programme. The experimental Navel block is surrounded by rocky outcrops and so is difficult for aerial releases of sterile FCM. This is particularly so now that releases by autogyro are no longer permitted. The fixed-wing aircraft currently in use would find it more difficult to turn within the limited space and this may influence the results. Nevertheless, the conditions were the same for all treatments and so a valid comparison can be made. Yellow delta traps loaded with Chempac FCM pheromone were placed

out in the treated and untreated (SIT only) blocks and monitored weekly. In addition, 10 trees per treatment (and control) were marked and all fallen fruit were collected weekly and examined for the presence of FCM. Despite the multiple extra MD treatments in the one half of the Navel block, under these high pressure conditions, fruit loss could not be reduced to below 0.7 fruit per tree per week by late April (with a mean of 1.9 fruit per tree per week). Indeed, there was very little difference in fruit loss between the single Isomate applied at end of January and the treatment that included 2 x Isomate and 3 Checkmate applications. It was only in the final two weeks that fruit loss in the multiple treatment block started to be significantly less than that in the 1 x Isomate block. In this experiment, it was not possible to drive fruit loss down to very low levels (e.g. <0.5 fruit per tree per week). This might be possible under conditions of lower FCM pressure. Under these high FCM conditions, total control of FCM could not be achieved by the excessive amount of MD products which were applied. As regards the local synthesis of FCM pheromone, Dr. Hennie Jordaan of Imagichem in the Eastern Cape prepared a detailed document (at no cost to CRI) on the subject. This document will be retained for future reference. Nevertheless, his opinion remained that the cost of local production would be too high at the relatively low volumes of product that would be required in this country. The experimental results obtained in the Western Cape in 2016 (see above) also have not yet shown that multiple applications of mating disruption products will significantly increase the level of FCM control. Positive results of chemical mating disruption "overkill" therefore remain unproven at this time.

Opsomming

Die doel van hierdie projek is om VKM beheer te ondersoek deur die verhoogde gebruik (meer as wat huidig geregistreer is) van paringsontwrigting produkte. Nog ŉ aspek is die gebruik van paringsontwrigting produkte in kombinasie met die Steriele Insek Tegniek. Albei hierdie aspekte, (indien van waarde) kan die moontlikheid van die plaaslike vervaardiging van VKM feromoon verhoog, deur die aanvraag van die feromoon te verhoog. Gedurende 2015/16 is werk by Bo-Bergvlei in die Citrusdal area voortgesit omdat hierdie ŉ VKM "hotspot" is.Op die plaas is 2 ha van ŉ 4 ha blok Washington Nawels met 2 x Isomate soos geregistreer behandel. Hierdie was deur 3 x 150 ml per ha Checkmate as ŉ ligtedek bespuiting op 2-weeklikse intervalle opgevolg. ŉ Verdere 1 ha van die blok is met ŉ enkele toediening van Isomate teen einde Januarie behandel. Die ander ha van die blok is as ŉ kontrole gelos. Loslatings van steriele VKM is elke week op alle dele van die 4 ha blok gedoen as deel van Xsit se kommersiële program. Die proef blok is deur rotskranse omring en dus moeilik vir lugloslatings. Die vastevlerk vliegtuig wat tans in gebruik is sal sukkel om te draai wat nie die geval was met die autogiro wat voorheen gebruik is. Nietemin, is die omstandighede vir al die behandelings dieselfde. Twee geel VKM delta valle is gebruik om motte weekliks in behandelde en onbehandelde blokke te monitor. Al die sanitasie vrugte is op ŉ weeklikse basis onder 10 bome in behandelde en kontrole blokke opgetel. Die vrugte is vir die teenwoordigheid van VKM nagegaan. Ten spyte van die addisionele paringsontwrigting behandelings (3 x Checkmate) in die helfde van die blok, onder die hoë infestasie toestande in die boord was vrugverlies nooit minder as 0.7 vrugte per boom per week met ŉ gemiddeld van 1.9 vrugte per boom per week (Feb – April). Daar was min verskil in vrugverlies tussen die 1 x Isomate behandeling (teen einde Januarie) en die 2 x Isomate behandeling met addisionele Checkmate toedienings. Net in die laaste 3 weke voor oes het vrugverlies in die 1 x Isomate blok betekenisvol toegeneem in verlyking met díe van die Checkmate blok. In die proef was dit nie moontlik om vrug verlies tot ŉ lae vlak (bv < 0.5 vrugte per boom per week) te verminder. Dit mag dalk moontlik wees onder laer VKM druk. Onder sulke hoë VKM druk kon paringsontwrigting produkte VKM nie voldoende beheer nie. Wat die plaaslike vervaarding van VKM feromoon betref, het Dr. Hennie Jordaan van Imagichem in die Oos Kaap ŉ volledige document (met geen koste teen CRI) op die onderwerp voorberei. Hierdie dokument sal vir toekomstige verwysing gehou word. Nieteenstaande het sy opinie dat die kostes van plaaslike produksie van VKM feromoon te hoog sal wees vir die lae volumes wat benodig sal wees die selfde gebly. Die 2016 proef resultate wat in die die Wes Kaap gektry is het ook nie getoon dat veelvoudige toedienings van paringsontwrigting produkte ŉ betekenisvolle verbetering van VKM beheer sal gee. Dus positiewe resultate van chemise paringsonwrigting "overkill" op hierdie stadium bly onbeslis.

3.2.13 Progress Report: Movement of false codling moth (FCM) and fruit flies (FF) in multi-crop (citrus, stone fruit, grape, pomegranate) systems

Project 1081 (2013/14 – 2016/7) by Martin Gilbert and Claire Love (CRI)

Summary

Fruit fly and false codling moth are polyphagous pests infesting many different cultivated fruit types as well as wild hosts. The aim of this project was to investigate these pests on farms where differing fruit types were grown close to each other. Monitoring continued on a weekly basis in 2015/16. In addition, a Porterville farm, where citrus and pomegranates, are grown close together was included in the project in 2015. Regarding fruit fly, as in 2014/15, initial peaks of 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 occur 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. 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.

Opsomming

Vrugtevlieg en valskodlingmot (VKM) is plae wat verskillende aangeplante vrugtesoorte sowel as wilde gasheerplante aanval. Die doel van hierdie projek is om hierdie peste te ondersoek op plase waar verskillende vrugtesoort naby aan mekaar voorkom. Moniteering is in 2015/16 op ʼn plaas in Riebeek-Kasteel voortgesit. ʼn Plaas op Porterville waar sitrus en granate naby aan mekaar verbou word, is ook in die projek ingesluit ten einde VKM en vrugtevlieg te monitor. Met vrugtevlieg, soos in 2014/15, het die eerste pieke van aktiwiteit met die rypwording van elke vrugtetipe saamgegaan. Moniteering in herfs / winter het ʼn soortgelyke tendens as 2014/15 gewys. Teen die einde Maart het Mediterreënse vrugtevlieg getalle in steenvrug boorde (nektarien, pruim en perske) geweldig toegeneem. Getalle in nektariens was besonders hoog, met ʼn piek van 313 vlieë per week (Biolure valletjies) in laat April. Hierdie nektarien, pruim en perske boorde was lankaal geoes. Die baie hoë getalle in nektarien boordemag dalk verband hou met afskuiding van nectar deur “extrafloral nectaries” wat op sekere kultivars verskyn. Sulke hoë getalle van vrugtevlieë ʼn groot risiko vir nabye sitrus blokke sal inhou. Op die minste die vermoë om vrugtevlieë te beheer in nabye boorde sal benadeel word. Hoë getalle vrugtevlieë deur die hele Mei in alle steenvrugte en sitrus boorde oorleef het. VKM mannetjie vlugpieke was weereens nie aan die rypwording van die vrugtipes gekoppel nie. Resultate van Riebeek Kasteel het duidelik getoon dat mot aktiwiteit pieke van Oktober 2015 tot April 2016 teen min of meer dieselfde tyd op elke gewas plaasgevind het, onafhangklik van die gewas waarin die valletjies geplaas is. Pieke van vlugaktiviteit in spesifieke vrugtesoorte, soos gemeet deur feromoön valletjies op veelvuldigegewaas, nie altyd saam met vrug rypheid gekoppel is nie. Vlugaktiwiteit van VKM kan soms baie hoog of laag wees in sekere weke in al die boorde, gëoes of nie. Vlugaktiwiteit mag soms in steenvrugboorde wat maande lank sonder vrugte staan hoog wees. Veelvuldigegewas produsente moet ʼn lokval stelsel in alle boorde behou ondanks die vrugte alreeds gëoes is of nie. Die risiko van hoë VKM tellings in boorde waar daar nie meer vrugte is nie kan net geskat word deur monsterneming van vrugte in aangrensende boorde wat nie gëoes is nie.

3.2.14 PROGRESS REPORT: Improving the cold tolerance of false codling moth (*Thaumatotibia leucotreta*) for improved performance in a sterile insect release programme

Project 1083 (Apr 2014 – Dec 2015) by Claire Daniel, Mat Goddard, Martin Hill (Rhodes University), Claire Love and Sean Moore (CRI)

Summary

Since its implementation in 2007, the sterile insect technique (SIT) programme for the control of false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), has generally been successful in the suppression of this pest. The mass-rearing process, and possibly also the irradiation, negatively affects their ability to compete successfully with wild males at cool temperatures. Flight ability of sterile males is reduced at temperatures below 20°C, while wild males have been recorded in active flight down to 12°C. This study aimed to improve upon the current cold tolerance of sterile FCM males by investigating the influence of a variety of cryoprotectant dietary additives, which may assist in improving cold tolerance. The larvae of FCM were reared on these augmented diets in the laboratory. Flight tests with adult males indicated that diets augmented with cholesterol and trehalose significantly increased male flight ability, with trehalose showing an average increase in flight activity of 41% and cholesterol an average increase of 36% at 15°C under laboratory conditions. Larvae reared on these diets also showed no negative impacts on physiology and development. In field flight tests, recaptures of trehalose-treated sterile males was 3-5 times higher than untreated moths at

temperatures below 20°C. Results with cholesterol have not been as promising. The usefulness of such dietary additives for improved cold tolerance of sterile males will be discussed.

Opsomming

Sedert die implementering in 2007 is die steriele insek tegniek (SIT) program vir die beheer van valskodlingmot (VKM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), oor die algemeen suksesvol in die onderdrukking van hierdie plaag. Die massatelingsproses en moontlik ook die bestraling, het ŉ negatiewe effek op die vermoë van die steriele mannetjies om suksesvol met wilde mannetjies teen koeler temperature te kompeteer. Die vlieg vermoë van steriele mannetjies word verlaag by temperature onder 20°C, terwyl wilde mannetjies in aktiewe vlug nog by 12°C aangeteken is. Die doel van hierdie studie is om die huidige koue toleransie van steriele VKM mannetjies te verbeter deur die invloed van ŉ verskeidenheid kouebeskermer byvoegings tot die dieet wat moontlik koue toleransie kan verbeter, te ondersoek. Die larwes van VKM is op hierdie aangepaste diëte in die laboratorium geteel. Vlugtoetse met volwasse mannetjies het aangedui dat ŉ dieet wat met cholesterol of trehalose kouebeskermers aangevul is, die vliegvermoë van mannetjies betekenisvol verhoog het. Die trehalose het ŉ gemiddelde verhoging van 41% in vliegaktiwiteit getoon, en cholesterol ŉ gemiddelde verhoging van 36% by 15°C onder laboratorium toestande. Larwes wat op hierdie diëte geteel is, het ook geen negatiewe impak op insekfisiologie en ontwikkeling getoon nie. In vlugtoetse in die veld is hervangs van trehalose-behandelde steriele mannetjie motte 3-5 maal hoër as onbehandelde steriele mannetjies teen temperature onder 20°C. Resultate met cholesterol is nie so belowend nie. Die bruikbaarheid van hierdie dieet byvoegings om koue toleransie van steriele mannetjies te verbeter sal bespreek word.

3.2.15 PROJECT REPORT: Verification of proposed inspections standards within an FCM systems approach

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

Summary

The International Plant Protection Convention describes different options to attaining requisite risk mitigation for pests of phytosanitary concern in international trade. These include the use of Pest Free Areas; Pest Free Places of Production; Pest Free Production Sites; Pest Free Consignments; Post harvest Disinfestation Treatments; Non Host Status; Areas of Low Pest Prevalence; and the Systems Approach to combining partial controls into a composite system that achieves the requisite level of phytosanitary security. The Systems Approach is a concept that has a high level of flexibility inherent in choice of components that can be combined. This allows for tailoring of a Systems Approach to a particular pest by selection of treatments that are of particular relevance to the pest, the production environment and the export country's infrastructural and regulatory capacity. A risk management framework was developed for FCM in citrus fruit for export from South Africa. A component of the framework was a Systems Approach that included a series of pre- and post-harvest controls and infestation assessments. The performance of the Systems Approach was evaluated by monitoring larval infestation of fruit weekly in 33 orchards, until the time of harvest, post-picking and post-packing into export cartons. Significant positive regressions were recorded between infestation of fruit during the full monitoring period in the orchard and the last four weeks before harvest, between the last four weeks before harvest and on delivery to the packinghouse, and on delivery to the packinghouse and in the packed carton. There was an improvement in the level of compliance with each of these successive steps in the system, thus verifying that the grading and inspection thresholds were appropriately sensitive and confirmed the effectiveness of the system. The overall risk mitigation efficacy of the systems approach was calculated. The calculation included several known compounding under estimations of efficacy. Nonetheless, the proportion of fruit that could be infested with *T. leucotreta* after application of the systems approach was between $P \leq 5.328 \times 10^{-6}$ and $P \leq 8.380 \times 10^{-7}$, 6 to 38 times less than the proportion associated with the probit 9 ($P \leq 3.2 \times 10^{-5}$) standard for a stand-alone cold treatment, being 3 survivors in 100,000 at the 95% confidence level.

Opsomming

Die Internasionale Plant Beskermings Konvensie beskryf verskillende opsies om nodige risko vermindering te bereik vir plae van fitosanitêre belang vir internasionale handel. Hierdie sluit in die gebruik van Plaagvrye Areas; Plaagvrye Plekke van Produksie; Plaagvrye Produksie Persele; Plaagvrye Besendings; Na-oes Ontsmettingsbehandelings; Nie Gasheer Status; Areas van Lae Plaag Voorkoms; en die Stelselsbenadering tot samestelling van onvoldoende beheer maatreels in ŉ saamgestelde stelsel wat die nodige vlak van fitosanitêre sekuriteit behaal. Die Stelselsbenadering is ŉ konsep wat ŉ hoë mate van buigsaamheid het in

die keuse van komponente wat gekombineer kan word. Hierdie maak dit moontlik om ŉ Stelselsbenadering aan te pas by ŉ spesifieke plaag deur seleksie van behandelings wat direk relevant is tot die plaag, die produksie omgewing en die uitvoerende land se infrastruktuur en regulatoriese kapasiteit. ŉ Risiko bestuursraamwerk is ontwikkel vir VKM in sitrusvrugte vir uitvoer uit Suid-Afrika. ŉ Komponent van die raamwerk was ŉ Stelselsbenadering wat ŉ reeks voor- en na-oes behandelings en besmetting analieses behels het. Die vertoning van die Stelselsbenadering is geëvalueer deur monitering van larwe besmetting van vrugte in 33 boorde tot oestyd, na-oes en na verpakking vir uitvoer. Betekenisvolle positiewe regressies is aangeteken in vrugbesmetting tussen die volle moniterings tydperk in die boord en die laaste vier weke voor oes, tussen die laaste vier weke voor oes en aflwering by die pakhuis, en op aflewering by die pakhuis en in die uitvoer karton. Daar is met elkeen van hierdie stappe in die stelsel, ŉ verbetering in die nakomings vlak, wat dus bevestig het dat die gradering en inspeksie drempelwaardes aanvaarbaar sensitief was en het die doeltreffendheid van die stelsel bevestig. Die algemene risko verminderings effektiwiteit van die stelsel is bereken. Die berekening het verskeie bekende onderskattings in effektiwiteit ingesluit. Nietemin was die proporsie vrugte wat na toepassing van die Stelselsbenadering met VKM besmet kon wees tussen $P \leq 5.328 \times 10^{-6}$ en $P \leq 8.380 \times 10^{-7}$, 6 tot 38 keer minder as die proporsie wat met die probit 9 ($P \leq 3.2 \times 10^{-5}$) standaard vir ŉ alleenstaande koue behandeling geassosieer is dws 3 oorlewendes in 100 000 teen ŉ 95% vlak van vertroue.

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

Project 1090 (April 2014 – March 2017) 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. Volatile compound detection is achieved by inserting this probe into a Gas Chromatography-Mass Spectrometry (GCMS) system. In a previous study, SPME detection of volatiles emitted by fruit and differences in emission profiles between healthy and infested fruit showed that volatile analysis has great potential as a post-harvest screening option. Five major volatile compounds of interest were released by the 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. Satsuma mandarins and Navel oranges were infested in the field between 1 and 20 days before harvest, but unfortunately the GCMS at Rhodes was not available at the time. Subsequently a GCMS unit has been made available, and W Kirkman has undergone extensive training on it, using 3,7-dimethyl-1,3,6-octatriene and naphthalene, two known volatiles affected by FCM infestation, as test substances. Trials on fruit will commence in the new season. W Kirkman visited RoboScientific at the University of Leeds, and received training on their "Bloodhound" electronic nose. The unit was successful in differentiating between injured and healthy fruit. The unit was brought back to South Africa, and preliminary trials were conducted on Navel and Valencia oranges. Results were variable, and efforts were made to standardise conditions in the laboratory to improve the reliability of results. Trials were conducted to see which of the individual sensors were the most suitable to detect the bouquet of volatiles emitted by FCM infested fruit. Trials will continue in the new season.

Opsomming

Soliede Fase Mikro-ekstraksie (SPME) het hoofruim vlugtigestowwe om vrugte effektief opgevang en gekonsentreer. Gaskromatografie-Massaspektrometrie (GCMS) analise is gedoen om die vlugtigestowwe van besmette en gesonde vrugte te meet. In ŉ vorige studie het SPME opsporing van vlugtigestowwe goeie potensiaal gewys vir na-oes bepaling van vlugtigestowwe wat afgeskei word. Vyf hoof vlugtigestowwe van belang is deur besmette vrugte afgeskei, naamliks D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen, kariofileen en naftaleen. Satsuma mandaryne en Nawellemoenne is tussen 1 en 20 dae voor oes in boorde kunsmatig met VKM besmet, maar ongelukkig was die GCMS eenheid nie op daardie stadium beskikbaar nie. ŉ GCMS eenheid is aan die begin van 2016 beskikbaar gestel, en W Kirkman het intensiewe opleiding daarop gekry, met die gebruik van 3,7-dimetiel-1,3,6-oktatrieen en naftaleen, twee vlugstowwe wat deur VKM-besmetting beinvloed word, as proefstowwe gebruik. Proewe sal in die nuwe seisoen voortduur. W Kirkman het RoboScientific by die Universiteit van Leeds besoek vir opleiding op hulle "Bloodhound" elektroniese neus. Die eenheid kon tussen beseerde en gesonde vrugte onderskei, en is na Suid Afrika toe teruggebring. Voorlopige proewe is op Nawel en Valencia lemoene gedoen. Resultate was wisselvallig, en pogings is aangewend om omgewingstoestande in die laboratorium te standardiseer, en sodoende die betroubaarheid van die resultate te verbeter. Proewe is gedoen om te bepaal watter individuele sensors die mees geskik is om die bos vlugtigestowwe op te spoor wat deur VKM-besmette vrugte afgeskei word. Proewe sal in die nuwe seisoen voortgesit word.

3.2.17 **PROJECT REPORT: The efficacy of registered treatments for FCM control in Limpopo**
Project 1112 (Apr 2015 – July 2016) by Sean Moore, Wayne Kirkman (CRI), Sean Thackeray (CRI/RU), Francois Joubert (RU), Jacolene Meyer and Marili Mouton (QMS Laboratories)

Summary

In this project we proposed to test and compare the efficacy of control measures that are registered for use against FCM on citrus. This is because it cannot be assumed that results generated from trials elsewhere in the country will be identical for Letsitele or any other region for that matter; and because anecdotal information indicates differences in efficacy of certain products between certain regions. Two trials were conducted: one involving mating disruption (MD) and attract and kill (A&K) products and one involving products registered as sprays. The MD and A&K trial was initiated in October 2015 and the spray trial was applied in December 2015. Spray treatments included Cryptogran, Cryptex, Meothrin, Cypermethrin, Delegate, Coragen, Runner, Alsystin and Broadband. The MD and A&K trial used Isomate, Checkmate and Last Call. Monitoring of traps and fruit infestation was conducted weekly until the end of March 2016. Monitoring was terminated at this time, as FCM infestation was too low to justify continuation thereof. FCM infestation was too low to establish any significant differences between spray treatments and the untreated control. Although FCM infestation of fruit in the MD and A&K trial was similarly low, there were large differences in trap catches, a good indication of treatment efficacy. Trap catches were lowest in the Isomate treatment, followed by Checkmate and Last Call, all of which were lower than the untreated control. If FCM levels justify it, the spray trial will be repeated in 2016/17.

Opsomming

In hierdie projek beplan ons om die werking van produkte wat op sitrus teen VKM geregistreer is te toets en met mekaar te vergelyk. Hierdie is omdat dit nie sommer aanvaar moet word nie dat restultate van proewe elders in die land identies sal wees vir Letsitele of enige ander streek. Boonop, anekdotiese inligting dui aan dat daar verskille in die werking van sekere produkte in sekere streke mag wees. Twee proewe is uitgevoer: een het paringsontwrigting (PO) en lok-en-vrek (L&V) produkte behels en die ander het geregistreerde spuit-produkte behels. Die PO en L&V proef is in Oktober 2015 geïnisieer, en die spuitproef is in Desember 2015 toegedien. Spuitbehandelings het Cryptogran, Cryptex, Meothrin, Cypermethrin, Delegate, Coragen, Runner, Alsystin en Broadband ingesluit. Die PO en L&V proef het Isomate, Checkmate en Last Call gebruik. Lokvalvangstes en vrugbesmetting is gemonitor tot einde Maart 2016. Monitering was toe gestaak omdat VKM besmetting te laag was. VKM besmetting was ook te laag om enige beduidende verskille tussen die spuitbehandelings en the onbehandelde kontrole te wys. Hoewel VKM vrugbesmetting baie laag in die PO en L&V proef was, was daar groot verskille in lokvalvangstes, wat ŉ goeie aanduiding van effeftwiteit is. Lokvalvangstes was die laagste vir die Isomate behandeling, gevolg deur Checkmate en Last Call, en almal was laer as die onbehandelde kontrole. Die proef sal in 2016/17 herhaal word as VKM vlakke dit regverdig.

3.2.18 **PROGRESS REPORT: FCM population ecology in citrus orchards: the influence of orchard age**
Project 1114 (2015/6 – 2017/8) by Sonnica Albertyn, Martin Hill (Rhodes University) and Sean Moore (CRI)

Summary

Anecdotal reports have been made of high populations of false codling moth, (FCM*) Thaumatotibia leucotreta*, during the first three to five harvesting years of citrus planted in virgin soil, after which, FCM numbers decline. The FCM population ecology of newly planted, juvenile (2 – 4 years) and established (9 years and older) orchards are being monitored to determine if and why juvenile orchards facilitate higher FCM infestation than established orchards. FCM trap catches were significantly higher in juvenile and established orchards than in newly planted and bushveld sites. FCM infestation was very low in all orchards, except for two Washington Navel orchards in the established orchard group. Washington Navels have been shown to be more susceptible to FCM infestation than the Cambria Navels in the juvenile orchard group that they were compared to. During the 2016 season one Cambria Navel orchard was replaced with an M7 Navel orchard. From after harvest around end July, FCM traps were monitored every second week until January 2016. Low FCM trap catches of <0.5 moths per trap per week persisted after harvest in both juvenile and established orchards. The average numbers of eggs were significantly higher in established orchards compared to juvenile orchards. Egg parasitism was however, also significantly higher in established orchards. Bushveld sites and newly planted orchards had the highest occurrence of entomopathogenic fungi (EPF) in soil samples. Entomopathogenic nematodes (EPN) were only found in 2% and 4% of juvenile and established orchard soil samples respectively. The susceptibility to FCM of fruit from a 12 year old (established) Washington Navel orchard and fruit from a four year (juvenile) Washington Navel orchard from the same farm, was compared. Fruit from young orchards were twice as susceptible (24% infestation) to FCM than fruit from established orchards (12% infestation). The

nutritional results of the fruit showed that the main differences between the two age groups were protein and ash content. The protein content was 1.3% higher and the ash content 3% higher in fruit from the young orchard in comparison to fruit from the established orchard.

Opsomming

Anekdotiese verslae is gemaak van hoë bevolkings van valskodling mot, (VKM) *Thaumatotibia leucotreta*, gedurende die eerste drie tot vyf oes jare van sitrus geplant in onversteurde grond, waarna VKM getalle verminder. Die VKM bevolkings ekologie van nuut geplant, jong (2-4 jaar) en gevestigde (9 jaar en ouer) boorde word gemonitor om te bepaal of en waarom jong boorde hoër VKM besmetting fasiliteer as gevestigde boorde. VKM lokval vangste was aansienlik hoër in jong en gevestigde boorde as in nuut geplante boorde en bosveld gebiede. VKM-besmetting was baie laag in alle boorde, behalwe vir twee Washington Nawel boorde in die gevestigde boord groep. Studies het al gewys dat Washington Nawels meer vatbaar vir VKM besmetting is as die Cambria Nawels in die jong boord groep waarmee hul vergelyk is. Gedurende die 2016 seisoen is een van die Cambria Nawel boorde vervang met ŉ M7 Nawel boord. Vanaf na-oes om einde Julie was VKM lokvalle elke tweede week gemonitor tot Januarie 2016. Lae VKM lokvalvangste van <0.5 motte per lokval per week was volgehou na oes in beide jong en gevestigde boorde. Die gemiddelde aantal eiers in gevestigde boorde was aansienlik hoër in gevestigde boorde in vergelyking met jong boorde. Eier parasitisme was egter ook aansienlik hoër in gevestigde boorde. Bosveld gebiede en nuwe boorde het die hoogste voorkoms van entomopatogeniese swamme in grondmonsters gehad. Entomopatogeniese nematodes is slegs in 4% van gevestigde boorde en 2% van jong boorde se grondmonsters gevind. Die vatbaarheid vir VKM besmetting vir vrugte vanaf ŉ 12 jarige, gevestigde Washington Nawel boord en ŉ jong 4 jarige Washington Nawel boord vanaf dieselfde plaas is vergelyk. Vrugte van jong boorde was twee keer so vatbaar (24% besmetting) vir VKM besmetting as vrugte vanaf gevestigde boorde (12% besmetting). Die voedingswaarde resultate van die vrugte het getoon dat die belangrikste verskille tussen die twee boord ouderdomsgroepe die proteïen en as inhoud is. Die proteïen inhoud was 1.3% hoër en die as inhoud 3% hoër in vrugte vanaf die jong boord in vergelyking met vrugte vanaf die ouer boord.

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

Project 1116 (2015-2017) by Francois Joubert, Unathi Heshula, Martin Hill (RU) and Sean Moore (CRI)

Summary

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

commercial blends and the Ivory Coast and Malawi blends being the least attractive. This hints to the possibility that the South African blend might be a better alternative for implementation in commercial lures and mating disruption products. Further replicates are still required and the trials are still ongoing. The results from the trials with the virgin females will hopefully shed light on the possibility of females from areas other than a male's area of origin being less attractive. After the completion of the field trials the laboratory trials will be revisited. A flight tube system will be implemented rather than the y-tube olfactometer method previously referred too.

Opsomming

Valskodling mot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Torticidae), is een van die belangrikste plae van sitrus in Suid-Afrika omrede hy endemies vir Afrika suid van die Sahara is en die feit dat die meerderheid Suid-Afrikaanse sitrus uitgevoer word. Die beheer van die plaag is dus noodsaaklik om markte vir uitvoer oop te hou. Een van die metodes van beheer is die steriele insek tegniek (SIT). Xsit (Edms) Bpk bedryf die SIT program vir VKM in Suid-Afrika. Hulle laat steriele mannetjie motte los, vanaf ŉ kultuur gevestig van motte van die Citrusdal area. Die vraag wat in die projek ontleed word is of die mannetjies in staat is om die wyfies van ander areas in Suid-Afrika net so doeltreffend op te spoor in vergelyking met hul area van herkoms (Citrusdal) deur middel van die feromone wat die wyfies afgee. Die doel van die projek is om dus vas te stel of daar enige feromoon variasies tussen verskillende VKM populasies deur Suid-Afrika is, en of hulle ŉ impak het op die opsporing van wyfies deur mannetjies motte. Dit kan belangrike implikasies hê vir die SIT program, bestuur deur Xsit, en het die potensiaal om die program te verbeter. Vroeg in 2015 is laboratorium werk begin deur van ŉ Y-buis olfaktometer gebruik te maak om die aantreklikheid van wyfie VKM te toets. Eerstens is die aantreklikheid van Addo wyfies tot Addo mannetjies getoets. Beide mannetjies en wyfies vanaf dieselfde populasie is gebruik vir die optimalisering van die olfaktometer opset. Die positiewe reaksie van mannetjies tot wyfies was tot 85%. Nietemin is die resultate steeds teenstrydig en daar is besluit om die eksperimente onder volkome donker te doen met net die gebruik van ŉ rooi lig om die mannetjies se gedrag waar te neem. Gedurende die eksperimente, het die mannetjies gedurende talle herhalings nie op die wyfies gereageer nie. Dit dui daarop dat die gedrag wat voorheen waargeneem is, dalk deur die kunsmatige lig in die laboratorium veroorsaak is. Na hierdie resultate is besluit om aan te beweeg na veldproewe. Die veldproewe bestaan uit twee verskillende proefnemings: 1) die aantreklikheid van maagd wyfies van vyf verskillende populasies (kulture word by Rhodes Universiteit gehandhaaf) tot steriele Citrusdal mannetjies (deur Xsit aangeteel) en 2) toets die aantreklikheid van drie kommersiële feromone (Checkmate, Pherolure en Isomate) en drie plaaslike mengsels (Suid-Afrika, Malawi en Ivoorkus) tot steriele Citrusdal mannetjies. ŉ Proef wat die aantreklikheid van wyfies van die 5vyfbeskikbare populasies toets tot die steriele Citrusdal mannetjies is voltooi. Die hervangs was baie laag en geen afleidings kon gemaak word nie. Die proef sal nog drie keer in ŉ poliëtileen tonnel herhaal word om beter beheer oor die eksperiment te verseker. Dit sal ook verseker dat die proewe uitgebrei kan word tot in die winter maande. Twee proewe met die kommersiële en plaaslike feromoon mengsels is ook voltooi. Op hierdie stadium lyk dit of die Suid-Afrikaanse plaaslike mengsel die mees aantreklik is, gevolg deur die kommersiële mengsels, en die Ivoorkus en Malawi mengsels die minste aantreklik. Dit sinspeel tot die moontlikheid dat die Suid-Afrikaanse mengsel dalk ŉ beter alternatief is vir implementering in kommersiële lokmiddels en paringsontwrigting produkte. Verdere herhalings word vereis en die proewe is nogsteeds aan die gang. Die resultate van die proewe met die maagd wyfies sal hopelik lig werp op die moontlikheid dat wyfies van areas anders as die van die mannetjies se herkoms minder aantreklik is. Na voltooiing van die veldproewe sal laboratorium proewe hervat word. ŉ Vlug buis sisteem sal geïmplementeer word eerder as die Y-pyp olfaktometer metode waarheen voorheen verwys is.

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

Project NMMU-1117 by Patrick Mwanza, Gill Dealtry (NMMU) and Sean Moore (CRI)

Summary

The use of baculoviruses as biopesticides continues to gain popularity as they appear to provide a more advantageous approach to combat crop pests than chemical pesticides do. As such they have become important in the agricultural economy. *Cryptophlebia leucotreta* granulovirus (CrleGV) has been formulated as a biocontrol agent against the common citrus pest, the false codling moth, (FCM). One of the major setbacks with the use of baculoviruses as biopesticides has been their susceptibility to ultraviolet (UV) radiation from the sun. When exposed to UV some baculoviruses lose their activity within 24 hours. This forces farmers to re-apply the biopesticides frequently. In this study various methods of protecting CrleGV-SA formulations against the effect of UV are being investigated. Naturally occurring UV-resistant strains are being selected for by exposing the virus to UV from an artificial UV source, propagating the virus in fifth instar FCM larvae and repeating the cycle several times. The first cycle of exposure to UV has been conducted and bioassay analysis

is being conducted. Molecular analysis will also be carried out to determine the genes conferring UV-resistance to CrleGV-SA. Propagation of CrleGV-SA for the second cycle is being carried out with FCM fifth instars. The effect of various artificial UV-protectants ranging from optical brighteners to UV absorbents will be investigated. Combinations of UV-resistant strains and most promising artificial protectants will be tested in the field. Molecular techniques such as qPCR and microscopic techniques such as Raman microscopy and transmission electron microscopy are being optimised.

Opsomming

Gebruik van bakuloviruse as biologiese plaagdoders het meer gewild geword omdat hulle ŉ meer omgewings vriendelike benadering as breë-spektrum chemiese plaagdoders vir die betryding van gewas plae voorsien. As sulks het hulle in die landbou ekonomie belangrik geword. *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) is as ŉ biologiese beheer agent geformuleer teen die sitrus plaag, valskodlingmot (VKM). Een van die grootste tekortkominge met bakuloviruse as biologiese plaagdoders is hulle vatbaarheid vir ultraviolet (UV) bestraling van die son. Na blootstelling aan UV bestraling verloor sekere bakuloviruse hulle virulensie binne 24 ure. Hierdie feit dwing produsente om die biologiese plaagdoders gereeld te hertoedien. In hierdie studie word verskeie metodes ondersoek om CrleGV-SA teen UV-bestraling te beskerm. UV-bestande isolate wat natuurlik voorkom word geselekteer deur blootstelling van die virus aan UV-bestraling van ŉ kunsmatige UV-bron, vermeenigvuldiging van die virus in vyfde instar VKM met verskeie herhalings van die siklus. Die eerste blootstellings-siklus is al uitgevoer en biotoets analise word uitgevoer Molekulêre analiese sal ook uitgevoer word om te bepaal watter genes verantwoordelik is vir UV-bestandheid in CrleGV-SA. Vermeenigvuldiging van CrleGV-SA vir die tweede siklus word tans met vyfde instar VKM uitgevoer. Die effek van verskeie kunsmatige UV-beskermers, insluitend optiese blinkmakers en UV-absorbeerders, sal ondersoek word. Kombinasies van UV-bestande isolate en die mees belowende kunsmatige beskermers sal in veldproewe getoets word. Molekulêre tegnieke soos qPCR en mikroskopiese tegnieke soos Raman mikroskopie en transmissie mikroskopie word tans geoptomaliseer.

3.2.21 PROGRESS REPORT: Developing and optimising automatic sorting equipment with focus on online detection of FCM

Project 1120 (April 2015 – March 2017) by Wayne Kirkman and Sean Moore (CRI)

Summary

False codling moth (FCM) is considered a phytosanitary organism by certain markets, and there is therefore an urgent need to investigate methods for its post-harvest detection in fruit. Collaborative research with manufacturers of automatic sorting equipment, to develop systems to detect FCM infested fruit in an online grading system, has been conducted over the past few years. Progress has been made, but no systems are able to detect FCM at all stages of infestation. The companies are based in Europe, and so are unable to conduct research on FCM there. However, CRI with its expertise on the pest, can conduct the research in South Africa, using equipment provided by the companies. Discussion and planning was conducted with two leading manufacturers (Company A and B). Company A has a sorting unit which employs four different visual detection technologies, which collectively take 80 images of each fruit at full packhouse line speed. Technicians and researchers from the company were present on two occasions where several hundred fruit (laboratory and naturally infested) were run through their unit. Where visible, penetrations were marked with a permanent marker, so that the programmers could see exactly where infestation took place, and could use these images to improve algorithms to detect FCM. Several other batches of fruit were run through this system, and images were downloaded in Europe. All fruit were dissected after scanning to verify infestation, and this information was sent to the programmers. Company A technology is advanced for visual aspects, but less so for early signs of decay. Two visits were made to the laboratories of Company B, where several hundred fruit were run through their demonstration unit. Two new technologies, which are not currently part of their automated system, were also tested on each fruit, with reasonable success. These technologies will be incorporated into their systems for trials in the new season. Towards the end of the 2016 season, the efficacy of the units from both companies will be evaluated for their ability to detect FCM infested fruit.

Opsomming

Valskodlingmot (VKM) word deur sekere markte as ŉ fitosanitêre plaag beskou, en daar is dus ŉ dringende behoefte om alle na-oes metodes wat VKM moontlik kan opspoor te ondersoek. Gesamentlike navorsing met vevaardigers van outomatiese sorteringstoerusting is vir die afgelope paar jaar uitgevoer. Vordering het plaasgevind, maar geen toerusting kan VKM teen alle lewensstadiums in vrugte opspoor nie. Die maatskapye is in Europa gebaseer, en kan dus nie daar navorsing op VKM doen nie. Nietemin, weens sy se kundigheid op VKM, kan CRI die navorsing in Suid-Afrika uitvoer met toerusting wat deur die vervaardigers verskaf is.

Onderhandelings en beplanning is met twee van die voorste maatskapye uitgevoer (Maatskapy A en B). Maatskapy A het ŉ sorteerings eenheid wat vier visuele opsporings tegnologieë gebruik om gesamentlik 80 beelde van elke vrug te neem, teen normale pakhuislyn spoed. Tegnikuste en vavorsers van die maatskapy was op twee geleenthede aanwesig waar ŉ paar honderd vrugte (natuurlik en kunsmatig besmet) deur hulle stelsel gevoer is. Waar sigbaar, is elke penetrasie merk met ŉ permanente merker aangedui, dat die programeerders presies kon sien waar die besmetting plaasgevind het, en die beelde kon gebruik om hulle algoritmes te verbeter om VKM op te spoor. Verskeie ander besendings vrugte is ook deur hulle sisteem gesit, en die beelde is in Europa afgelaai. Alle vrugte is na skandeering gedissekteer om besmetting te verifieer. Maatskapy A se visuële tegnologie is vêr gevorder, maar hulle spoor nie maklik vroeë tekens van verotting op nie. Twee besoeke is aan die laboratoriums van Maatskapy B gemaak. Honderde vrugte is deur hulle demonstrasie-eenheid gesit, en twee nuwe tegnologieë wat nie tans deel van hulle eenheid is nie, is op elke vrug getoets, met redelike sukses. Hierdie tegnologieë sal vir proewe in hulle eenheid ingesluit word in die nuwe seisoen. Die doeltreffendheid van die eenhede van albei maatskapeie sal laat in die 2016 seisoen geëvalueer word.

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

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

Summary

The use of baculoviruses as biocontrol agents has become an essential component of integrated pest management programmes for the control of a variety of agricultural pests around the world. In South Africa, the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) has been commercially formulated into the biocontrol product Cryptogran for the control of *Thaumatotibia leucotreta*, more commonly known as the false codling moth (FCM). FCM is a major pest in the citrus industry in South Africa and raises phytosanatory concerns regarding the export of citrus to European and other international markets. A recent study at the University of Gdansk and Medical University of Gdansk in Poland on homogenates of infected FCM larvae was carried out to produce a complete genome sequence of CrleGV. During this study a second unknown baculovirus was detected in the homogenate samples alongside CrleGV, with this novel virus identified as a nucleopolyhedrovirus (NPV). The identification of a novel baculovirus which infects FCM allows for a new avenue of research regarding the production of biocontrol agents for the control of this pest. The primary aim of this project is to isolate the NPV from FCM homogenates in order to evaluate its virulence against FCM larvae. This will further be expanded to evaluate whether a combination of the NPV and CrleGV can increase the virulence of these viruses as well as determine the ratio required to achieve high levels of mortality. To date, the virus has been successfully identified in and isolated from FCM homogenate samples. A multiplex PCR assay has been developed to screen these and any future samples for the presence of either the GV or NPV. Furthermore, a quantitative PCR analysis has been established in order to accurately quantify virus occlusion bodies recovered from larval cadavers. These cadavers will be collected from a series of bioassays which are currently underway to test for any potential synergism between these viruses. The results of this project will provide an indication of whether the NPV can form the primary component of a new biopesticide and whether it can be used in combination with CrleGV to achieve greater levels of FCM control in the field.

Opsomming

Die gebruik van bakulovirusse as biologiese beheer agente het ŉ essensiële komponent geword van geïntegreerde plaagbestuur programme vir die beheer van ŉ verskeidenheid landbou plae regoor die wêreld. In Suid-Afrika, is die bakulovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) kommersieel geformuleer as die biologiese beheer produk Cryptogran vir die beheer van *Thaumatotibia leucotreta*, meer algemeen bekend as valskodlingmot (VKM). VKM is ŉ belangrike plaag in die sitrus bedryf in Suid-Afrika en is ŉ fitosanitêre kommernis met betrekking tot die uitvoer van sitrus na Europa en ander internasionale markte. In ŉ onlangse studie by die Universiteit van Gdansk en die Mediese Universiteit van Gdansk in Poland is homogenates van geïnfekteerde VKM larwes gemaak om ŉ volledige genoom van CrleGV te produseer. Gedurende hierdie studie is ŉ tweede onbekende bakulovirus in die monsters ontdek saam met CrleGV, en hierdie nuwe virus is ŉ nukleopolyhedrovirus (NPV) geïdentifiseer. Die identifikasie van ŉ nuwe bakulovirus wat VKM besmet maak voorsiening vir nuwe navorsing oor produksie van biologiese beheer agente vir die beheer van die plaag. Die primêre doel van die projek is om die NPV van VKM homogenate te isoleer en om sy virulensie teen VKM larwes te evalueer. Die studie sal verder uitgebrei word om te bepaal of ŉ kombinasie van die NPV en CrleGV die virulensie van die virusse verhoog asook om die ideale verhouding te bepaal om hoë vlakke van mortaliteit te kry. Tot op hede is die virus suksesvol van VKM homogenaat monsters geïsoleer en geïdentifiseer. ŉ Multipleks PCR toets is ontwikkel om hierdie en enige toekomstige monsters vir die teenwoordigheid van óf

die GV of NPV te skandeer. ʼn Kwantitatiewe PCR analise is ook ontwikkel om virus partikels van larwale kadawers akkuraat te kwantifiseer. Hierdie kadawers sal van ʼn reeks biotoetse versamel word wat tans aan die gang is om moontlike sinergisme tussen hierdie virusse te ondersoek. Die resultate van hierdie projek sal ʼn aanduiding verskaf of die NPV die primêre komponent kan wees in ʼn nuwe bio-plaagdoder, en of dit gebruik kan word in kombinasie met CrleGV om ʼn hoër vlak van VKM beheer in die veld te bekom.

3.3 PROGRAMME: FRUIT FLY

Programme Coordinator: Aruna Manrakhan (CRI)

3.3.1. Programme summary

The three fruit fly species which are problematic to the citrus industry of Southern Africa are: *Ceratitis capitata* (Medfly), *Ceratitis rosa s.l* (Natal fly) and *Bactrocera dorsalis* (Oriental fruit fly). The Oriental fruit fly is a relatively new pest in South Africa, being present in the northern parts of the country since 2013. To date, the Oriental fruit fly is mainly restricted to the northern and north eastern parts of South Africa. There is likely to be a taxonomic change with regards to the Natal fly in the near future. The two genotypes and morphotypes of the Natal fly would be considered as two species. However, irrespective of species, fruit fly pests have to be effectively controlled in citrus orchards during the fruiting season due to zero tolerance of eggs and larvae in fruit being exported. The focus of the fruit fly programme for 2015-2016 has been on optimising pre-harvest control measures for fruit fly pests and understanding the biology and ecology of the new fruit fly pest- the Oriental fruit fly. In order to enable applied and fundamental research studies, fruit flies were reared at the CRI facility in Nelspruit. Five fruit fly colonies: Medfly, Natal fly (two types), *Ceratitis cosyra* (marula fly) and Oriental fruit fly were maintained (3.3.2). Fruit fly materials were used in CRI funded projects (3.3.2). Management of fruit fly pests consists of two components: monitoring and control. New and existing fruit fly monitoring tools were evaluated as part of a project funded by the Department of Science and Technology (3.3.7). The 3-component Biolure was found to be the most effective fruit fly attractant for females of Medfly, Natal fly and Oriental fruit fly (3.3.7). EGO Pherolure was also found to be a promising new attractant for *Ceratitis* species (3.3.7). Currently only three protein based baits are registered and widely used across South Africa for fruit fly control: a mixture of protein hydrolysate and organophosphate (malathion or trichlorfon), the formulated and ready to use GF-120 and the M3 fruit fly bait stations. The low tolerance of insecticide residues in fruit has prompted further research into development of a new bait station. A paper based bait station, Tephri cone, is currently being developed by CRI (3.3.3). In field trials, the Tephri cone compared well with the M3 fruit fly bait stations for up to 5 weeks. Changes were made to the Tephri cone to improve field longevity and economic viability. The new version of the Tephri cone was tested in the laboratory and field cages on Medfly, Natal fly (*C. rosa s.s.* or *C. rosa* hot type) and Oriental fruit fly (3.3.3). The Tephri cone will be tested in the field in three climatic areas of South Africa in 2016-2017. For the Oriental fruit fly, control using the male annihilation technique (MAT) is required in addition to application of protein baits and orchard sanitation. Different MAT products are commercially available in South Africa. The more widely used ones are fibre board blocks containing methyl eugenol and malathion. There are three types of MAT blocks which are available in the country: Invader-b-lok, Dorsalure blocks and B.I. Toolkit. Two other MAT products which are available are drop applications of STATIC Spinosad ME and Last Call B.I. In 2014 and 2015, these five different MAT products were tested in the field and compared against each other (3.3.8). Two densities of Invader-b-lok were also tested (4 per ha versus 12 per ha). All MAT products were tested in combination with a standard fruit fly bait – M3 fruit fly bait station. In the field trials, the control treatment consisted of no MAT and only M3 fruit fly bait station. The field trials demonstrated clearly the advantage of having MAT in fruit fly control programmes against the Oriental fruit fly (3.3.8). The MAT products were equally effective in the 2014 field trial whilst in the 2015 field trial STATIC Spinosad ME was not as effective as the other treatments (3.3.8). In areas where the Oriental fruit fly is present, MAT blocks at 12 per ha would be more effective than MAT blocks at 4 per ha in suppressing levels of the pest (3.3.8). The chemosterilisation of *B. dorsalis* males using methyl eugenol and lufenuron (an insect growth regulator) was explored in laboratory trials. The highest concentration of lufenuron tested (120 mg per g of methyl eugenol) conferred partial male sterility (3.3.8). Studies on the chemosterilisation of the Oriental fruit fly was not pursued further. Research conducted on the biology and ecology of the Oriental fruit fly, aimed at determining the dispersal capacity of the pest (3.3.4), its invasion patterns (3.3.5) and its utilisation of citrus and other hosts (3.3.6). Mark-release-recapture experiments using sterile Oriental fruit fly are being carried out in three commercial orchards in the Vhembe District, Limpopo Province in order to determine the effects of fly physiology and environmental conditions (biotic/host and abiotic/climate) on field dispersal of the Oriental fruit fly (3.3.4). In laboratory tests, optimal locomotor activity of *B. dorsalis* was found to be between 24ºC and 32ºC (3.3.4). The host range of the Oriental fruit fly to date is still limited. The Oriental fruit fly was reared from cashew, guava, mango, orange (cv. Valencia), *Solanum mauritianum* and the indigenous fruit *Xylotheca kraussiana* (3.3.6).

Programopsomming

Die drie vrugtevliegspesies wat problematies vir die sitrusbedryf in suidelike Afrika is, is: *Ceratitis capitata* (Meditereense vrugtevlieg), *Ceratitis rosa s.l* (Natalse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg). Die Oosterse vrugtevlieg is ŉ relatief nuwe plaag in Suid-Afrika, en is sedert 2013 in die noordelike dele van die land aanwesig. Tot op datum is die Oosterse vrugtevlieg hoofsaaklik tot die noordelike en noord-oostelike dele van Suid-Afrika beperk. Daar gaan moontlik in die nabye toekoms, ŉ mate van verandering in die taksonomie van die Natalse vlieg plaasvind. Die twee genotipes en morfotipes van die Natalse vlieg gaan as twee spesies beskou word. Ongeag spesies, moet vrugtevliegplae effektief in sitrusboorde beheer word, veral tydens die vrugteseisoen, weens nul toleransie van eiers en larwes in vrugte wat uitgevoer word. Die fokus van die vrugtevliegprojek vir 2015-2016 was om voor-oes beheermaatreëls vir vrugtevliegplae te optimaliseer, en om die biologie en ekologie van die nuwe vrugtevliegplaag, die Oosterse vrugtevlieg, te verstaan. Ten einde toegepaste en basiese navorsingsstudies moontlik te maak, is vrugtevlieë by die CRI fasiliteit in Nelspruit geteel. Vyf vrugtevliegkolonies: Meditereense vrugtevlieg, Natalse vlieg (2 tipes), *Ceratitis cosyra* (marula vlieg) en Oosterse vrugtevlieg, is in stand gehou (3.3.2). Vrugtevliegmateriaal is in CRI-befondste projekte gebruik (3.3.2). Bestuur van vrugtevliegplae bestaan uit twee dele: monitering en beheer. Nuwe en bestaande vrugtevlieg moniteringsgereedskap is as deel van ŉ projek wat deur die Departement van Wetenskap en Tegnologie (3.3.7) befonds is, geëvalueer. Die 3-komponent Biolure was die mees effektiewe vrugtevlieg lokmiddel vir wyfies van die Meditereense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg (3.3.7). EGO Pherolure is ook as ŉ belowende nuwe lokmiddel vir *Ceratitis* spesies (3.3.7) geïdentifiseer. Tans is slegs drie proteïen-gebaseerde lokmiddels geregistreer, en word wyd oor Suid-Afrika vir vrugtevliegbeheer gebruik: ŉ mengsel van proteïen-hidrolisaat en organofosfaat (malathion of trichlorfon), die geformuleerde en gereed om gebruik te word GF-120 en die M3 vrugtevlieg lokaasstasies. Die lae toleransie vir chemiese residue in vrugte, het verdere navorsing in die ontwikkeling van ŉ nuwe lokaasstasie nodig gemaak. ŉ Papier-gebaseerde lokaasstasie - Tephri Cone, word tans deur CRI (3.3.3) ontwikkel. Die Tephri Cone het in veldproewe goed met die M3 vrugtevlieg lokaasstasies vir ŉ periode van tot 5 weke vergelyk. Veranderinge is aan die Tephri Cone gemaak ten einde veld lewensduur en ekonomiese lewensvatbaarheid te verbeter. Die nuwe weergawe van die Tephri Cone is in die laboratorium en in veldhokke op die Meditereense vrugtevlieg, Natalse vlieg (*C. rosa s.s.* of *C. rosa* "warm" tipe) en Oosterse vrugtevlieg (3.3.3) getoets. In 2016-2017 gaan die Tephri Cone in die veld in drie klimaatsareas van Suid-Afrika getoets word. Beheer van die Oosterse vrugtevlieg vereis die mannetjie uitwissingstegniek ("male annihilation technique" - MAT), tesame met die toediening van proteïen lokase en boordsanitasie. Verskillende MAT produkte is tans kommersieel in Suid-Afrika beskikbaar. Die mees algemeen gebruikte produkte is veselbordblokke, bevattende metiel-eugenol en malathion. Daar is drie tipes MAT blokke wat in die land beskikbaar is: Invader-b-lok, Dorsalure blokke en B.I. Toolkit. Twee ander MAT produkte wat beskikbaar is, is druppeltoedienings van STATIC Spinosad ME en Last Call B.I. In 2014 en 2015 is hierdie vyf verskillende MAT produkte in die veld getoets en met mekaar vergelyk (3.3.8). Twee digthede van Invader-b-lok is ook getoets (4 per ha teenoor 12 per ha). Alle MAT produkte is in kombinasie met ŉ standaard vrugtevlieg lokaas (M3 vrugtevlieg lokaasstasie), getoets. In die veldtoetse het die kontrole behandeling uit geen MAT en slegs M3 vrugtevlieg lokaasstasies bestaan. Die veldproewe het duidelik die voordeel gedemonstreer om MAT in vrugtevlieg beheerprogramme teen die Oosterse vrugtevlieg in te sluit (3.3.8). Die MAT produkte was net so effektief in die 2014 veldproewe, terwyl STATIC Spinosad ME in die 2015 veldproef nie so effektief in vergelyking met die ander behandelings was nie (3.3.8). In areas waar die Oosterse vrugtevlieg aanwesig is, is MAT blokke teen 12 per ha meer effektief om die vlakke van die plaag te onderdruk as MAT blokke teen 4 per ha (3.3.8). Die chemo-sterilisasie van *B. dorsalis* mannetjies deur gebruik te maak van metiel-eugenol en lufenuron (ŉ insek groeireguleerder) is in laboratoriumproewe ondersoek. Die hoogste konsentrasie van lufenuron wat getoets is (120 mg per g metiel-eugenol) het tot gedeeltelike manlike steriliteit gelei (3.3.8). Studies rakende die chemo-sterilisasie van die Oosterse vrugtevlieg is nie verder ondersoek nie. Navorsing rakende die biologie en ekologie van die Oosterse vrugtevlieg het ten doel gehad om die verspreidingsvermoë van die pes te bepaal (3.3.4), sy indringingspatrone (3.3.5) en die benutting van sitrus en ander gashere deur die plaag (3.3.6). Merk-vrylaat-hervang eksperimente met die steriele Oosterse vrugtevlieg, word in drie kommersiële boorde in die Vhembe Distrik, Limpopo Provinsie uitgevoer, ten einde effekte van vliegfisiologie en toestande (bioties/gasheer en abioties/klimaat) op veldverspreiding van die Oosterse vrugtevlieg (3.3.4) te bepaal. In laboratoriumtoetse is gevind dat optimale lokomotoriese aktiwiteit van *B. dorsalis* tussen 24ºC en 32ºC plaasvind (3.3.4). Die gasheerreeks van die Oosterse vrugtevlieg is tot op datum steeds beperk. Die Oosterse vrugtevlieg is vanaf kasjoe, koejawel, mango, lemoen (cv. Valencia), *Solanum mauritianum* en die inheemse vrug, *Xylotheca kraussiana*, geteel (3.3.6).

3.3.2. **PROGRESS REPORT: Fruit fly rearing**

Project 407 (1999/2000 – 2016/17) by A. Manrakhan, J-H. Daneel, R. Beck and G. Shongwe (CRI)

Summary

Currently at CRI, colonies of five fruit fly species: *Ceratitis capitata* (Medfly), two species under *Ceratitis rosa s.l.* (Natal fly), *Ceratitis cosyra* (marula fly) and *Bactrocera dorsalis* (Oriental fruit fly) are being maintained. Materials were made available for other CRI funded fruit fly projects: project 915 (Fruit fly bait), project 1107 (Utilisation of citrus and other hosts by *B. dorsalis*), project 1093 (Male annihilation treatments for control of *B. dorsalis*), project 1075 (Dispersal capacity of *B. dorsalis*) as well as for the DST funded project ERAfrica (Fruit fly attractants and monitoring). Colonies of Medfly, *Ceratitis rosa s.l.* (two species) and Oriental fruit fly were refreshed by addition of wild males collected from fruit and traps.

Opsomming

Kolonies van vyf vrugtevliegspesies: *Ceratitis capitata* (Meditereense vrugtevlieg), twee spesies onder *Ceratitis rosa s.l.* (Natalse vlieg), *Ceratitis cosyra* (maroela vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg) word tans by CRI in stand gehou. Materiaal is vir ander CRI befondste vrugtevliegprojekte beskikbaar gestel: projek 915 (Vrugtevlieglokaas), projek 1107 (Benutting van sitrus en ander gashere deur *B. dorsalis*), projek 1093 (Mannetjie uitwissingsbehandelings vir beheer van *B. dorsalis*), projek 1075 (Verspreidingsvermoë van *B. dorsalis*), asook vir die DST befondste projek ERAfrica (Vrugtevlieg lokmiddels en monitering). Kolonies van die Meditereense vrugtevlieg, *Ceratitis rosa s.l.* (twee spesies) en die Oosterse vrugtevlieg is aangevul deur die toevoeging van wilde mannetjies wat van vrugte en lokvalle versamel is.

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

Project 915 (2008/9 – 2016/17) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck and Glorious Shongwe (CRI)

Summary

Laboratory, semi-field and field tests were carried out to compare the attract and kill efficacy of the Tephri cone, a paper based bait station being developed by CRI, with standard baits used for fruit fly control in South Africa. In tests with fresh standard baits (M3 fruit fly bait station, GF-120 at 20% dilution with water, HymLure and malathion mixture with HymLure at 0.4% and malathion at 0.175% dilution with water), Tephri cones with a malathion-based bait caused the highest fly mortality among the four fruit fly species tested: Medfly, Natal fly (*C. rosa s.s*), marula fly and Oriental fruit fly. Fresh M3 bait stations were on the other hand more attractive than the other baits tested for most fly species. Flies of all species responded more to a fresh Tephri cone with spinosad-based bait than to a fresh Tephri cone with a malathion-based bait. Field testing of the Tephri cone with a spinosad-based bait (at 160 stations per ha) was carried out in commercial Star-Ruby grapefruit orchards between February and April 2015 and compared with the M3 fruit fly bait stations (at 240 stations per ha). The Tephri cone with a spinosad-based bait was able to effectively control field populations of Medfly and the Oriental fruit fly for a period of five weeks. Thereafter the M3 bait stations were more effective in controlling pest fruit fly populations. Changes had to be made to the Tephri cone with a spinosad-based bait in order to improve the efficacy of the Tephri cone and render it economically viable for fruit fly control. An extension of the field longevity of the Tephri cone beyond five weeks was required. An altenative toxicant to spinosad was required in order to make the Tephri cone more economically viable. A polyacrylamide polymer, Stockosorb, was added to the bait mixture in the Tephri cone to determine if this would improve field longevity. Malathion was chosen as an alternative toxicant to spinosad based on previous published studies. The efficacy of Tephri cone containing a malathion based bait (at 50 parts attractant to 1 part malathion) and spinosad based bait (at 10 parts attractant to 1 part spinosad) with and without Stockosorb Micro 660 was determined in laboratory and field cage assays for three fruit fly pest species of citrus: Medfly, Natal fly and Oriental fruit fly. The Tephri cones were evaluated at different ages: fresh, 2 weeks, 4 weeks, 8 weeks and 12 weeks. The stations were aged outdoors. For Medfly and Natal fly, there were no significant differences in fly responses to the different types of the Tephri cone. For the Oriental fruit fly, spinosad and malathion Tephri cones containing Stockosorb were more attractive than the Tephri cones without Stockosorb. Fly attraction to Tephri cones with and without Stockosorb was significantly reduced when the stations were 8 weeks old and above. Mortality of female flies following exposure to the four types of Tephri cones varied between species. Medfly was the most susceptible fly species to all types of the Tephri cones tested, with mortality levels remaining above 70% despite the aging of the Tephri cones for up to 12 weeks. For Medfly, there was no significant difference in mortality between the four types of Tephri cones tested. For Natal fly, the malathion based Tephri cone without Stockosorb was the least effective. Mortality of Natal fly dropped to below 50% on average when the four types of Tephri cones were 8 weeks old and above. For the Oriental fruit fly, Tephri cones with Stockosorb were the least effective

in terms of female fly kill. Mortality levels of *B. dorsalis* dropped to below 50% when Tephri cones were 2 weeks and older. The series of laboratory and semi field assays showed that malathion would be an effective alternative to spinosad in the Tephri cone. Stockosorb improved attraction of the Tephri cones in particular for the Oriental fruit fly at least for up to 4 weeks. The Tephri cone should be further optimised in order to improve its efficacy against the Oriental fruit fly.

Opsomming

Laboratorium-, semi-veld- en veldtoetse is uitgevoer om die lok- en doodmaak-effektiwiteit van die Tephri Cone, ʼn papier-gebaseerde lokaasstasie wat deur CRI ontwikkel is, met standaard lokaas wat vir vrugtevliegbeheer in Suid-Afrika gebruik word, te vergelyk. In toetse met vars standaard lokase (M3 vrugtevlieg lokaasstasie, GF-120 teen 20% verdunning met water, HymLure en malathion mengsel met HymLure teen 0.4% en malathion teen 0.175% verdunning met water), het Tephri Cones met ʼn malathion-gebaseerde lokaas, die hoogste vrugtevlieg mortaliteit tussen die vier vrugtevliegspesies wat getoets is, veroorsaak: Meditereense vrugtevlieg, Natalse vlieg (*C. rosa s.s*), maroela vlieg en die Oosterse vrugtevlieg. Vars M3 lokaasstasies was aan die ander kant meer aanloklik in vergelyking met die ander lokase wat vir die meeste vrugtevlieë getoets is. Vlieë van alle spesies het meer op ʼn vars Tephri Cone met spinosad-gebaseerde lokaas gereageer as ʼn vars Tephri Cone met ʼn malathion-gebaseerde lokaas. Veldtoetsing van die Tephri Cone met ʼn spinosad-gebaseerde lokaas (teen 160 stasies per ha) is in kommersiële Star-Ruby pomelo boorde tussen Februarie en April 2015 uitgevoer en met die M3 vrugtevlieg lokaasstasies vergelyk (teen 240 stasies per ha). Die Tephri Cone met ʼn spinosad-gebaseerde lokaas was in staat om veldpopulasies van die Meditereense vrugtevlieg en die Oosterse vrugtevlieg effektief vir ʼn periode van vyf weke te beheer. Daarna was die M3 lokaasstasie meer effektief in die beheer van vrugtevliegplaagpopulasies. Veranderinge moes aan die Tephri Cone met ʼn spinosad-gebaseerde lokaas aangebring word ten einde die effektiwiteit van die Tephri Cone te verbeter en dit ekonomies lewensvatbaar vir vrugtevliegbeheer te maak. ʼn Verlenging van die veld lewensduur van die Tephri Cone tot langer as vyf weke was nodig. ʼn Alternatiewe gifstof vir spinosad was nodig ten einde die Tephri Cone ekonomies meer lewensvatbaar te maak. ʼn Poliakrielamied polimeer, Stockosorb, is tot die lokaas-mengsel in die Tephri Cone gevoeg om te bepaal of dit die veld lewensduur sou verbeter. Malathion is as ʼn alternatiewe gifstof vir spinosad gekies, gebaseer op vorige gepubliseerde studies. Die effektiwiteit van die Tephri Cone, bevattende ʼn malathion-gebaseerde lokaas (teen 50 dele lokmiddel tot 1 deel malathion) en spinosad-gebaseerde lokaas (teen 10 dele lokmiddel tot 1 deel spinosad) met en sonder Stockosorb Micro 660, is in laboratorium- en veldhoktoetse vir drie vrugtevlieg plaagspesies van sitrus, bepaal: Meditereense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg. Die Tephri Cone is by verskillende ouderdomme geëvalueer: vars, 2 weke, 4 weke, 8 weke en 12 weke. Die stasies is buite verouder. Vir Meditereense vrugtevlieg en Natalse vlieg was daar geen betekenisvolle verskille in vliegreaksies op die verskillende tipes Tephri Cone nie. Vir die Oosterse vrugtevlieg, was spinosad en malathion Tephri Cones, bevattende Stockosorb, meer aanloklik as die Tephri Cones sonder Stockosorb. Vliegaantrekking na Tephri Cones met en sonder Stockosorb is betekenisvol verminder wanneer die stasies 8 weke oud en ouer was. Mortaliteit van wyfievlieë ná blootstelling aan die vier tipes Tephri Cones, het tussen spesies gevarieer. Die Meditereense vrugtevlieg was die vatbaarste vliegspesie vir alle tipes Tephri Cones wat getoets is, met mortaliteitvlakke wat bo 70% gebly het, ongeag die veroudering van die Tephri Cones, vir tot 12 weke. Vir die Meditereense vrugtevlieg was daar geen betekenisvolle verskil in mortaliteit tussen die vier tipes Tephri Cones wat getoets is nie. Vir die Natalse vlieg was die malathion-gebaseerde Tephri Cone sonder Stockosorb die minste effektief. Mortaliteit van die Natalse vlieg het tot onder 50% gemiddeld geval wanneer die vier tipes Tephri keëls 8 weke oud en ouer was. Vir die Oosterse vrugtevlieg was Tephri keëls met Stockosorb die minste effektief wat betref wyfievlieë wat gedood is. Mortaliteitvlakke van *B. dorsalis* het onder 50% geval wanneer Tephri Cones 2 weke oud en ouer was. Die reeks laboratorium- en semi-veldtoetse het getoon dat malathion ʼn effektiewe alternatief vir spinosad in die Tephri Cone kan wees. Stockosorb het aantrekking van die Tephri Cones verhoog, veral vir die Oosterse vrugtevlieg, vir ten minste 4 weke. Die Tephri Cone moet verder geoptimaliseer word ten einde sy effektiwiteit teen die Oosterse vrugtevlieg te verbeter.

3.3.4. PROGRESS REPORT: Dispersal capacity of *Bactrocera dorsalis*

Project 1075 (2013/14 – 2016/17) by C.W. Weldon (University of Pretoria), R. Anguelov (University of Pretoria) and A. Manrakhan (CRI)

Summary

The key outcome of this project is to establish the dispersal capacity of the Oriental fruit fly, *Bactrocera dorsalis*, with regard to environmental and physiological variables. The PhD student assigned to the project, Mrs Louisa Makumbe, has established that a pigment dose of at least 2 g/L pupae is suitable for marking *B. dorsalis*. The pigment colours Astral Pink and Blaze (orange) are particularly effective. These results were used to inform the marking of sterile *B. dorsalis* in mark-release-recapture experiments to determine the effects of fly maturity,

sex and availability of host fruit on dispersal. Two of four releases of sterile *B. dorsalis* have been completed for the dispersal study at three study sites near Louis Trichardt in Limpopo Province. Results are still too preliminary to draw any conclusions, but recaptures indicate a characteristic spread of flies over space as time lapses after release. The remaining releases are scheduled for May and October 2016. To explore the physiological basis for field dispersal results, Mrs Makumbe has determined the influence of temperature on locomotor activity. To date, she has found that *B. 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 begins to increase, which may indicate the onset of thermal stress. This work will be continued in 2016 with the use of flight mills to determine flight speed, distance and periodicity in response to temperature.

Opsomming

Die hoofdoel van die projek is om die verspreidingsvermoë van die Oosterse Vrugtevlieg, *Bactrocera dorsalis*, te bepaal met betrekking tot die omgewings en fisiologiese veranderlikes. Me Louisa Makumbe, die Doktorale student aan wie die projek toegeken is, het bepaal dat die optimale pigment dosis om *B. dorsalis* te merk minstens 2 g/L papies is. Dit is bevind dat die pigment kleure Astral Pink en Blaze (oranje) beter as die ander werk. Die resultate was in ag geneem tydens die merk-vrylaat-hervangs eksperiment van steriele *B. dorsalis*, waar die uitwerking van volwassenheid, geslag en vrug beskikbaarheid op verspreiding bepaal is. Twee van die vier vrylatings is reeds by studie areas naby Louis Trichardt in Limpopo voltooi. Alhoewel resultate steeds bestudeer word, wil dit voorkom of hervangs ŉ kenmerkende verspreiding van vlieë oor tyd na loslating aandui. Die oorblywende vrylatings sal in Mei en Oktober 2016 plaasvind. Me Makumbe het die invloed van temperatuur op lokomotoriese aktiwiteit gebruik om die fisiologiese basis van die verspreiding resultate te ondersoek. Tot op hede het sy gevind dat *B. dorsalis* meestal onaktief is teen temperature onder 20˚C. Die frekwensie en duur van loop en vlug aktiwiteit neem tussen 24-32˚C toe. Van 36˚C begin daar ŉ toename in die duur van rusperiodes, wat moontlik die begin van temperatuur spanning aandui. Dié werk sal in 2016 voortgesit word, en vlug meule sal gebruik word om die vlugspoed, afstand en periodisiteit teen verskeie temperature te bepaal.

3.3.5. PROGRESS REPORT: Invasion and expansion of *Bactrocera dorsalis* in South Africa: a genetic analysis

Project 1105 (2014/15 – 2015/16) by Caroline Knox (Rhodes University), Melissa Lloyd (Rhodes University), Aruna Manrakhan (CRI), Minette Karsten (University of Stellenbosch), Pia Addison (University of Stellenbosch), Vaughan Hattingh (CRI) and Jan Hendrik Venter (Department of Agriculture Forestry and Fisheries)

Summary

There have been a number of incursions of *Bactrocera dorsalis* followed by several eradication campaigns in the northern parts of South Africa since 2010. In 2010 and 2011, all eradication campaigns were declared successful following no detections of the pest in affected areas for a period of more than 12 weeks (approximately three generations). However, incursions of *B. dorsalis* recurred in areas where the pest was previously eradicated. In 2013, the pest was declared present in the Vhembe district, Limpopo Province. In 2015, *B. dorsalis* was reported as present in other districts in Limpopo and in a number of areas in other northern and north eastern provinces: Mpumalanga, North-West, Gauteng and Kwa-Zulu Natal. The main aim of this project was to understand the *B. dorsalis* incursion patterns observed in the northern parts of South Africa through genetic analysis of the specimens captured in the various areas across the years. Samples of *B. dorsalis* were collected from 32 affected locations in South Africa over a period of four years (2010-2014). The objective was to genotype individual flies of the samples at 11 previously characterized polymorphic microsatellite loci, and then to derive genetic diversity estimates for each sample. These estimates would then be used to determine the relatedness of *B. dorsalis* populations. The relatedness of *B. dorsalis* populations across the years within one area in South Africa, Weipe, Limpopo Province, will be determined. The relatedness of the *B. dorsalis* populations between locations within South Africa will also be determined. In 2015, the project was extended to include samples of *B. dorsalis* populations in selected African countries and selected countries outside of Africa where the pest is present. These samples were included in order to understand the invasion patterns of the pest into South Africa. There was a delay in delivering the final project outputs due to a change in leadership of the project at the end of 2014 and due to difficulties experienced in the population genetic structure analysis at Rhodes University. In September 2015, the project execution was transferred to Dr Minette Karsten, Stellenbosch University. At Rhodes University, the DNA extraction protocol was optimised The primers for the 11 microsatellite loci were synthesized, and the PCR protocol for the primers for four of these loci was optimised. Samples of *B. dorsalis* were obtained from two Asian countries and 10 African countries including three southern African countries. All *B. dorsalis* samples were processed by Dr

Karsten, Stellenbosch University. Microsatellite primers were multiplexed into two sets, one containing five primer pairs and the other containing six primer pairs. About 300 individuals have been genotyped for the 11 microsatellite markers. Further samples of *B. dorsalis* from other locations have also been requested. All individuals will be genotyped and data will be analysed. A final report will be provided in 2017.

Opsomming

Sedert 2010 was daar ŉ aantal gevalle van *Bactrocera dorsalis* indringings, gevolg deur uitwissingsveldtogte, in die noordelike dele van Suid-Afrika. In 2010 en 2011 was die uitwissingpogings suksesvol verklaar nadat die plaag vir ŉ periode van meer as 12 weke (drie generasies) nie in die geaffekteerde areas waarneembaar was nie. Herindringings van *B. dorsalis* het egter daarna plaasgevind in die areas waar dit voorheen uitgewis was. In 2013 was die plaag teenwoordig verklaar in die Vhembe distrik van die Limpopo Provinsie en in 2015 was dit in ander distrikte van Limpopo gerapporteer en in ŉ aantal areas in die noordelike en noord-oostelike provinsies: Mpumalanga, Noord-Wes, Gauteng en KwaZulu-Natal. Die hoofdoel van hierdie projek was om die *B. dorsalis* indringingspatrone waargeneem in die noordelike dele van Suid-Afrika te ondersoek en verstaan deur middel van genetiese analises van monsters wat deur die jare in die onderskeie gebiede versamel is. Monsters van *B. dorsalis* was in 32 geaffekteerde gebiede oor ŉ periode van vier jaar (2010-2014) versamel. Die doelwit was om indiwiduele vlieë in die monsters genotipies te ontleed op 11 voorheen gekarakteriseerde polimorfiese mikrosatelliet lokusse, en dan genetiese diversiteitsberamings vir elke monster te bepaal. Hierdie beramings sou dan gebruik word om verwantskappe in die *B. dorsalis* populasies te bepaal. Die populasieverwantskappe in *B. dorsalis* versamelings versamel deur die jare in een area van Suid-Afrika (Weipe, Limpopo) sal bepaal word. Die verwantskappe tussen *B. dorsalis* populasies in verskillende dele van Suid-Afrika sal ook bepaal word. In 2015 is die projek uitgebrei om monsters van geselekteerde gebiede in ander Afrikalande, sowel as lande buite Afrika waar te plaag teenwoordig is, in te sluit. Hierdie mosters is ingeluit om die indringingspatrone van die plaag in Suid-Afrika beter te begryp. Daar was ŉ vertraging in die bereiking van die finale projekuitsette a.g.v. ŉ verandering in projekleierskap teen die einde van 2014 en verdere probleme ervaar met die populasie genetiese struktuuranalises by Rhodes Universiteit. In September 2015 is projekleierskap aan Dr Minette Karsten by Stellenbosch Universiteit oorgedra. Die DNS ekstraksieprosedure was by Rhodes Universiteit geoptimiseer. Die voorvoerders vir die 11 mikrosatelietlokusse is gesintetiseer en die PKR protokol vir vier van hierdie lokusse is geoptimaliseer. Monsters van *B. dorsalis* is uit twee Asiese en 10 Afrikalande verkry, insluitende drie suidelike Afrikalande. Alle monsters is deur Dr Karsten, Stellenbosch Universiteit, verwerk. Mikrosatelietvoorvoerders was in twee multiplex PKR reaksies gebruik, met vyf voorvoerderpare in die een stel en ses in die ander. Ongeveer 300 indiwiduele vlieë is vir die 11 mikrosatelietmerkers genotipies ontleed. Verdere *B. dorsalis* monsters van ander gebiede is ook aangevra. Alle indiwiduele organismes sal genotipies ontleed word en die data geanaliseer word. ŉ Finale verslag sal in 2017 verskaf word.

3.3.6. PROGRESS REPORT: Utilisation of citrus and other fruit grown in South Africa by *B. dorsalis* previously recognized as *B. invadens*

Project 1107 (2014/15 – 2016/17) by Christopher Weldon (University of Pretoria) and Aruna Manrakhan (CRI)

Summary

The key outcomes of this project are to determine the fruit species and varieties used by the Oriental fruit fly, *Bactrocera dorsalis*, with a particular emphasis on citrus, and the properties of citrus cultivars that make them more or less susceptible to attack. The PhD student assigned to the project, Ms Charmaine Theron, has completed field work to determine the host range of *B. dorsalis* within its current distribution in South Africa. Between July 2014 and June 2015, Ms Theron conducted monthly fruit sampling in tandem with adult trapping in agricultural and untransformed areas of Limpopo and Mpumalanga. Hosts used in South Africa are currently more limited than in other African countries (cashews, guavas, mangoes, Valencia oranges, *Solanum mauritianum*, and *Xylotheca kraussiana*). Infestation of Valencia oranges occurred only when there was a high adult population. *Bactrocera dorsalis* was most abundant in fruit found on the ground and in damaged fruit on the tree. These results suggest that orchard sanitation and removal of the invasive *S. mauritianum* should complement management of *B. dorsalis* with bait sprays and mass trapping. The oviposition propensity and egg-laying behaviour of females is currently being performed with regard to fruit ripeness and damage. To date, these experiments have been completed on green Eureka lemons, green and colour-break Star Ruby grapefruit, and green Nadorcott mandarins. Eggs have not been found in intact fruit. Host recognition and oviposition has occurred only in response to damaged, colour-break Star Ruby grapefruit.

Opsomming

Die hoofdoel van die projek is om te bepaal watter vrugtesoorte deur die Oosterse Vrugtevlieg, *Bactrocera dorsalis*, gebruik word, met spesifieke klem op sitrus, asook die eienskappe van die sitrus kultivars wat hul meer of minder vatbaar maak vir aanvalle. Me Charmaine Theron, die doktorale student aan wie die projek toegeken is, het veldwerk voltooi om die gasheer spektrum van *B. dorsalis* in sy huidige verspreiding in Suid-Afrika te bepaal. Monsterneming van vrugte, met gelyktydige monitering van volwasse vlieg lokval-vangste, is tussen Julie 2014 en Junie 2015 deur Me Theron in landbou –en ongerepte areas in Limpopo en Mpumalanga uitgevoer. Gasheerplante in Suid-Afrika word tans meer beperk as in ander Afrika-lande (kasjoeneute, koejawels, mango's, Valencia lemoene, *Solanum mauritianum* en *Xylotheca kraussiana*). Besmetting van Valencia lemoene was slegs opgemerk waar groot bevolkings vlieë voorgekom het. *Bactrocera dorsalis* was meestal in vrugte op die grond en in beskadigde vrugte aan die boom gevind. Dié resultate dui daarop dat boord-instandhouding en verwydering van die indringer, *S. Mauritianum*, die huidige bestuursprosesse teen *B. dorsalis* moet komplimenteer. Die waarskynlikheid van eierlegging en die eierleggingsgedrag van wyfies word tans getoets deur te kyk na vrugrypheid en -skade. Tans is eksperimente op Eureka suurlemoene, groen en kleurbreuk "Star Ruby" pomelo's en groen "Nadorcott" naartjies voltooi. Tot dus ver is daar nog geen eiers in ongeskonde vrugte gevind nie. Gasheer-herkenning en eierlegging het net in beskadigde kleurbreuk "Star Ruby" pomelo's plaasgevind.

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

Project ERAfrica (project funded by Department of Science and Technology: Jan 2014- Jan 2017) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Christopher Weldon (University of Pretoria), Louisa Makumbe (University of Pretoria), Caroline Knox (Rhodes University), Minette Karsten (Stellenbosch University), Pia Addison (Stellenbosch University), Marc De Meyer (Royal Museum for Central Africa), François Hala N'Klo (Centre National de Recherche Agronomique), Helene Delatte (CIRAD) and Pierre Francois Duyck (CIRAD)

Summary

Ten fruit fly attractants were evaluated in four commercial citrus orchards and two natural areas between September 2014 and September 2015. The 3-component Biolure was found to be the most effective attractant for females of Medfly, Natal fly and Oriental fruit fly. Throughout the study period, Medfly responded equally well to the new attractant EGO Pherolure and to Trimedlure. Although numerically more Natal fly were caught in EGO Pherolure traps than in Trimedlure traps, differences between the two attractants were not statistically significant for this pest group. In comparison, for the marula fly, EGO Pherolure was significantly more attractive to the pest than Trimedlure. The distance-dependent responses of male Medfly, Natal fly and Marula fly to an EGO Pherolure baited trap were determined using mark-release-recapture methods in December 2015 and January 2016 in three commercial orchards near Nelspruit. Recaptures are currently being analysed. Based on results of the field experiments, the sensitivity of the EGO Pherolure trapping system for pest fruit flies, Medfly, Natal fly and Marula fly will be estimated.

Opsomming

Tien vrugtevlieg lokmiddels is in vier kommersiële sitrusboorde en twee natuurlike areas tussen September 2014 en September 2015 geëvalueer. Daar is gevind dat die 3-komponent Biolure die mees effektiewe lokmiddel vir wyfies van die Meditereense vrugtevlieg, Natalse vlieg en die Oosterse vrugtevlieg was. Die Meditereense vrugtevlieg het reg deur die studie tydperk op die nuwe lokmiddel, EGO Pherolure, en op Trimedlure ewe goed gereageer. Hoewel meer Natalse vlieë in EGO Pherolure lokvalle gevang is in vergelyking met Trimedlure lokvalle, was verskille tussen die twee lokmiddels nie statisties betekenisvol vir hierdie pesgroep nie. In teenoorstelling, was EGO Pherolure betekenisvol meer aanloklik as Trimedlure vir die Maroela vlieg. Die afstand-afhanklike reaksies van mannetjie Meditereense vrugtevlieg, Natalse vlieg en die Maroela vrugtevlieg teenoor 'n EGO Pherolure gelaaide lokval, is in Desember 2015 en Januarie 2016 in drie kommersiële boorde naby Nelspruit bepaal, deur gebruik te maak van 'n merk-vrylaat-hervang metode. Hervangsdata word tans geanaliseer. Gebaseer op resultate van die veldproewe, sal die sensitiwiteit van EGO Pherolure lokval sisteme vir vrugtevlieg plae, Meditereense vrugtevlieg, Natalse vlieg en die Maroela vrugtevlieg, vasgestel word.

3.3.8. **PROGRESS REPORT: Evaluation of male annihilation treatments for control of *Bactrocera dorsalis***

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

Summary

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

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

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

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

Opsomming

Die mannetjie-uitwissingstegniek (MAT) word aanbeveel vir die beheer van die Oosterse vrugtevlieg, *Bactrocera dorsalis*. *Bactrocera dorsalis* word in MAT geteiken deur ontplooiing van stasies of substrate bevattende ŉ mengsel van die mannetjie lokmiddel, metiel-eugenol (ME), en ŉ insekdoder. Die doel van MAT is om hoë getalle van die mannetjies dood te maak en daardeur die aantal parings en vrugtevlieg populasievlak te verlaag. In Suid-Afrika is ŉ aantal MAT produkte kommersieel beskikbaar vir die beheer van die Oosterse vrugtevlieg. In 2014 en 2015 is proewe in Star Ruby pomelo boorde in Constantia, Limpopo Provinsie, uitgevoer om die werking van hierdie verskillende MAT produkte vir *B. dorsalis* beheer te bepaal. Drie tipes veselbordblokke, bevattende ME en malathion: Invader-b-lok, Dorsalure blok en B.I. Toolkit, is met druppeltoedienings van SPLAT Spinosad ME (ME en spinosad) en Last Call B.I. (ME en permethrin) vergelyk. Twee toedieningstempo's van Invader-b-lok is addisioneel geëvalueer: 4 blokke per ha teenoor 12 blokke per ha. Alle MAT behandelings is in kombinasie met ŉ standaard vrugtevlieg proteïen lokaas geëvalueer (M3

vrugtevlieg lokaasstasie) wat hoofsaaklik wyfies teiken. As ʼn kontrole (geen MAT), is ʼn behandeling met slegs M3 vrugtevlieg lokaasstasies in die veldstudies ingesluit. In hierdie veldproewe is die effektiwiteit van alle behandelings geëvalueer deur gebruik te maak van ME lokvalle en valle wat met voedsel-gebaseerde lokmiddels gelaai is. Vrugtevlieg besmetting van vrugte is ook by oestyd uitgevoer.
In ʼn afsonderlike studie onder hierdie projek, is ʼn nuwe metode van beheer deur die gebruik van ME ondersoek. Die effektiwiteit van die insek groeireguleerder, lufenuron, in kombinasie met ME in die indusering van steriliteit van *B. dorsalis* mannetjies is in laboratoriumstudies by CRI ondersoek. Tien-dae-oue ongepaarde *B. dorsalis* mannetjies is blootgestel aan tegniese graad lufenuron (99.9% suiwerheid) gemeng met ME teen 6 konsentrasies van lufenuron: 0.0 (kontrole), 5.0, 10.0, 30.0, 60.0 en 120.0 mg, per g van ME. Mannetjies is vir 2 ure aan die verskillende behandelings blootgestel. Een dag ná blootstelling aan die lufenuron en ME mengsel, is behandelde mannetjies met ongepaarde wyfies van dieselfde ouderdom gepaar. ʼn Mengsel van suiker en gishidrolisaat en water is daarna aan die vlieë verskaf. ʼn Eierleggingsapparaat is daagliks in elke hok vir eierlegging geplaas. Vliegmortaliteit en vrugbaarheid is daagliks vir die verskillende behandelings vir ʼn tydperk van 2 weke bepaal.

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

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

3.4 PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS

Programme coordinator: Sean D Moore (CRI)

3.4.1 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 have been experienced in Letsitele over the last three years. A project was initiated to survey for parasitoids attacking this species (3.4.4). However, *D. aberiae* infestation was a lot lower during the 2015/16 season and consequently no parasitoids were found and it was not possible to establish a laboratory culture. Another insect which is considered to be a phytosanitary organism by China is carob moth. A comprehensive study was conducted on this pest (3.4.3), establishing a monitoring technique and confirming completion of the carob moth life cycle in Navel oranges, usually in association with mealybug. A mating disruption product was shown to suppress carob moth and in spray trials, Delegate, Runner and Dipel were effective. Two parasitoid species were also recovered from carob moth larvae. The final trial in the programme evaluated GRAS fumigants for post-harvest disinfestation of fruit of a series of phytosanitary pests (3.4.2). Vapormate proved to be a useful disinfestation treatment for external insects and mites that are not in the egg stage, whereas CO_2 proved more effective against FCM in fruit. A combination treatment of 60% CO_2 followed by cold (2°C for 6 d) gave a mean mortality of 96% for the third instars and 82% for the fifth instars.

Programopsomming

Alhoewel dit beskou word dat die twee hoof fitosanitê plae op sitrus in suidelike Afrika VKM en vrugtevlieë is, sekere witluis spesies wat op sitrus voorkom het ook fitosanitêre status vir sekere markte. Een van hierdie

spesies is *Delotococcus aberiae*, waarvan opvallende uitbrake te Letsitele oor die laaste drie jaar opgelet is. ʼn Projek is begin om ʼn opname te doen vir parasiete wat hierdie spesie aanval (3.4.4). *Delotococcus aberiae* besmetting is egeter heelwat laer gedurende die 2015/16 seisoen en gevolglik is geen parasiete gekry nie en dit was ook nie moontlik om ʼn laboratorium kultuur te stig nie. Nog ʼn insek wat as ʼn fitosanitêre organisme deur China beskou word is karobmot. ʼn Omvattende studie is op hierdie plaag uitgevoer (3.4.3). ʼn Moniterings tegniek is ontwikkel en dit is bevestig dat karobmot sy lewenssiklus in Nawellemoene kan voltooi, gewoonlik met witluis geassosieer. ʼn Paringsontwrigting produk het karobmot onderdruk en in spuitproewe was Delegate, Runner en Dipel doeltreffend. Twee karobmot spesies is ook van karobmot larwes gekry. Die finale proef in die program het GRAS na-oes berokingsmiddels ontleed vir ontsmetting van vrugte vir ʼn reeks fitosanitêre plae (3.4.2). Dit is gewys dat Vapormate ʼn waardevolle ontsmettings behandeling vir eksterne plae en myte kan wees, indien hulle nie in die eier stadium is nie, maar vir VKM in vrugte was CO_2 meer doeltreffend. ʼn Kombinasie behandeling van 60% CO_2 gevolg deur koue (2°C vir 6 d) het ʼn gemiddelde mortaliteit van 96% vir derde instars en 82% vir vyfde instars veroorsaak.

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

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

Summary

Further research was conducted with Vapormate and carbon dioxide with the assistance of Postharvest Innovation funding from DST. Vapormate at 250 g/m^3 for 4 h at 15°C was previously found to completely eliminate grain chinch bug adults and this treatment was also shown to control the oribatulid mite *Siculobata sicula* with 100% mortality of 2 659 mites. Vapormate at the same dosage/time combination also killed all adult California red scale *Aonidiella aurantii* and adult mussel scale *Lepidosaphes beckii* on 100 fruit. This dosage/time combination of Vapormate can therefore serve as a useful disinfestation treatment for external insects and mites that are not in the egg stage. Eggs are less susceptible and 100% mortality could not be achieved with eggs of *Planococcus citri* or *P. ficus* at 250 g/m^3 for 24 h at 25°C. No trials could be conducted against eggs of Fullers rose beetle because inadequate numbers of the beetle could be found. Vapormate efficacy against internal fruit pests has been shown to be too variable to warrant further evaluation, so control of false codling moth (FCM) using CO_2 fumigation at 60% for 24 h followed by a short cold treatment was investigated further in different citrus cultivars. The CO_2 fumigation alone causes from 30 to 76% mortality of third instar FCM and a 7-day cold treatment alone at 2°C may cause 79% mortality. When these treatments immediately follow one another 100% mortality is obtained. A comparison between third and fifth instars following CO_2 fumigation at 60% and 6 days at 2°C, gave a mean mortality of 96% for the third instars and 82% for the fifth instars. Waxing fruit between fumigation and cold treatment had no effect on mortality. Delaying the cold treatment after fumigation lowers the mortality achieved by the combination treatment but if the fruit can be dropped to around 6°C within 12 h and reaches 2°C within 24 h the combined effect of both treatments can be maintained. Research will now focus on slightly longer cold treatments of 9 days after CO_2 fumigation to ensure 100% mortality of fifth instar FCM and also evaluate this combination treatment against fruit fly *Ceratitis capitata.* All cultivar types will also be evaluated for internal and external quality after these treatments.

Opsomming

Verdere navorsing is met Vapormate en koolstofdioksied gedoen met ondersteuning van die Postharvest Innovation Fund van die DST. Vapormate teen 250 g/m^3 vir 4 ure teen 15°C het in vorige proewe volwasse graanstinkbesies heeltemal uitgewis en hierdie behandeling het ook 100% mortaliteit van 2 659 oribatulid myte, *Siculobata sicula*, veroorsaak. Vapormate teen dieselfde dosis/tyd kombinasie het ook alle volwasse rooidopluis, *Aonidiella aurantii*, en volwasse mosseldopluis, *Lepidosaphes beckii*, op 100 vrugte doodgemaak. Hierdie dosis/tyd kombinasie van Vapormate kan dus dien as ʼn nuttige ontsmettings behandeling vir eksterne insekte en myte wat nie in die eier stadium is nie. Eiers is minder vatbaar en 100% mortaliteit kon nie bereik word met eiers van *Planococcus citri* of *P. ficus* by 250 g/m^3 vir 24 h by 25°C. Geen toetse kon teen eiers van Fuller se Rooskewer gedoen word nie omdat dit nie moontlik was om genoegsame kewers te versamel nie. Doeltreffendheid van Vapormate teen interne vrugte plae is reeds gewys om wisselvallig te wees en dus regverdig nie verdere evaluasie nie. Daarom is verdere evalusie van die beheer van valskodlingmot (VKM) met CO_2 beroking teen 60% vir 24 ure, gevolg deur ʼn kort koue behandeling in verskillende sitrus kultivars ondersoek. Die CO_2 beroking alleen kon 30-76% mortaliteit van derde instar VKM veroorsaak en ʼn 7-dae koue behandeling alleen by 2°C kon 79% mortaliteit veroorsaak. Wanneer hierdie behandelings onmiddellik mekaar volg, is 100% mortaliteit verkry. ʼn Vergelyking tussen die derde en vyfde instars na CO_2 beroking teen 60% en 6 dae teen 2°C, het ʼn gemiddelde mortaliteit van 96% vir die derde instar en 82% vir die vyfde instar getoon. Verwaksing van vrugte tussen beroking en koue behandeling het geen effek op mortaliteit gehad nie. Uitstelling van die koue behandeling na beroking het die mortaliteit wat deur die kombinasie behandeling bereik is

verlaag, maar as die vrugte binne 12 ure tot sowat 6°C verkoel kan word en tot 2°C binne 24 ure, kan die gekombineerde effek van beide behandelings gehandhaaf word. Navorsing sal nou op effens langer koue behandeling van 9 dae na CO_2 beroking fokus om 100% mortaliteit van vyfde instar VKM te verseker. Hierdie kombinasie behandeling sal ook teen vrugtevlieg *Ceratitis capitata* ondersoek word. Alle kultivars sal ook vir interne en eksterne gehalte na hierdie behandelings geëvalueer word.

3.4.3 PROGRESS REPORT: Establishment of a monitoring system and control practices for carob moth on citrus

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

Summary

Country-wide monitoring of traps and infestation was conducted successfully over the 2014-15 and 2015-16 seasons, with male carob moth being caught in all provinces monitored. Limpopo, Mpumalanga and the Northern Cape are the only provinces where meaningful levels of fruit infestation were recorded. The standard method of evaluating infestation of dropped fruit, as is done for FCM, does not seem to be effective for accurately assessing carob moth infestation and was revisited in the 2015/16 season. It was observed that carob moth is able to complete its life cycle in the navel end of the fruit and its presence seems to be associated with fungus and mealybug. A mating disruption trial using SPLAT® was completed at Schoeman Boerdery in the 2014-2015 season; low infestation levels resulted in no firm results being produced. However, a second trial with increased replicates at Rosle Boerdery has yielded promising results that suggest the product is effective in reducing infestation levels and trap catches. Spray trials were successfully conducted at Rosle Boerdery in the 2014-2015 and 2015-16 seasons with Delegate and Runner reducing infestation by 89.5% compared to the untreated control. In the 2015-16 season Dipel also performed relatively well. In a search for parasitoids of carob moth in citrus, larvae from four farms in the Loskop Valley production region were collected and two parasitoids emerged in very low numbers. These were sent for Identification.

Opsomming

Landswye monitering van lokvalle en besmetting is suksesvol oor die 2014-15 en 2015-16 seisoene uitgevoer, met die mannetjie karobmotte gevang in alle provinsies waar monitering gedoen is. Limpopo, Mpumalanga en die Noord-Kaap is die enigste provinsies waar betekenisvolle vlakke van vrug besmetting aangeteken is. Die standaarde metode vir die evaluering van besmetting in gevalde vrugte, wat algemeen vir VKM gebruik word, blyk nie om doeltreffend te wees vir akkurate ontleding van karobmot besmetting nie, en is in die 2015/16 seisoen hersien. Daar is waargeneem dat karobmot sy lewenssiklus in die nawel-end van die vrug kan voltooi, en dit wil voorkom of daar ŉ verband tussen karobmot en swamme of witluis is. ŉ Paringsontwrigting proef met die gebruik van SPLAT® is by Schoeman Boerdery in die 2014-2015 seisoen voltooi; lae besmettings vlakke het gelei tot geen vaste resultate nie. Daar is wel ŉ tweede proef met meer herhalings by Rosle Boerdery gedoen, met belowende resultate wat aandui dat die produk doeltreffend was in die vermindering van besmettings vlakke en lokval vangste. ŉ Suksesvolle spuitproef is by Rosle Boerdery in die 2014-2015 en 2015-2016 seisoene uitgevoer, met Delegate en Runner wat besmetting met 89.5% verlaag het in vergelyking met die onbehandelde kontrole. In die 2015-16 seisoen het Dipel ook relatief goed gevaar. In die soektog na parasitoïdes vir karobmot in sitrus, is larwes van vier plase in die Loskop Vallei produksie streek ingesamel. Twee parasitoïdes het in baie lae getalle uitgekom. Hulle is vir identifikasie wegestuur.

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

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

Summary

Delottococcus aberiae, previously incorrectly identified as *D. elizabethae*, one of the seven mealybug species recorded to infest citrus in South Africa, has been considered extremely rare and very difficult to find, with no more than nominal pest status. However, since around 2012 fairly dramatic outbreaks of this species have been recorded on citrus in the Letsitele region, with an apparent gradual spread of the pest in the area. Due to the limited knowledge of the pest and its biological control, we proposed to regularly collect samples from infested orchards in Letsitele with the objective of monitoring the species of parasitoids and the levels of parasitism. Three to 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. The study will be continued during the 2016-17 season.

Opsomming

Delottococcus aberiae, voorheen bekend as *D. elizabethae*, een van die sewe witluis spesies wat op sitrus in Suid-Afrika aangeteken is, is as baie skaars en moeilik om te vind beskou, en het daarom net nominale plaagstatus. Van omtrent 2012 is redelike dramatiese uitbrake van die spesie op sitrus in die Letsitele streek egeter plaasgevind, met ʼn oënskynlike geleidelike verspreiding van die plaag in die omgewing. As gevolg van die beperkte kennis van die plaag en sy biologiese beheer, ons het voorgestel om gereeld monsters van besmette boorde in Letsitele te versamel met die doel om parasiet spesies en vlakke van parasitisme to monitor. Drie tot vier versamelings van witluis besmette pomelos is van Desember 2015 tot Februarie 2016 gemaak. Hierdie is gebruik albei om ʼn *D. aberiae* laboratorium kultuur op sitrus saailinge te probeer stig en vir ondersoek vir parasitisme deur om individuele monsters in uitbroeings kaste te sit. *Delotococcus aberiae* besmetting was laer as wat dit vir die vorige drie jaar was en dus was dit nie moontlik om ʼn laboratorium kultuur te vestig. Daar is ook geen parasiete gekry nie. Die studie sal geduurende die 2016-17 seisoen voortgesit word.

3.5 PROGRAMME: NON-PHYTOSANITARY KEY PESTS

Programme Coordinator: Tim G Grout (CRI)

3.5.1 Programme summary

Although most IPM research is focussed on finding solutions for phytosanitary pests, there are key pests that can prevent fruit from meeting export standards and others that can result in a loss of production or even the ultimate death of the tree, if not controlled. Research on IPM-compatible treatments for late season populations of citrus thrips and mealybug was completed and written up as a final report (3.5.2). The impact of entomopathogenic fungi against these pests was disappointing and short-lived but some products may be evaluated further in other projects. No further research was conducted on possible late-season treatments for citrus psylla, woolly whitefly and leafhoppers due to a need to conduct unplanned research on cold treatment of false codling moth (FCM), but this research will continue in the future (3.5.4). A two-year project on the use of mating disruption for the control of red scale showed that when one dispenser was used per tree the efficacy was similar to that of a winter oil spray (1%). In the northern production areas, the disruptant was most effective when hung out during bloom, but in the Eastern Cape it was more effective when hung after petal fall (3.5.3). The use of imidacloprid as a standard in this research showed that red scale in Limpopo and Mpumalanga has developed a degree of resistance to this chemical such that efficacy was no better than a 1% oil spray. In the Eastern Cape, imidacloprid was still significantly more effective than a winter oil spray. Although the mating disruptant is a perfect treatment for maintaining commercial control of red scale without causing other pest repercussions, its cost will have to be competitive with horticultural mineral oil.

Programopsomming

Die grootste gedeelte van IPM navorsing is gefokus op oplossings vir fitosanitêre plae, maar daar is ook belangrike plae wat kan verhoed dat vrugte uitvoerstandaarde kan nakom, en ander wat produksieverliese, of selfs afsterf van bome kan veroorsaak as hulle nie beheer word nie. Navorsing op IPM-verenigbare beheermaatreëls vir laat-seisoen bevolkings van sitrus-blaaspooitjie en witluis is afgehandel en as ʼn finale verslag opgeskryf (3.5.2). Die impak van entomopatogeniese swamme teen hierdie plae was teleurstellend met kort nawerking, maar sekere produkte sal in die toekoms in ander projekte geêvalueer word. Geen verdere navorsing is uitgevoer op moontlike laat-seisoen beheermaatreëls van sitrusbladvlooi, wollerige-witvlieg of bladspringers nie, omdat onbeplande addisionele proewe met kouebehandeling van valskodlingmot (VKM) moes gedoen word. Die navorsing sal in die toekoms hervat word (3.5.4). Twee jaar se navorsing om paringsontwrigting vir die beheer van rooidopluis het gewys dat as een vrysteller per boom gehang word, word soortgelyke beheer van rooidopluis as met ʼn winter-oliebespuiting (1%) gekry. In die noordelike produksiestreke was die paringsontwrigting meer effektief toe dit tydens blom toegedien is, maar in die Oos-Kaap was dit meer effektief wanneer dit na blomblaarval toegedien is (3.5.3). Die gebruik van imidaklopried in die projek het gewys dat rooidopluis tot ʼn sekere mate weerstand teen die produk ontwikkel het in Limpopo en Mpumalanga, en is nou net so effektief as ʼn 1% oliebespuiting. In die Oos-Kaap was imidaklopried steeds beduidend meer effektief as ʼn winter-oliebespuiting. Hoewel die paringsontwrigting die perfekte produk is om rooidopluis komersieël te beheer sonder om ander plaagreperkussies te veroorsaak, sal die koste daarvan met spuitolies mededingend moet wees.

3.5.2 FINAL REPORT: Evaluation of entomopathogenic fungi and new chemicals against thrips and mealybug

Project 1029 (2011/2-2014/5) by Tim G Grout, Sean D Moore, Peter R Stephen and Wayne Kirkman (CRI)

Summary

There is an urgent need for plant protection products that can be used in summer for the control of citrus thrips and mealybug without disrupting natural enemies of key pests such as false codling moth. In 2011/2 the evaluation of two dosages of a commercially formulated entomopathogenic fungus (EPF) in different parts of the country gave disappointing results so in the 2013/4 season two experimental EPF isolates were evaluated at two dosages in northern and southern citrus regions. The results were again disappointing, although some thrips suppression was recorded for a week in the south. In the 2014/5 season, results from Limpopo Province showed that a *Metarhizium anisopliae* isolate caused an initial reduction in larval thrips numbers with a corresponding reduction in early scarring that was similar to that achieved with abamectin plus oil, but it was short-lived. A commercial biological product TripStop + BFA gave similar results against thrips but BioCure was almost ineffective for thrips. None of these products showed significant efficacy against mealybug. A new chemical product DPX8723, with a good IPM profile, gave excellent control of thrips for a month but also had no impact on mealybug.

In the Eastern Cape, two trials were applied in spring 2014. Neither *M. anisopliae* nor *Beauveria bassiana* (applied with and without oil as a tank mix) appeared to have any effect on thrips. However, in one of the trials, the EPFs appeared to cause some suppression of mealybug when applied with oil. As both trials were applied on an organic farm, the only standard that could be applied was Entrust (spinosad), which was the most effective treatment against thrips and mealybug. In mid to late summer, two corrective trials were applied against mealybug. In the first trial, *M. anisopliae* reduced mealybug infestation by 26%, whereas Applaud and Closer reduced mealybug infestation by 56% and 53%, respectively. In the second trial *M. anisopliae* reduced mealybug infestation by 33%.

This project has now been terminated, although promising products or improved EPF formulations will be evaluated in other projects.

Opsomming

Daar bestaan ŉ dringende behoefte vir plantbeskermings produkte wat in die somer gebruik kan word vir die beheer van sitrusblaaspootjie en witluis sonder om natuurlike vyande van sleutel plae soos valskodlingmot te versteur. In 2011/2 is twee dosise van ŉ kommersieel beskikbare entomopatogeniese swam (EPS) in verskillende dele van die land geëvalueer en het teleurstellende resultate gegee. Daarom het ons in 2013/4 twee eksperimentele EPSe teen twee verskillende dosise geëvalueer in die noordelike en suidelike sitrus streke. Resultate was weer teleurstellend al is daar ŉ mate van onderdrukking van blaaspootjie vir ŉ week in die suide aangeteken. In die 2014/5 seisoen het resultate van Limpopo Provinsie gewys dat ŉ *Metarhizium anisopliae* isolaat ŉ aanvanklike afname in blaaspootjie larwe getalle veroorsaak het met ŉ gesaamentlike vermindering in vroë skade, wat vergelykbaar was met abamektien en olie, maar was kort van werking. ŉ Kommersiële biologiese produk, TripStop + BFA, het eenselwige resultate teen blaaspootjie gegee. Nie een van die twee produkte het ŉ beduidende werking teen witluis getoon nie. ŉ Nuwe chemiese produk, DPX8723, met ŉ goeie IPM profiel, het uitstekende beheer van blaaspootjie vir ŉ maand gegee maar het ook geen impak op witluis gehad nie.

In die Oos-Kaap is twee proewe in die lente toegedien. Nie *M. anisopliae* of *Beauveria bassiana* (met en sonder olie as ŉ tenk-mengsel toegedien) het gelyk of hulle teen blaaspootjie doeltreffend was nie. In een van die proewe het die EPSe egter gelyk of hulle ŉ mate van onderdrukking van witluis veroorsaak het wanneer hulle met olie toegedien is. Omdat albei proewe op ŉ organiese plaas toegedien is is Entrust (spinosad) die enigste standaard behandeling wat ons kon toedien. Dit is ook die mees doeltreffende behandeling teen beide blaaspootjie en witluis. In die somer is twee korektiewe proewe teen witluis uitgevoer. In die eerste proef het *M. anisopliae* witluis besmetting met 26% verminder, waar Applaud en Closer witluis besmetting met onderskeidelik 56% en 53% veroorsaak het. In die tweede proef het *M. anisopliae* witluis besmetting met 33% verminder.

Hierdie projek het nou tot einde gekom maar belowende produkte of verbeterde EPS formulasies sal in ander projekte evalueer word.

Introduction

Over the last few years many requests have been made for research on alternatives to the diminishing number of products available for the control of citrus thrips (*Scirtothrips aurantii*) and mealybug (various spp.) that would not be disruptive to IPM. Tartar emetic is no longer available as an IPM-compatible treatment for thrips control and thrips populations are becoming increasingly tolerant to abamectin. Buprofezin is the only relatively IPM-compatible treatment that is registered for the control of mealybug after petal fall, although Movento may provide some suppression.

Two previous attempts have been made to try entomopathogenic fungi (EPF) against citrus thrips. Attempts were made in 2000 (Project 525) to import *Metarhizium anisopliae* Strain ICIPE 69 from Kenya and Mycotal (*Verticillium lecanii*) from Koppert in Holland, but both ground to a halt in the bureaucratic process of acquiring government permits for importation and environmental impact assessments before field trials could be conducted. The approach of searching for South African strains of EPF that would be effective against citrus thrips was therefore used in Project 879 in 2009 but no useful fungi could be isolated from soil samples without using the *Galleria* technique and when we obtained some isolates that Tarryn Goble had obtained using the *Galleria* technique, we could not maintain a citrus thrips culture long enough to screen the different isolates in the laboratory. We terminated this research with the statement that if an EPF became commercially available in South Africa we would evaluate it against citrus thrips.

A commercially formulated entomopathogenic fungus (EPF) *Metarhizium anisopliae* did become available in 2011 and was included in the first trials described below at two different dosage rates, in addition to a product containing oxymatrine and emamectin benzoate (Biocure). These were compared with other standard registered treatments in the Eastern Cape and the Mpumalanga Lowveld, but the results were disappointing. Research was then postponed for a year and resumed in 2013/4 with EPF isolates that were found and tested in the laboratory at Rhodes University by Goble et al. (2010 & 2011) and had been increased to sufficient volumes for field work. The final report of all research conducted in this project from 2011 to 2015 follows.

Stated objective

Compare the thrips efficacy of EPF spray options with thripicides that can be used after petal fall and evaluate direct or indirect effects on mealybug populations.

Materials and methods

2011/12

Mpumalanga

The first site used was a Valencia orchard at Crocodile Valley Citrus Co. at Nelspruit. Plans were made to use a grapefruit orchard at Malalane for a second site but these were abandoned due to excessive fruit drop. Eventually we had to conduct a second trial with a reduced number of treatments close to the other trial in Valencias at Nelspruit. Each trial site was divided in two and treatment blocks randomly allocated for each treatment within each half of the orchard (2 replicate blocks per treatment). Replicate blocks were 4 rows wide and at least 6 trees long so that 8 data trees could be selected from the centre 2 rows. All treatments below were applied as light cover, film-wet sprays on 3 November 2011 using hand guns at high pressure (20 bar). The thrips populations built slowly in this orchard after petal fall and the grower was concerned about citrus psylla so the whole orchard was sprayed with mevinphos EC at 20 ml/hl water on 12 October. The first trial was evaluated 6 and 25 days after treatments were applied and the second trial was evaluated once, 19 days after treatment. Evaluations were based on fruit infestation only because there were already slight thrips scars before the treatments were applied. Infestation for both thrips and mealybug was determined by inspecting 20 fruit (including under the calyx) on each of 8 trees per replicate and recording the number infested with thrips larvae, thrips adults and mealybug. Infestation proportions were transformed with arc sine square root before two-way analysis of variance was conducted.

Eastern Cape

Two orchards were demarcated for trials – one Navel orange (Newhall Navels) and one Valencia. Both were on Avoca Farm near Summerville in the Sundays River Valley. Unfortunately, it was a very low thrips pressure season, and thrips levels never picked up adequately in the Valencia orchard in order to justify a trial. Two blocks in the Navel orchard were sprayed with each treatment. Each block consisted of four rows by six trees and positioning of blocks relative to one another within the trial orchard was random. Treatments were applied in the Navel orange orchard on 26 October 2011, when a precount indicated 4% fruit infestation with thrips nymphs. Comm-EPF was sprayed after 17h30, due to its UV-sensitivity. Sprays were applied using hand

guns, fitted with 2 mm orifice nozzles and at a pressure of 20 bar. This resulted in an application of an average of 13.5 L per tree. The first evaluation of the trial was conducted on 2 November 2011 (7 days after treatment) and the second on 14 November 2011 (19 days after treatment). A third evaluation, only of mealybug infestation, was conducted on 25 January 2012 (67 days after treatment). Infestation for both thrips and mealybug was determined by inspecting 10 fruit (including under the calyx) on each of 8 trees per replicate (16 per treatment) and recording the number infested with thrips larvae, thrips adults and mealybug. Thus, a total of 160 fruit were inspected per treatment. On 19 April 2012, thrips damage was evaluated by inspecting 20 randomly selected fruit on each of 8 trees in the middle of each block, thus 16 trees and 320 fruit per treatment. Fruit were classified as clean, blemished or culled (pertaining specifically to thrips induced damage).

As mealybug infestation was quite high and of a similar level in several treatment blocks, it was decided to spray another trial in these blocks with some of the same products previously used. The trial was to test the efficacy against only mealybug, as it was speculated that a high pest density might be important for good efficacy with an entomopathogenic fungus. Five of the previously listed treatments were used i.e. untreated control, the two Comm-EPF rates, Bio-Cure and Dursban. Comm-EPF was sprayed after 18h00. All treatments were mixed with 3 ml per 100 L Break-Thru. Six trees in the middle of each of two blocks were sprayed with each treatment on 6 February 2012. Sprays were applied in the same manner as previously described, at an average of 12.5 L per tree. Mealybug infestation was evaluated 22 days later on 28 February 2012.

Treatments applied in both regions in 2011

1. Untreated control
2. Abamectin (18 g/L EC) 20 ml plus medium horticultural mineral oil 300 ml/hl water
3. Delegate (spinetoram 250 g/kg WG) at 10 g/hl water
4. Comm-EPF 5 ml/hl water
5. Comm-EPF 10 ml/hl water
6. Bio-Cure (oxymatrine 6 g/L and emamectin benzoate 2.4.g/L SL) at 150 ml/hl water
7. Dursban (chlorpyrifos 750 g/kg WG) 64 g/hl water or EC 100 ml/hl water
8. Movento (spirotetramat 240 g/L SC) 20 ml plus medium hort. oil 300 ml/hl water

2013/14

Three trials were conducted on oranges in spring of 2013/4, two in the Eastern Cape (Huguenot Farm and Avoca Farm in the Sundays River Valley) and one in Limpopo Province (Olifants River Estate). The layout of these trials was as follows. The orchard was split in two and each half contained one block per treatment. These blocks comprised at least 4 rows of 6 trees each or 3 rows of 10 trees each (if interrow spacing was 7 m or trees were large) so that 2 rows of 4 data trees or 1 row of 8 data trees, respectively, could be used within the centre of each block. The treatments were assigned randomly to the blocks in each half of the orchard. If necessary, the orchard was sprayed at petal fall with abamectin 15 ml/hl plus medium horticultural mineral oil 150 ml/hl in order to prevent excessive thrips numbers and early damage shortly after petal fall. The following treatments were then applied by hand approximately 3 weeks after petal fall as full cover, film wet sprays in order to reach mealybug on the tree framework.

1. Untreated control
2. Abamectin (18 g/L EC) 20 ml plus medium horticultural mineral oil 300 ml/hl water
3. Closer (sulfoxaflor 240 g/L SC) 12 ml/hl water
4. G ll 3 L6 (*Metarhizium anisopliae*) at 1.65 g/hl water ($1x10^{13}$ spores/ha) plus 5 ml BreakThru
5. G ll 3 L6 (*Metarhizium anisopliae*) at 16.53 g/hl water ($1x10^{14}$ spores/ha) plus 5 ml BreakThru
6. FCM AR 23 B3 (*Metarhizium anisopliae*) at 1.75 g/hl water ($1x10^{13}$ spores/ha) plus 5 ml BreakThru
7. FCM AR 23 B3 (*Metarhizium anisopliae*) at 17.51 g/hl water ($1x10^{14}$ spores/ha) plus 5 ml BreakThru
8. Dursban (chlorpyrifos 750 g/kg WG) 64 g/hl water or 480 g/L EC at 100 ml/hl water

Evaluation of thrips and mealybug infestation under the calyx was made 7 days after treatment using 20 outside fruit per data tree and rating fruit as clean, infested with thrips larvae, infested with thrips adults or infested with mealybug. A second fruit evaluation was made 3-4 weeks after treatment and the orchard was sprayed out by the grower. Approximately one month later a third evaluation was made of mealybug infestation and thrips scarring (slight or severe).

A late season trial was conducted in the Gamtoos River Valley, Eastern Cape, for corrective control of mealybug. On 10 February 2014, the two *M. anisopliae* isolates, the *B. bassiana* isolate and Applaud were

sprayed with Break Thru, as described above, at 10^{14} spores/ha. Mealybug infestation was evaluated on 11 March 2014.

2014/15

During 2014/15 a further three early season trials were conducted, again with two in the Eastern Cape (two orchards at Jurgens Farm, Sundays River Valley) and one in Limpopo Province (Olifants River Estate). In Limpopo, *M. anisopliae* (FCM AR 23 B3) was the only EPF used, whereas in the Eastern Cape, an isolate of *Beauveria bassiana* (G AR 17 B3) was also used. In Limpopo, oil, abamectin, Pyrinex, DPX and TripStop plus BFA were included in the trial. The Eastern Cape trials were conducted in organic orchards, so only Entrust (spinosad) could be included as a standard. In the Eastern Cape, a further two late season corrective trials were conducted at Kleinplaas, Sundays River Valley), aimed against mealybug.

Results and discussion

2011/12

Mpumalanga

Both dosages of Comm-EPF had no significant impact on adult citrus thrips or mealybug in any of the three evaluations in the two trials (Tables 3.x.x.1 and 3.x.x.2). This fungus also did not appear to have any effect on thrips larvae 6 days after treatment (DAT) but when evaluated 19 or 25 DAT it lowered the infestation significantly ($P<0.05$), but not enough to provide control. Biocure caused no significant reduction in mealybug infestation and in the supplementary trial caused a significant increase, perhaps due to a detrimental effect on natural enemies. However, Biocure always caused a significant reduction in thrips larval infestation and in two out of three evaluations it reduced infestation by adult thrips significantly. In most cases this impact on thrips was significantly greater than that of Comm-EPF but significantly worse than abamectin against the larvae. As Biocure contains various fertilisers it is possible that it is causing an antifeedant effect in addition to some degree of direct mortality. Reports of thrips suppression from the field after spraying Movento were confirmed in the main trial and this high rate of Movento (20 ml) gave similar control to Delegate of citrus thrips in all evaluations, even 6 DAT. Movento's efficacy against mealybug was slow at first due to minimal contact toxicity but by 25 days after treatment it gave similar control to Dursban. Delegate appeared slightly superior to abamectin against citrus thrips larvae, although not significantly so. However, it resulted in significantly more mealybug than abamectin. The increased level of mealybug was not significantly more than in the control 25 DAT, but infestation levels after 6 days in the control, abamectin and Comm-EPF treatments had all declined by the 25 DAT evaluation, whereas the levels in the Delegate treatment increased from 12% to 22%. This therefore indicates that although Delegate may be used late in the season from a residue viewpoint, it is more detrimental to natural enemies than abamectin at its registered dosage of 20 ml/hl.

Table 3.5.2.1. Results from Valencias at Nelspruit, Mpumalanga

Treatments	6 Days after treatment			25 Days after treatment		
	Fruit with thrips larvae (%)	**Fruit with thrips adults (%)**	**Fruit with mealybug (%)**	**Fruit with thrips larvae (%)**	**Fruit with thrips adults (%)**	**Fruit with mealybug (%)**
Control	35.6 a	19.1 a	20.0 a	23.4 a	10.3 a	15.0 ab
Abamectin	5.9 c	4.1 bc	14.7 a	2.5 d	2.2 b	10.6 b
Delegate	1.9 c	5.3 bc	12.2 a	0.9 d	1.3 b	21.9 a
Comm-EPF 5 ml	40.6 a	17.2 a	22.2 a	14.7 b	9.4 a	10.9 b
Comm-EPF 10 ml	30.9 a	14.4 a	14.4 a	16.9 b	9.4 a	9.4 b
Dursban	3.8 c	0.6 c	1.6 b	0.0 d	0.3 b	0.9 c
Bio-Cure	17.5 b	3.1 bc	16.6 a	10.0 c	3.1 b	16.3 ab
Movento 20 ml	5.9 c	7.2 b	10.6 a	0.9 d	2.8 b	1.9 c

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

Table 3.5.2.2. Supplementary trial in Valencias at Nelspruit, Mpumalanga

Treatments	19 Days after treatment		
	Fruit with thrips larvae (%)	**Fruit with thrips adults (%)**	**Fruit with mealybug (%)**
Control	20.0 a	6.3 a	10.6 b
Comm-EPF 10 ml	10.9 b	3.8 ab	8.4 bc
Dursban	0.9 d	0.9 b	2.8 c
Bio-Cure	5.0 c	3.4 ab	21.3 a

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Eastern Cape

None of the treatments had any significant impact on thrips adults (Table 3.5.2.3). The possibility for detecting any differences may have been reduced by the low level of thrips presence. Differences in infestation with thrips larvae only became evident at the second evaluation, 19 days after spraying (Table 3.5.2.3). Although none of the treatments differed significantly from the untreated control, three of the treatments were significantly superior to the higher Comm-EPF treatment. Delegate was the most effective treatment, followed by Dursban and Biocure. Abamectin performed disappointingly, whereas the two Comm-EPF treatments appeared to have no effect on thrips at all. Entomopathogenic fungi are known to be density-dependent and thrips presence may have been too sparse, but this may indicate that EPFs are not practical options for controlling citrus thrips which can cause economic damage at low infestation levels.

Dursban was the only treatment which significantly reduced mealybug infestation, and this was only recorded at the first evaluation, 7 days after treatment (Table 3.5.2.3). At 19 days after treatment, mealybug infestation was notably, but not significantly, higher for the two Comm-EPF treatments and for Biocure than for the untreated control. There is no clear explanation for this. By 67 days after treatment, Movento showed its worth as a mealybug suppressant, being similar in efficacy to Dursban (Table 3.5.2.3).

Table 3.5.2.3. Results from Navels at Sundays River Valley, Eastern Cape

Treatments	7 Days after treatment			19 Days after treatment			67 Days after treatment
	Fruit with thrips larvae (%)	**Fruit with thrips adults (%)**	**Fruit with mealybug (%)**	**Fruit with thrips larvae (%)**	**Fruit with thrips adults (%)**	**Fruit with mealybug (%)**	**Fruit with mealybug (%)**
Control	2.5a	1.3a	8.1ab	10.0ab	1.3a	11.3ab	41.9b
Abamectin	1.3a	0.6a	3.8ab	6.9ab	0a	13.8b	31.9b
Delegate	0.6a	1.9a	5.6ab	0.6a	1.9a	11.3ab	40.6b
Comm-EPF 5 ml	1.9a	2.5a	6.9ab	8.1ab	2.5a	16.9b	34.4b
Comm-EPF 10 ml	3.1a	0a	10.0b	15.0b	1.3a	18.1b	40.6b
Dursban	1.3a	0.6a	0a	3.1a	1.9a	2.5a	8.1a
Movento 20 ml	2.5a	0.6a	4.4ab	7.5ab	0a	10.6ab	7.5a
Bio-Cure	1.3a	2.5a	9.4b	3.1a	2.5a	18.1b	49.4b

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

As thrips infestation was not high, neither was the level of damage recorded, particularly damage which would be severe enough to lead to the fruit being culled. The only two treatments for which there was significantly less damage than the untreated control were abamectin and Delegate (Table 3.5.2.4). It is a bit surprising that this was not also the case for Dursban, as it appeared to be one of the more effective treatments in reducing thrips numbers.

Table 3.5.2.4. Thrips scarring recorded on Navels at Sundays River Valley, Eastern Cape, on 19 April 2012

Treatments	Fruit with thrips scarring (%)	Fruit which would be culled for thrips (%)	Total fruit with any thrips damage (%)
Control	17.2a	7.8a	25.0a
Abamectin	7.7b	3.0a	10.7b
Delegate	8.4b	3.8a	12.2b
Comm-EPF 5 ml	12.3ab	6.7a	19.0ab
Comm-EPF 10 ml	14.6ab	5.0a	19.6ab
Dursban	12.3ab	5.7a	18.0ab
Movento	11.6ab	3.4a	15.0ab
Bio-Cure	14.7ab	6.0a	20.7ab

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Dursban was the only treatment which significantly reduced mealybug infestation in the second trial (Table 3.5.2.5). The other treatments seemed to have no effect at all. This was disappointing, as a trial conducted with Comm-EPF against a high level of mealybug in the previous season showed significant reduction in numbers (Wayne Kirkman, pers comm.). It was not clear why this did not happen again, as great care was taken to apply the Comm-EPF in the cool of the evening and mealybug infestation was such that it was clearly exposed to the sprays.

Table 3.5.2.5. Supplementary trial in Navels at Sundays River Valley, Eastern Cape

Treatments	22 Days after treatment
	Fruit with mealybug (%)
Control	34.2b
Dursban	2.5a
Comm-EPF 5 ml	36.7b
Comm-EPF 10 ml	42.5b
Bio-Cure	43.3b

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

2013/4

In the north, the abamectin treatment performed very well and gave similar results to Closer against thrips and mealybug (Table 3.5.2.6). Dursban on the other hand performed poorly, although it was significantly more efficacious than the control for both pests. Only the higher dose of the G113L6 isolate gave some efficacy against mealybug and reduced thrips scarring but none of the EPF treatments reduced numbers of thrips significantly (P>0.05).

In the south, only the higher concentration of FCM Ar23 B3 significantly reduced infestation of thrips adults, relative to the control. This was also only at one of the trial sites and did not last longer than one week (Table 3.5.2.7). Neither of the fungal isolates provided any significant control of mealybug at either of the trial sites (Table 3.5.2.7 & 3.5.2.8). At one of the sites, abamectin, sulfoxaflor and Dursban significantly reduced thrips larvae and/or adults, with abamectin and sulfoxaflor showing significantly superior efficacy against larvae (Table 3.5.2.7). Although there were marked differences in thrips damage, these differences were surprisingly not different. At the second trial site, there were again very few significant differences between the efficacy of products, although abamectin and sulfoxaflor did appear to be the most efficacious against thrips (Table 3.5.2.8). Sulfoxaflor was the most effective product against mealybug.

In the late season corrective trial applied in the Gamtoos River Valley, none of the fungal isolates provided any notable reduction in mealybug infestation. However, Applaud reduced mealybug infestation by 44%.

2014/5

In the north, the *Metarhizium* isolate without oil had some efficacy against thrips but when used with oil it had no significant effect. Perhaps this was due to too much run-off (Table 3.5.2.9). When used alone, *Metarhizium* had similar efficacy to abamectin and another biological called TripStop, but by the second evaluation all these treatments were overrun with thrips and only DPX and Pyrinex were providing control. DPX had no effect on mealybug.

In the south, Entrust (spinosad) was the most effective product against thrips, significantly reducing infestation and damage (Table 3.5.2.10). *Beauveria* plus oil also notably, but not significantly, reduced thrips infestation. However, this did lead to a significant reduction in thrips damage. Mealybug infestation was lower for all treatments than the untreated control at one of the sites (Table 3.5.2.10). However, this was only so for the first evaluation. At the other site, Entrust was surprisingly the only treatment that significantly reduced mealybug infestation (Table 3.5.2.11). In two late season corrective trials, *Metarhizium* reduced mealybug infestation by 26% (Table 3.5.2.12) and 33% (Table 3.5.2.13). In both cases, infestation was significantly lower than the control and not dissimilar to buprofezin and sulfoxaflor (in one of the trials – Table 3.5.2.12).

Conclusion

Results with entomopathogenic fungi against both thrips and mealybug were generally disappointing and perhaps the density-dependent response of EPFs is incompatible with citrus thrips populations that can cause a lot of damage at low levels. However, a couple of late season corrective treatments did result in a significant reduction in mealybug infestation.

Future research

No further research is planned with EPFs for thrips control but they will be investigated for the control of other insect pests or life stages that are less exposed to the sun. Additionally, another project has been registered to establish whether any of other EPF isolates are more suited to use against thrips and mealybug i.e. less UV- and moisture-dependent; and to improve formulation of EPFs for foliar application.

Technology transfer

As trial results with the entomopathogenic fungi were not compelling, no technology transfer was conducted.

References cited

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GOBLE, TA, DAMES, JF, HILL, MP & MOORE, SD. 2011. Investigation of Native Isolates of Entomopathogenic Fungi for the Biological Control of Three Citrus Pests. *Biocontrol Science and Technology* 21(10): 1193-1211.

Table 3.5.2.6. 2013 results from trial at Olifants River Estate, Limpopo Province

			Count: 13/11/2013 (7 DAT)						Count: 25/11/2013 (19 DAT)			
Sprayed: 05&06/11/2013			**%Thrips larvae**		**%Thrips Adults**		**Mealybug %**		**%Thrips scars**		**% Thrips cull**	
1	Control		28.8	a	23.1	a	43.4	a	22.8	a	2.8	a
2	Abamectin + oil	20 ml+300ml	1.9	c	4.1	c	5.6	d	3.1	d	0.0	b
3	Sulfoxaflor (Closer) +BreakThru	12 ml + 5ml	2.5	c	4.1	c	6.6	d	4.7	d	0.0	b
4	G113L6+BreakThru	10^{13} + 5ml	20.9	ab	15.0	ab	31.3	abc	14.1	ab	0.6	ab
5	G113L6+BreakThru	10^{14} + 5ml	17.8	ab	15.0	ab	27.2	bc	12.5	bc	1.3	ab
6	FCM Ar23B3+BreakThru	10^{13} + 5ml	25.0	a	17.2	ab	36.6	ab	20.9	ab	1.6	ab
7	FCM Ar23B3+BreakThru	10^{14} + 5ml	23.8	ab	19.4	ab	34.7	ab	13.8	ab	0.6	ab
8	Dursban	100 ml	10.6	bc	10.3	bc	19.1	c	5.6	cd	0.0	b

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Table 3.5.2.7. 2013/14 results from trial at Huguenot Farm, Eastern Cape Province

			28/10/2013 (19 DAT)				04/11/2013 (26 DAT)				13/01/2014 (96 DAT)					
Sprayed: 09/10/2013			**%Thrips larvae**		**%Thrips adults**		**Thrips larvae**		**%Thrips adults**		**%Thrips cull**		**% Thrips scars**		**% Mbug infested**	
1	Control		12.5	a	15.6	a	-	-	-	-	11.56	a	23.44	a	2.5	a
2	Abamectin + hort mineral oil	20 ml + 300ml	0	c	0.6	b	2.8	a	1.9	b	0.94	a	9.38	a	11.2	a
3	Sulfoxaflor (Closer) + BreakThru	12 ml + 5 ml	0	c	2.5	bc	5.3	a	8.1	ab	2.81	a	11.88	a	4.69	a
4	G113L6+BreakThru	10^{13} + 5 ml	10.6	ab	8.1	ab	-	-	-	-	3.13	a	17.5	a	3.13	a
5	G113L6+BreakThru	10^{14} + 5 ml	3.1	abc	5.0	ab	11.3	a	6.9	ab	6.56	a	14.06	a	8.13	a
6	FCM Ar23B3 + BreakThru	10^{13} + 5 ml	10.0	a	6.3	ab	-	-	-	-	4.69	a	18.75	a	2.19	a
7	FCM Ar23B3 + BreakThru	10^{13} + 5 ml	1.9	ab	4.4	b	10.6	a	15.0	a	6.56	a	17.19	a	10.00	a
8	Dursban	100 ml	2.5	ab	2.5	b	8.8	a	10.0	a	5.63	a	15.00	a	2.19	a

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Table 3.5.2.8. 2013/14 results from trial at Avoca Farm, Eastern Cape Province

			30/10/2013 (16 DAT)				06/11/2013 (23 DAT)				13/01/2014 (91 DAT)					
Sprayed: 14/10/2013			**%Thrips larvae**		**%Thrips adults**		**Thrips larvae**		**%Thrips adults**		**%Thrips cull**		**% Thrips scars**		**%Mbug infested**	
1	Control		15.9	a	9.1	a	-	-	-	-	10.31	a	28.75	a	25.00	a
2	Abamectin + hort mineral oil	20ml+300ml	1.9	a	2.5	a	4.1	a	3.1	a	8.13	ab	26.25	a	10.00	a
3	Sulfoxaflor(Closer)+BreakThru	12ml + 5ml	1.9	a	1.6	a	3.4	a	2.5	a	9.06	b	22.50	a	0.94	a
4	G113L6+BreakThru	10^{13} + 5ml	13.4	a	5.0	a	-	-	-	-	18.13	ab	24.63	a	19.38	a
5	G113L6+BreakThru	10^{14} + 5ml	7.2	a	3.8	a	9.7	a	9.7	b	21.56	ab	21.25	a	15.00	a
6	FCM Ar23B3+BreakThru	10^{13} + 5ml	12.2	a	8.1	a	-	-	-	-	10.31	ab	25.04	a	19.38	a
7	FCM Ar23B3+BreakThru	10^{14} + 5ml	12.5	a	4.1	a	-	-	-	-	6.88	ab	28.75	a	10.31	a
8	Dursban	100ml	4.1	a	1.6	a	-	-	-	-	1.56	ab	20.00	a	4.06	a

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Table 3.5.2.9. 2014 results from trial at Olifants River Estate, Limpopo Province

			Count: 06/11/2014 (14 DAT)						Count: 19/11/2014 (27 DAT)						27/11/2014 (35 DAT)	
Sprayed: 22&23/10/2014			**%Thrips larvae**		**%Thrips Adults**		**Thrips Scars**		**%Thrips larvae**		**%Thrips Adults**		**Thrips Scars**		**% Mbug infested**	
1	Control		5.6	a	12.5	a	12.8	a	23.4	b	19.1	b	35.9	a	7.2	ab
2	Medium oil	300ml	0.9	b	10.6	ab	6.9	b	36.3	a	27.8	a	38.8	a	1.9	bc
3	Abamectin+oil	20ml+300ml	0.3	b	7.5	ab	1.6	bc	22.5	b	19.4	b	19.7	b	3.4	abc
4	Metarhizium	10^{14}	0.6	b	8.4	ab	4.1	bc	17.8	b	10.9	b	18.1	b	4.4	abc
5	Metarhizium+oil	10^{14} + 300ml	5.0	a	11.9	ab	13.1	a	27.5	ab	15.9	b	31.6	ab	1.9	bc
6	DPX	100 ml	0.0	b	1.9	c	0.6	c	0.0	d	3.4	c	0.9	c	9.7	a
7	Pyrinex 250 CS	200 ml	0.6	b	5.3	b	4.7	bc	6.9	c	12.8	b	7.8	c	0.6	c
8	TripStop + BFA	5 ml + 100g	0.3	b	8.1	ab	3.4	bc	21.3	b	9.7	b	22.2	b	4.1	abc

Means in the same column followed by the same letter were not significantly different at α=0.05 (SNK)

Table 3.5.2.10. 2014/15 results from trial in orchard 8 at Jurgens, Eastern Cape Province.

			06/11/2014 (8 DAT)						14/11/2014 (16 DAT)						13/01/2015 (76 DAT)		27/05/2015 (210 DAT)			
Sprayed: 29/10/2014			%Thrips larvae		%Thrips adults		%Mbug inf		Thrips larvae		%Thrips adults		%Mbug infested		%Mbug inf		%Thrips cull		% Thrips scars	
1	Control		7.3	a	2.0	ab	2.7	a	6.0	a	0	a	31.3	a	31.3	a	33.1	a	46.3	a
2	Entrust+oil	9g+300ml	0.7	b	0	a	0.7	a	2.7	a	0	a	25.6	a	25.6	a	10.0	c	42.5	a
3	Metarhizium	10^{14}	5.0	ab	3.1	b	0.6	a	10.0	a	1.9	b	36.9	a	36.9	a	29.4	ab	48.1	a
4	Metarhizium +oil	10^{14} + 300ml	10.6	a	1.3	ab	0	a	5.0	a	1.3	ab	25.0	a	25.0	a	26.3	ab	47.5	a
5	Beauveria	10^{14}	5.6	ab	2.5	ab	1.3	a	6.3	a	0.6	ab	31.3	a	31.3	a	28.1	ab	40.6	a
6	Beauveria + oil	10^{14} + 300ml	3.8	ab	0.6	ab	1.3	a	5.6	a	0.6	ab	31.9	a	31.9	a	18.8	bc	48.1	a

Table 3.5.2.11. 2014/15 results from trial in orchard 7 at Jurgens, Eastern Cape Province.

			14/11/2014 (8 DAT)						15/05/2015 (190 DAT)			
Sprayed: 06/11/2014			%Thrips larvae		%Thrips adults		%Mbug inf		%Thrips cull		% Thrips scars	
1	Control		14.4	a	1.9	a	7.5	a	43.75	a	41.9	ab
2	Entrust+oil	9g+300ml	8.8	a	0	a	0.6	c	28.75	c	41.2	ab
3	Metarhizium	10^{14}	16.3	a	2.5	a	7.5	abc	30.0	c	47.5	ab
4	Metarhizium +oil	10^{14} + 300ml	18.1	a	0.6	a	6.3	bc	33.1	bc	48.1	ab
5	Beauveria	10^{14}	31.3	a	1.3	a	4.4	abc	36.3	abc	48.8	b
6	Beauveria +oil	10^{14} + 300ml	23.1	a	1.3	a	8.8	ab	41.25	ab	38.8	a

Table 3.5.2.12. 2015 results from trial 1 at Kleinplaas, Eastern Cape Province.

			11/03/2015 (41 DAT)		
Sprayed: 29/01/2015			% Mbug inf		% reduction
1	Control		62.0	a	
2	Applaud	30g	27.0	b	56.5
3	Metarhizium	10^{14}	46.0	b	25.8
4	Closer	12ml	29.0	b	53.2

Table 3.5.2.13. 2014 results from trial 2 at Kleinplaas, Eastern Cape Province.

			11/03/2015 (41 DAT)		
Sprayed: 29/01/2015			**% Mbug inf**		**% reduction**
1	Control		29.0	a	
2	Metarhizium + oil*	10^{14} + 300 ml	18.0	b	33.3

*Fungal spores pre-mixed with the oil.

3.5.3 FINAL REPORT: Mating disruption for red scale control

Project 1076 (2014/5 – 2015/6) by T G Grout, P R Stephen and W Kirkman (CRI)

Summary

Mating disruption for red scale control was proposed more than 25 years ago but has not been cost effective until the recent development of a mesoporous dispenser used in a system called Saturel CRS. We wanted to evaluate this system at three sites in the northern production regions in 2014/5 but lost one site after applying treatments because the orchard was top-worked. The remaining two sites were in Mpumalanga and Limpopo provinces and included an imidacloprid soil drench and winter medium grade horticultural oil spray at 1% as standards. These were compared to two treatments of Saturel CRS starting either during bloom or at petal fall, in addition to a combination treatment of a winter oil spray followed by petal fall Saturel CRS. Fruit infestation of all treatments was evaluated in summer. In 2015/6 the trials were repeated at the same sites in Mpumalanga and Limpopo and an additional site was included in the Eastern Cape. Saturel CRS treatments were applied to the same blocks as before, as requested by the manufacturer, but the control blocks and 1% oil blocks were swopped. Generic imidacloprid was used in 2014/5 but after its poor performance at both sites in the north, it was replaced with Confidor WG at all sites in 2015/6. The results after the two seasons show that red scale has developed resistance to imidacloprid in the northern production areas to the extent that this treatment gives similar efficacy to a winter oil spray (1%). In the Eastern Cape, imidacloprid was still effective and provided significantly better control than a winter oil spray (1%). In the northern regions, Saturel CRS applied during bloom gave similar efficacy to the winter oil spray and was more effective than when applied after petal fall. In the Eastern Cape the later application of Saturel CRS was more effective than the early application and gave similar efficacy to the winter oil spray. Saturel CRS could be a useful tool for maintaining red scale populations at low densities in IPM orchards, but the price will have to be competitive with alternative products that also have a low impact on natural enemies.

Opsomming

Paringsontwrigting vir die beheer van rooidopluis is meer as 25 jaar gelede al voorgestel. Dit was egter tot onlangs nie koste-effektief nie. Dit het onlangs verander met die ontwikkeling van ʼn mesoporieuse vrysteller wat in ʼn sisteem, bekend as Saturel CRS, gebruik word. Ons wou die sisteem by drie persele in die noordelike produksie-areas evalueer, maar het een perseel verloor na die behandelings toegedien is omdat die boord oorgewerk is. Die oorblywende twee persele was in die Mpumalanga en Limpopo provinsies, en het ʼn imidaclopried grondtoediening en winter medium graad tuinbou-olie bespuiting teen 1% as standaarde behandelings ingesluit. Hierdie was met twee behandelings van Saturel CRS vergelyk, toegedien óf tydens blom of blomblaarval, asook ʼn kombinasie van ʼn winter-oliebspuiting en Saturel CRS toediening teen blomblaarval. Vrugbesmetting is vir alle behandelings in die somer geêvalueer. In Mpumalanga en Limpopo is dieselfde persele gebruik as die vorige jaar, asook ʼn adisionële perseel in die Oos-Kaap. Saturel CRS is in dieselfde blokke as voorheen toegedien, soos deur die vervaardiger versoek, maar die kontrole- en 1% olie-blokke is omgeruil. By alle persele is Confidor WG in 2015/6 gebruik, weens die swak resultate wat in 2014/5 in die noordelike produksiegebide met generiese imidaklopried verkry is. Twee jaar se resultate wys dat rooidopluis weerstand teen imdaklopried in die noordelike produksiegebiede ontwikkel het, waar dit nou soortgelyke beheer gee as ʼn winter-oliebepuiting (1%). In die Oos-Kaap was imidaklopried steeds effektief, en het beduidend beter beheer gegee as ʼn winter-oliebespuitng (1%). In die noordelike gebiede het Saturel CRS, toegedien tydens blom, soortgelyke beheer gegee as die winter-oliebespuiting, en was meer effektief as die toediening na blomblaarval. In die Oos-Kaap was die latere toediening van Saturel CRS meer effektief as die vroë toediening, en het soortgelyke beheer gegee as die winter-oliebespuiting. Saturel kan ʼn handige opsie wees om rooidopluis-populasies laag te hou in IPM-boorde, maar die prys sal moet kompterend wees met alternatiewe produkte wat ook ʼn lae impak het op natuurlike vyande.

Introduction

Red scale, *Aonidiella aurantii*, populations in commercial citrus orchards are largely being controlled by the use of preventive imidacloprid applications due to concerns about the risks of horticultural mineral oils and the direct and indirect costs of spirotetramat (Movento) and pyriproxyfen, respectively. If the current concerns about the impact of imidacloprid on honeybees result in the cancellation of the registration of this active ingredient on citrus, the costs of controlling this pest will escalate. Mating disruption as a means of controlling red scale was first proposed by Hefetz et al. (1988) after field work in citrus orchards in Israel but due to relatively poor efficacy and high cost, no further research was done on this technique for almost 20 years when the product Red Scale Down appeared on the market in California. Sousa et al. (2008) evaluated this product in Portugal at 250 dispensers/ha and concluded that it was promising if applied to low red scale populations but could not control populations requiring a pesticidal spray. Gonzalez et al. (2008) were also not impressed with the efficacy of 500 dispensers/ha but suggested that application earlier in the season may improve efficacy. A change to mesoporous dispensers containing either 8 or 20 mg pheromone had more of an impact on trapped male numbers but not enough to reduce fruit damage (Vacas et al. 2009), but 50 mg per dispenser at 400 dispensers/ha reduced fruit infestation by 60 and 80% compared to two untreated blocks and 70% compared to a block receiving a 1% oil spray at 3 500 L/ha (Vacas et al. 2010). These dispensers were provided by Ecologia y Proteccion Agricola in Valencia, Spain. Further field trials with these dispensers loaded with 70 mg pheromone were conducted by Syngenta and provided scale control when placed in the orchard in time for the first flight of the season. These dispensers lasted more than 195 days (Mas et al. 2012). Research results with these dispensers were presented at the International Citrus Congress (Navarro-Llopis et al. 2012) and showed that a 90% reduction in the percentage of fruit with more than 5 red scale could be obtained with a minimum of 35 g pheromone/ha or 450 dispensers/ha. If the dispensers contain 70 mg pheromone this would require 500/ha. The dispensers last at least 150 days so if hung in late August they would be effective until late January when numbers of natural enemies increase. The trade name for this dispenser is Saturel CRS.

Stated Objective

Evaluate the Saturel CRS mating disruptant against red scale populations in the field in South Africa.

Materials and methods

Research in Spain has led to the recommendation of at least 35 g/ha of red scale pheromone (a diastereomeric mixture of 3S,6R and 3S,6S forms of 3-methyl-6-isopropenyl-9-decen-1yl acetate) in order to control red scale when at low to moderate population levels. We therefore did not experiment with different rates but only evaluated what was recommended in Spain, with or without an oil spray before the treatment. The trials were to be conducted on orange trees at three sites in Limpopo and Mpumalanga in the first year and at another three sites in the second year that included one site in the Eastern Cape.

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Trial layout required large treatment blocks of approximately 0.5 ha for the mating disruption blocks to ensure pheromone saturation in the middle of the block. Two such blocks of approximately 8 rows x 16 trees were used per treatment and 8 data trees in the centre of the 2 middle rows were evaluated for infestation. Other treatment blocks were smaller (6 rows x 10 trees) but still had 8 data trees each. Pretreatment infestation levels on fruit were determined before harvest in order to select a suitable orchard and determine whether the red scale infestation across the block was not too clumped. Two red scale pheromone traps were then placed in each untreated control block to monitor flight peaks at the site. Pheromone traps were not used to determine treatment infestation levels because this has been found to be unreliable in the past (Grout and Richards 1991) and trap shutdown is expected in the Saturel CRS treatment blocks. However, they were placed in some Saturel CRS blocks prior to hanging the mating disruptants to demonstrate the trap shut-down effect. The orchards were sprayed by the growers with standard treatments for thrips, FCM, black spot and fruit fly that were considered to have a negligible effect on red scale. The following red scale treatments were evaluated:

1. Untreated control
2. Saturel CRS at 500/ha hung out first week September
3. Saturel CRS at 500/ha hung out first week October
4. Medium horticultural oil 1% spray last week July or first week August
5. Confidor 700 WG at 4.5 g per tree or imidacloprid 350 SC 9 ml per tree at white bud in August
6. Treatment 4 followed by Treatment 3

In 2014/5 one trial was established at Schoeman Boerdery near Marble Hall, Limpopo Province in a Bahianinha navel orange orchard on drip irrigation and a second trial was established at Vergenoeg, Golden Frontiers Citrus near Komatipoort, Mpumalanga in a Turkey Valencia orchard. A third site was established at another site close to Nelspruit but after applying some of the treatments the grower decided to cut the trees down and top-work the orchard so it could not be used. Medium grade horticultural oil was sprayed at 1% with the growers' automatic spray machines at a slow enough ground speed and high enough spray volume to provide full cover film wetting. Volumes of approximately 6 300 L/ha were used at Vergenoeg and 8 500 L/ha at Schoeman Boerdery. Imidacloprid treatments were mixed in 1 L water and poured on each dripper where drip irrigation was used with half the dose being given to each dripper where there were two drippers per tree. In the case of microsprinkler irrigation the imidacloprid was mixed in 1 L water and slowly poured around the base of the tree. At Schoeman Boerdery Bandit 350 SC was used and at Vergenoeg GFC, Kohinor 350 SC was used. The tree spacing at Schoeman Boerdery was 3.8 m x 6 m resulting in approximately 440 trees per hectare and at Vergenoeg spacing was 3 m x 7 m resulting in 476 trees per hectare. Blocks treated with Saturel CRS therefore received one dispenser per tree which was hung inside the canopy near the centre of the tree 1.8 to 2.2 m above ground. Two pheromone traps (White Scale Cards with CRS PheroLures, Insect Science, Tzaneen) were hung inside the canopy of two trees in the centre row of each control block. Two pheromone traps were also hung in each of the early Saturel CRS only blocks for a few weeks before and after the release to demonstrate trap shut-down when the dispensers were hung in the trees.

Fruit infestation was determined in January to March when there was an adequate fruit infestation level in the control to show treatment differences. Twenty fruit were inspected on each of 8 data trees per block and the percentages of clean fruit, those with 1-10 red scale and those with more than 10, was determined. Trap peaks were transcribed to a phenological time scale based on a lower developmental threshold of 11.7°C after using iButtons in citrus trees to gather temperature data in each orchard.

<u>2015/6</u>
During the second season the timing and dose of the treatments were kept similar to those used in 2014/5 in order to provide replication. The same two orchards used in Limpopo and in Mpumalanga were re-used with the Saturel treatments in the same blocks at the request of the manufacturer but the control blocks and 1% oil blocks were swopped. A third trial site at Dunbrody Estate in the Sundays River Valley in the Eastern Cape was used as a replacement for the Mpumalanga site that was lost in 2014/5. At this site the two replicates were in two adjacent blocks of navel orange trees planted in 2006; one block was Powell navels and the other was Autumn Gold navel orange. There were 555 trees per hectare. Treatments, monitoring and evaluations were conducted in the same way as for 2014/5. However, Confidor WG was used in all imidacloprid treatments rather than the generic SC. BP Medium oil was sprayed at 8 763 L/ha in the Eastern Cape. Volumes sprayed in the northern orchards were approximately 7700 L/ha at Vergenoeg and 9100 L/ha at Schoeman Boerdery. The later treatment of Saturel CRS dispensers at Schoeman Boerdery and in the Eastern Cape was later than planned because of a delay in the supply of dispensers. Fruit infestation was determined in January as for 2014/5 except that categories were clean fruit, 1-5 red scale and 6 or more red scale per fruit.

Degree days were calculated from daily maxima and minima at each site using the calculator at http://www.ipm.ucdavis.edu/WEATHER/#DEGREEDAYS with a single sine-wave model, intermediate cut-off, a lower developmental threshold of 11.7°C and an upper developmental threshold of 37.8°C. These were used to plot trap catches of male red scale on a physiological time scale.

Results and discussion

<u>2014/5</u>
Results from the two sites in the first season showed few significant differences (Tables 3.5.3.1 and 3.5.3.2) but the best treatments at both sites were those receiving the 1% oil spray in winter which at Komatipoort was more effective than the imidacloprid. The later applied Saturel (Treatment 3) was inferior to the earlier applied pheromone, although this difference was only significant at Marble Hall when evaluated in February 2015. Despite this result the manufacturer wanted us to apply the pheromones later in 2015/6. At both sites in 2014/5 the early Saturel treatment during bloom gave the same results as registered generic imidacloprid drenches which were not very effective (Tables 3.5.3.1 and 3.5.3.2).

Table 3.5.3.1. Results from Golden Frontiers Citrus, Vergenoeg, Komatipoort, Mpumalanga in 2014/5

Treatments		Evaluated 18/03/2015	
		Fruit infested (%)	Fruit with 11+ (%)
1	Control	47.5 a	16.8 a
2	Saturel one per tree (8 Sep)	19.3 b	4.8 bc
3	Saturel one per tree (29 Sep)	21.8 b	6.0 bc
4	Med oil 1% (23 Jul)	8.5 c	2.0 bc
5	Imidacloprid (Kohinor 350 SC) at 9 ml/tree	21.8 b	7.8 b
6	Med oil 1% (23 Jul) + Treatment 3	5.8 c	1.5 c

Means in the same column followed by the same letter were not significantly different at α = 0.05 (SNK test)

Table 3.5.3.2. Results from Schoeman Boerdery, Marble Hall, Limpopo Province in 2014/5

Treatments		07/01/2015	Evaluated 12/02/2015	
		Fruit infested (%)	Fruit infested (%)	Fruit with 11+ (%)
1	Control	17.5 a	25.5 a	2.8 a
2	Saturel one per tree (15 Sep)	9.4 ab	15.3 b	2.0 a
3	Saturel one per tree (6 Oct)	13.8 ab	25.5 a	2.5 a
4	Med oil 1% (5 Aug)	7.2 bc	14.0 b	1.5 a
5	Imidacloprid (Bandit 350 SC) at 9 ml/tree	11.3 ab	14.3 b	2.8 a
6	Med oil 1% (5 Aug) + Treatment 3	2.5 c	11.8 b	0.3 a

Means in the same column followed by the same letter were not significantly different at α = 0.05 (SNK test)

The pheromone traps in the control clearly caught more red scale males than in the Saturel treatment due to the Saturel pheromone dispensers largely cancelling out the attraction of the traps (Figs. 3.5.3.1 and 3.5.3.2). The degree-days were accumulated from an arbitrary date but the time between peaks did not correspond well with generation and inter-cohort intervals (Grout et al. 1989) due possibly to thrips sprays or bad weather stopping some flights. The highest peak in the control at Komatipoort occurred on 22 Jan 2015 and at Marble Hall on 28 Jan 2015. Both of these were below 1 000 males per trap per week. The peak number of males trapped at Marble Hall was slightly higher than that at Komatipoort but the fruit infestation levels in the controls were clearly higher at Komatipoort (Table 3.5.3.1) which once again showed that the numbers of males trapped on pheromone traps are not a good indicator of expected fruit infestation levels (Grout and Richards 1991).

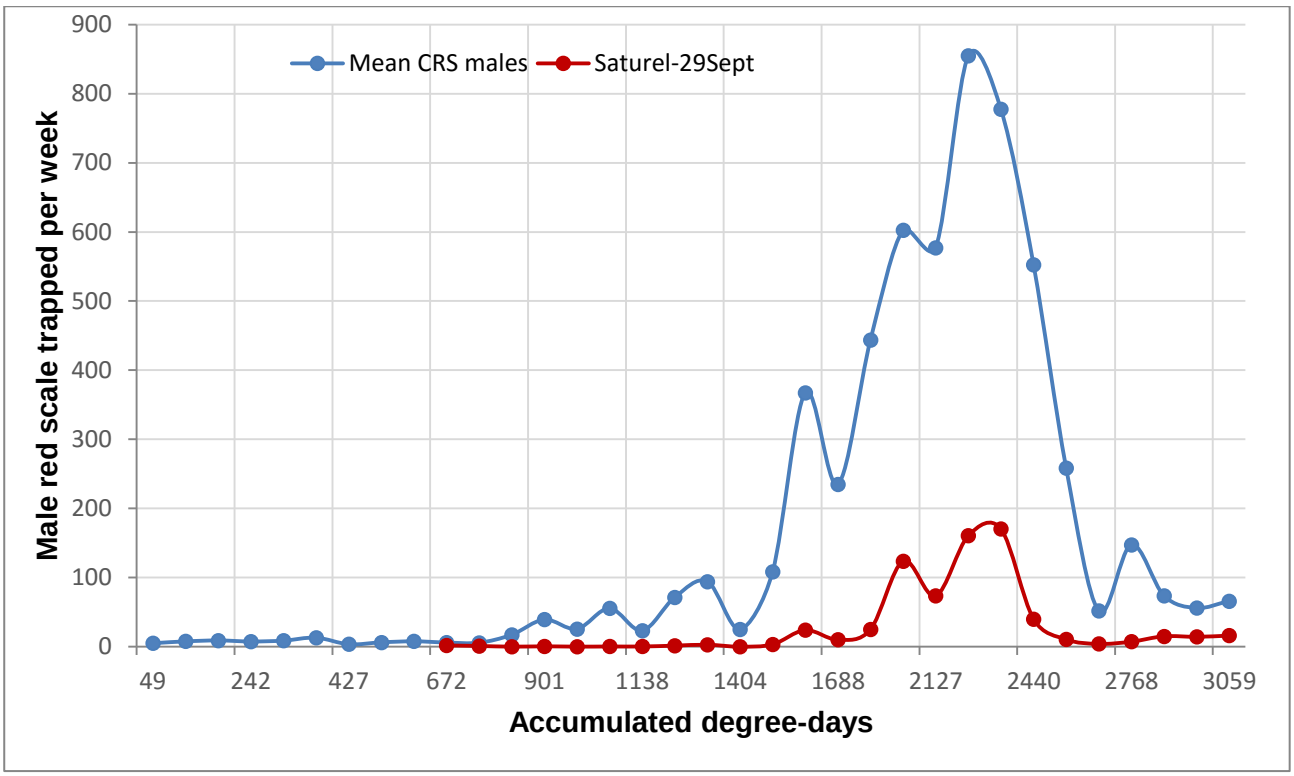

Figure 3.5.3.1. Mean numbers of male red scale trapped per week in the trial at Golden Frontiers Citrus, Vergenoeg, Komatipoort, Mpumalanga in 2014/5.

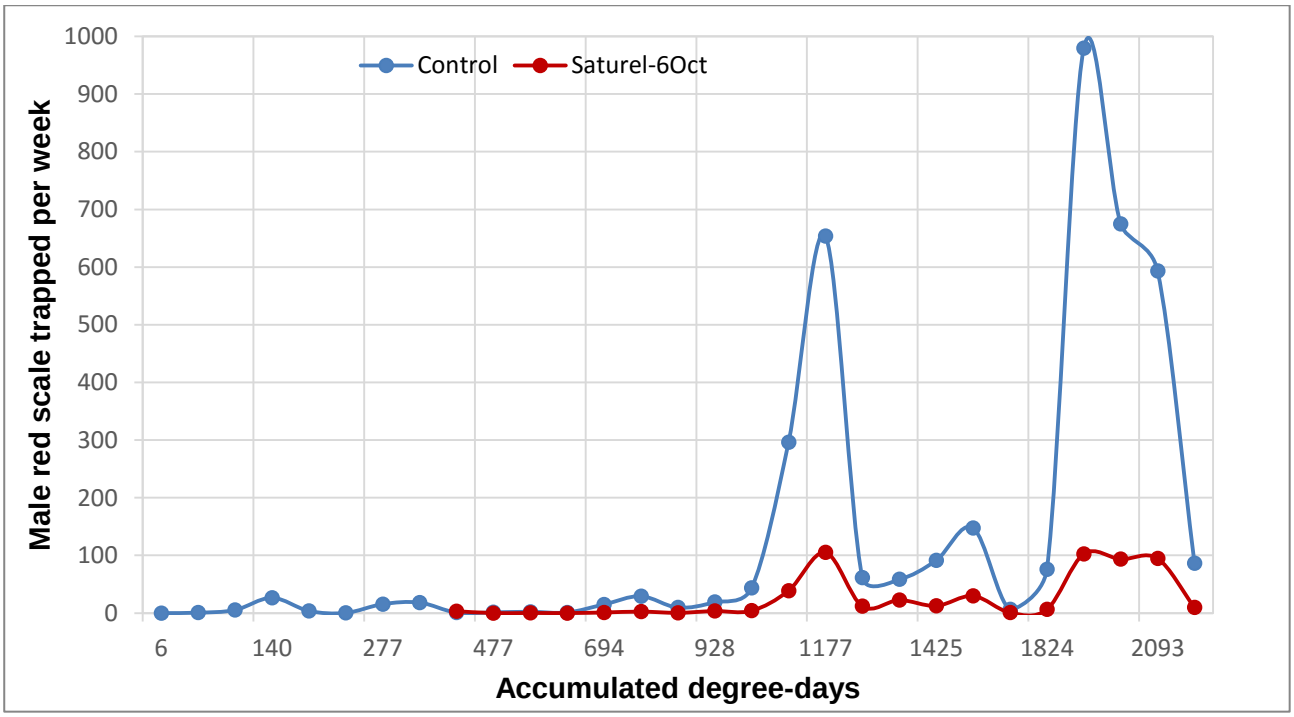

Figure 3.5.3.2. Mean numbers of male red scale trapped per week in the trial at Schoeman Boerdery, Marble Hall, Limpopo Province in 2014/5

<u>2015/6</u>

In the second season, the later Saturel application was again inferior to the early application in both northern sites and using the original Bayer Confidor rather than generic imidacloprid made no difference to the poor efficacy experienced (Tables 3.5.3.3 and 3.5.3.4). It is therefore apparent that red scale has developed a significant level of resistance to imidacloprid in the northern areas. In the northern areas a winter oil spray followed by a Saturel treatment starting in bloom would probably be the most effective combination. In the Eastern Cape Province, Confidor was very effective (Table 3.5.3.5) and significantly better ($P<0.05$) than a

winter oil spray, but results from the early Saturel application were poor and for this cooler climate this was probably too early. The post-petal fall application of Saturel in the Eastern Cape was equivalent in efficacy to a winter oil spray whereas in the four northern trials the post-petal fall Saturel application was always inferior to the winter oil spray.

Pheromone traps in the Saturel blocks in the northern trial sites were not maintained for as long as in the previous season but they once again demonstrated that fewer males in the treatment blocks were finding the traps (Fig. 3.5.3.3 and 3.5.3.4). The traps in the control blocks were also removed earlier, just after the late January peak flight which at Komatipoort occurred on 12 Jan 2016 and at Marble Hall on 26 Jan 2016. Numbers of males trapped in the control at Komatipoort were generally lower than those trapped at Marble Hall but the infestation levels on fruit were similar (Tables 3.5.3.3 and 3.5.3.4). In the Sundays River Valley, traps were not placed in the Saturel blocks but in both control replicates. The flight peaks in both replicates correspond well (Figure 3.5.3.5) and the last peak recorded was on 23 Dec 2015. Petal fall was around 300 DD so once again it looks as if the male flights are suppressed by thrips sprays for at least 6 weeks after petal fall. This effect of thripicides on the use of red scale pheromone traps was published in 1991(Grout and Richards 1991).

From these results over the two seasons it is clear that red scale has developed resistance to imidacloprid in the northern areas and perhaps the trend that occurred with organophosphate resistance is being repeated where field failures occurred in the northern areas approximately 10 years before they started to occur in the Eastern Cape. With these results and the increasing negative publicity about possible effects of imidacloprid on bees, imidacloprid applications could be moved to petal fall in order to provide more control of citrus psylla later in the season and perhaps some mealybug suppression in November. Alternatives to neonicotinoids would have to be used for red scale control such as oil sprays or Movento. In the Cape provinces it would be advisable to use imidacloprid less frequently so that resistance can be delayed.

Saturel at one dispenser per tree offers a perfect IPM solution for maintenance of low density red scale populations and its use after a winter oil spray would be a good IPM approach for higher red scale population densities. However, the cost would have to be competitive with oil alone at 1% and Movento plus oil for it to be attractive to use in citrus IPM.

Table 3.5.3.3. Results from Golden Frontiers Citrus, Vergenoeg, Komatipoort, Mpumalanga in 2015/6

Treatments		**Evaluated 13/01/2016**	
		Fruit infested (%)	**Fruit with 6+ (%)**
1	Control	31.8 a	6.3 a
2	Saturel one per tree (27 Aug)	16.5 b	5.0 ab
3	Saturel one per tree (6 Oct)	33.0 a	6.8 a
4	Med oil 1% (3 Aug)	11.8 b	2.0 bc
5	Imidacloprid (Confidor 700 WG) at 4.5 g/tree (12 Aug)	17.8 b	3.5 abc
6	Med oil 1% (3 Aug) + Treatment 3	2.8 c	0.5 c

Means in the same column followed by the same letter were not significantly different at α = 0.05 (SNK test)

Table 3.5.3.4. Results from Schoeman Boerdery, Marble Hall, Limpopo Province in 2015/6

Treatments		**Evaluated 26/01/2016**	
		Fruit infested (%)	**Fruit with 11+ (%)**
1	Control	41.3 a	16.8 a
2	Saturel one per tree (1 Sep)	2.8 d	0.3 c
3	Saturel one per tree (29 Oct)	8.0 c	1.0 c
4	Med oil 1% (5 Aug)	2.0 d	0.5 c
5	Imidacloprid (Confidor 700 WG) at 4.5 g/tree (18 Aug)	27.5 b	12.0 b
6	Med oil 1% (5 Aug) + Treatment 3	0.3 d	0.0 c

Means in the same column followed by the same letter were not significantly different at α = 0.05 (SNK test)

Table 3.5.3.5. Results from Dunbrody Estates, Sundays River Valley, Eastern Cape Province in 2015/6

Treatments		Evaluated 18/01/2016			
		Fruit infested (%)		Fruit with 6+ (%)	
1	Control	44.5	a	13.0	a
2	Saturel one per tree (25 Aug)	31.8	b	5.3	b
3	Saturel one per tree (29 Oct)	16.8	c	2.0	b
4	Med oil 1% (11 Aug)	15.0	c	1.8	b
5	Imidacloprid (Confidor 700 WG) at 4.5 g/tree (8 Sep)	1.3	d	0.0	b
6	Med oil 1% (11 Aug) + Treatment 3	13.5	c	3.0	b

Means in the same column followed by the same letter were not significantly different at α = 0.05 (SNK test)

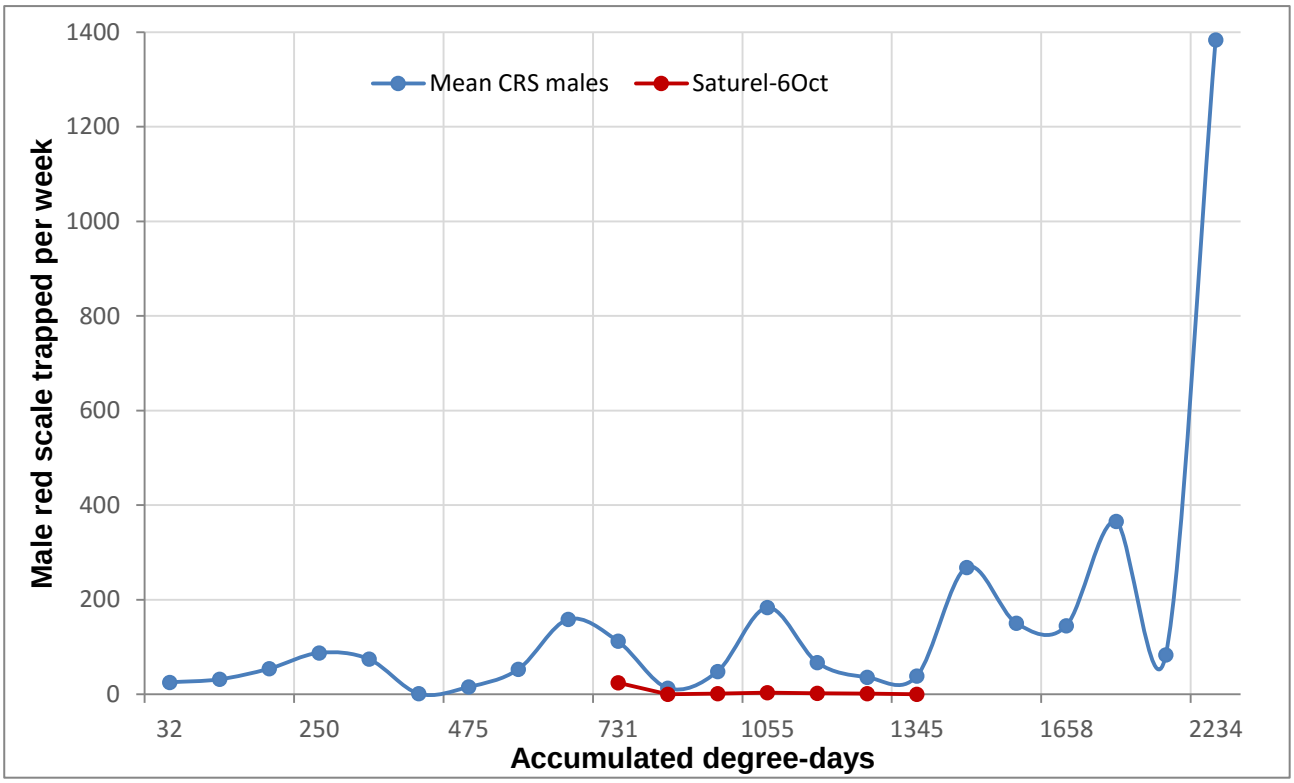

Figure 3.5.3.3. Mean numbers of male red scale trapped per week in the trial at Golden Frontiers Citrus, Vergenoeg, Komatipoort, Mpumalanga in 2015/6.

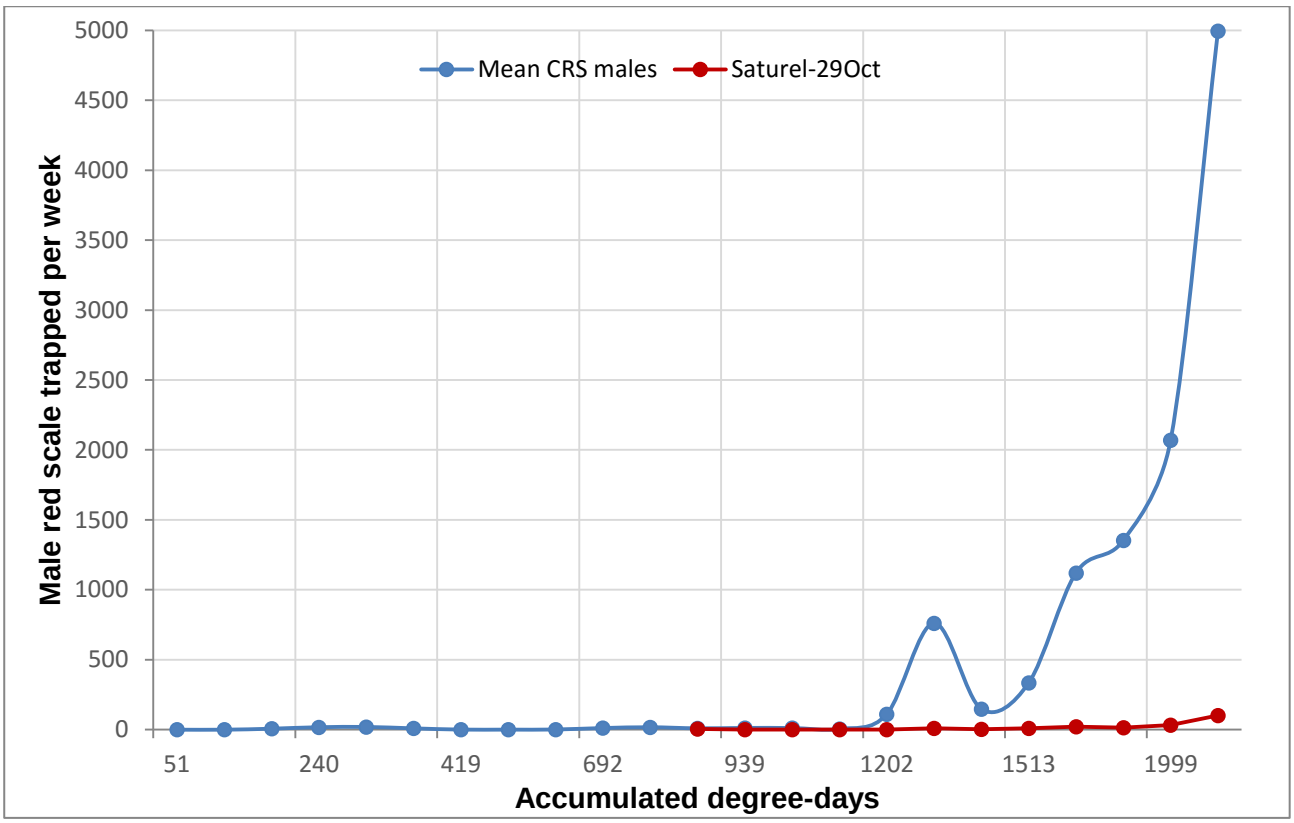

Figure 3.5.3.4. Mean numbers of male red scale trapped per week in the trial at Schoeman Boerdery, Marble Hall, Limpopo Province in 2015/6.

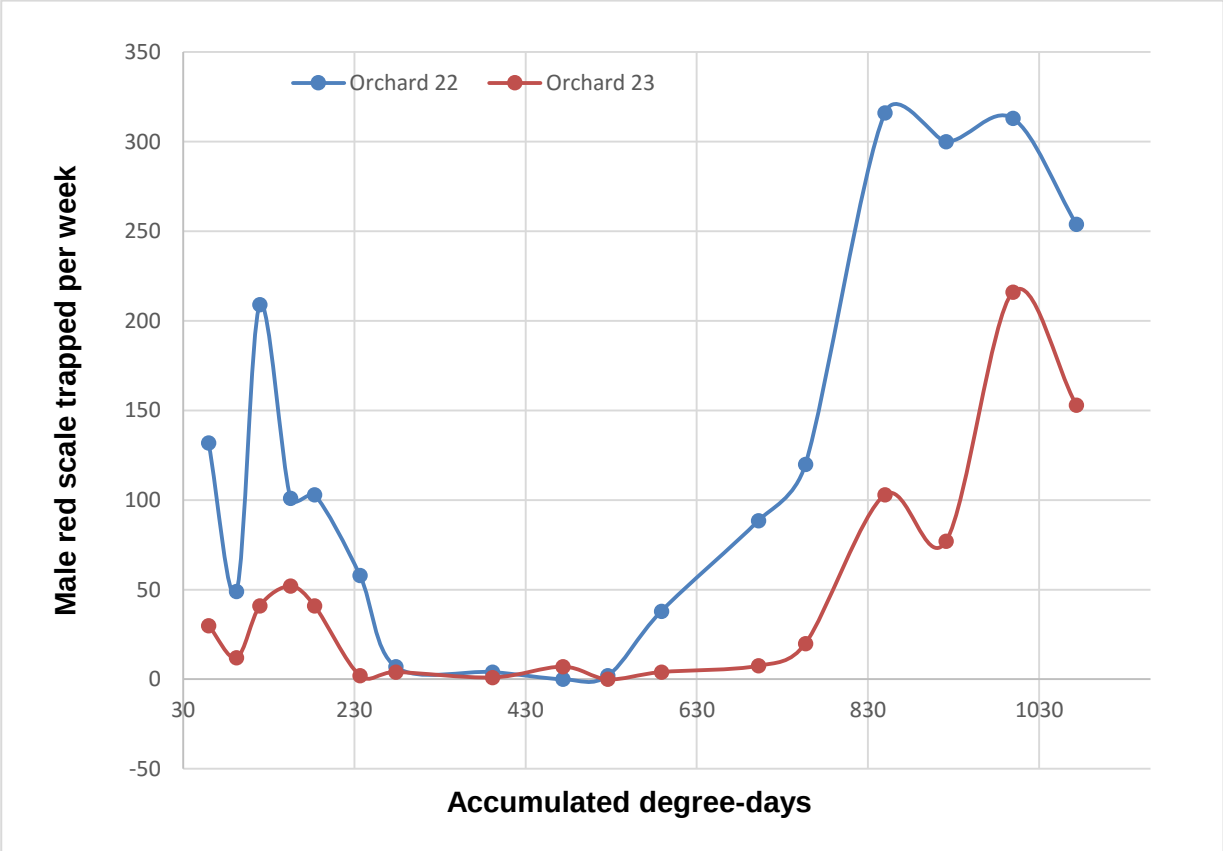

Figure 3.5.3.5. Numbers of male red scale trapped per week in the control of the two replicate orchards in the Sundays River Valley.

Conclusion

Saturel mating disruption dispensers hung during bloom in the northern regions had the same effect as imidacloprid applications in controlling red scale, but this was because red scale has developed some resistance to imidacloprid. Imidacloprid is still effective in the Eastern Cape where a later application of Saturel was significantly more effective than an early application and provided similar control to a winter oil spray. Numbers of red scale males trapped on pheromone traps in controls that did receive thripicides once again gave a poor reflection of expected red scale fruit infestation levels in summer.

Technology transfer

Citrus Research Symposium, August 2016

Future research

No further research is planned with the mating disruptant but it may be commercialised in South Africa if it is economical to do so.

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

Project 1061 (2013/4-2016/7) by Tim G Grout and Peter R Stephen (CRI)

Summary

There is a shortage of registered control options that can be used for late season control of thrips, citrus psylla, leafhoppers and woolly whitefly. The objective of this research is to evaluate unregistered products, and products that have been recently registered on citrus against other pests, that are likely to have short preharvest intervals. Unfortunately, due to the need to work on cold treatment of false codling moth (FCM), a lack of trial sites late in the season with the required pest infestations, and the inability to rear woolly whitefly for bioassays, no further research was conducted in this project. Once the FCM cold treatment research is complete there will be time to address the challenges of this project.

Opsomming

Daar is ŉ tekort aan geregistreerde beheermaatreëls om blaaspotjie, sitrusbladvlooi, bladspringer en wollerige-witvlieg (WWV) laat in die seisoen te beheer. Die doel van hierdie navorsing is om ongeregistreerde middels en middels wat onlangs teen ander sitrusplae geregistreer is, met moontlike kort vooroes intervalle, te evalueer. Ongelukkig, as gevolg van die behoefte om aan koue behandeling van valskodlingmot (VKM) te werk, ŉ tekort aan geskikte persele met die vereiste plae laat in die seisoen, en die onvermoë om wollerige-witvlieg vir laboratoriumproewe te teel, was geen verdere navorsing in hierdie projek gedoen nie. Sodra die VKM koue behandeling navorsing voltooi is, sal daar tyd wees om terug te keer na die uitdagings van die projek.

3.6 PROGRAMME: MINOR PESTS AND MITES

Programme Coordinator: Tim G Grout (CRI)

3.6.1 Programme summary

Some pests may be considered of minor importance by most growers but in certain situations or climates they can be extremely problematic. This is the case for woolly whitefly (WWF) which is becoming a serious pest where growers apply few pesticides for sucking insects. The WWF parasitoid *Cales noacki* was successfully imported from Spain and released in the North-West Province, Mpumalanga and the Eastern Cape where WWF was present. However, the natural enemy does not appear to have established and the culture of WWF at CRI has collapsed (3.6.3). The reason for this collapse must be determined before starting a new culture and considering the importation of *Cales noacki* again. Natural banana is a useful attractant for monitoring fruit-piercing moths but the most important species *Serrodes partita* is usually present in small numbers relative to the harmless fruit-sucking moths that may also be trapped in orchards, and the different types can be difficult to distinguish (3.6.2). No artificial attractants were found to be as effective as natural banana so a cost-effective monitoring system and a species-selective bait will both be difficult to develop. Further research on fruit-piercing moth is therefore not planned.

Programopsomming

Sekere plae word beskou as van geringe belang deur meeste produsente maar in sekere situasies of klimate kan hulle baie problematies wees. Dit is wel die geval vir wollerige witvlieg (WWF) wat besig is om as ŉ ernstige plaag te ontwikkel waar produsente minder plaagdoders vir suigende insekte toedien. Die WWF parasitoïed *Cales noacki* is suksesvol van Spanje ingevoer en in die Noordwes-Provinsie, Mpumalanga en die Oos-Kaap vrygestel waar WWF teenwoordig is. Dit blyk dat die natuurlike vyand egter nie gevestig het nie en die kultuur van die WWF by CRI het ineengestort (3.6.3). Die rede vir die ineenstorting moet bepaal word voor die aanvang van ŉ nuwe kultuur en oorweging van die herinvoer van *Cales noacki*. Natuurlike piesang is ŉ nuttige lokmiddel vir die monitering van vrugte-steek motte, maar die belangrikste spesie *Serrodes partita* is gewoonlik in klein getalle teenwoordig in vergelyking met die onskadelike vrugte-suig motte wat ook in boorde gevang kan word, en die verskillende tipes is moeilik onderskeibaar (3.6.2). Geen kunsmatige lokmiddels was so effektief soos natuurlike piesang nie, dus sal ŉ koste-effektiewe moniteringstelsel en ŉ spesie-selektiewe lokaas albei moeilik wees om te ontwikkel. Verdere navorsing oor vrugte-steek motte word dus nie beplan nie.

3.6.2 FINAL REPORT: Using banana odour as an attractant for monitoring fruit-piercing moth in citrus orchards

Project RU 1058 (2013 – 2016) by Mathew Goddard, Martin Hill (RU) and Sean Moore (CRI)

Summary

Fruit-piercing moths are a sporadic pest of citrus, especially in the Eastern Cape Province of South Africa, where the adults can cause significant damage in outbreak years. Currently the only way in which to successfully control fruit-feeding moths within the orchards is the use of repellent lights. However, growers confuse fruit-piercing moths with fruit-sucking moths, the latter not causing primary damage. There is also currently no way of monitoring which moth species are present in orchards and potentially attacking the fruit during the night. In a previous study, banana was shown to be the most attractive bait for a variety of fruit-feeding moth species. Therefore, the aim of this study was to determine the population dynamics of fruit-feeding moths and to develop a cost-effective alternative to the use of fresh banana as a bait for fruit-piercing moths. Fresh banana was compared to nine alternative synthetic attractants, frozen banana and a control under field conditions in several orchards in the Eastern Cape Province. Banana was shown to be the most

attractive bait. Some 23 species of fruit-feeding moth species were sampled in the traps, but there were only two fruit-piercing species, *Serrodes partita* (Fabricius) (Lepidoptera: Noctuidae) and *Eudocima* sp. Surprisingly *S. partita*, believed to be the main pest, comprised only 6.9% of trap catches. *Serrodes partita* is a sporadic pest, only becoming problematic every five to 10 years after good rainfall in the Little Karoo region, which causes flushing of its larval host, the wild plum, *Pappea capensis* (Ecklon & Zeyher). During these outbreaks, damage to fruit can range from 70 to 90% and this is especially so for soft skinned citrus. A study on the morphology of the proboscis confirmed that only two species of fruit-piercing moths were present. Trap catches over three citrus growing seasons were linked to fruit damage found within several orchards. Fruit-piercing moth damage was relatively low in comparison to other types of damage, such as mechanical and undefined damage. There was a very weak correlation between *S. partita* trap catches and damage, but generally damage was recorded two to three weeks after a peak in *S. partita* trap catches. Climatic conditions were also recorded and compared to weekly trap catches of *S. partita*, and while temperature and wind direction had no influence on moth populations, precipitation in the orchards was weakly correlated with trap catches. This study has shown that in non-outbreak seasons, the main fruit-piercing moth, *S. partita*, comprises a small percentage of fruit-feeding moths in citrus orchards, but that growers are unable to determine the difference between fruit-piercing species and the harmless fruit-sucking species. Further, fresh banana remains the best method for attracting fruit-piercing moths to traps, but this is not cost effective and thus a commercially viable protocol for monitoring these species remains elusive.

Opsomming

Vrugte-steek motte is ʼn sporadiese plaag van sitrus, veral in die Oos-Kaap Provinsie van Suid-Afrika, waar die volwassenes beduidende skade in uitbreek jare kan veroorsaak. Die enigste manier om vrugte-voedende motte tans suksesvol in boorde te beheer is deur die gebruik van afstotende ligte. Produsente verwar vrugte-steek motte met vrugte-suig motte wat nie primêre skade veroorsaak nie. Daar is tans geen manier om te bepaal watter mot spesies in die boorde teenwoordig is nie en dus moontlik die vrugte gedurende die nag aanval. In ʼn vorige studie, is piesang gewys as die mees aantreklike lokaas vir ʼn verskeidenheid vrugte-voedende mot spesies. Daarom was die doel van hierdie studie om die bevolkingsdinamika van vrugte-voedende motte te bepaal, asook die ontwikkeling van ʼn koste-effektiewe alternatief vir die gebruik van vars piesang as ʼn lokaas vir vrugte-steek motte. Vars piesang is vergelyk met nege alternatiewe sintetiese lokmiddels, bevrore piesang en ʼn kontrole onder veldtoestande in verskeie boorde in die Oos-Kaap Provinsie. Weereens is getoon dat piesang die mees aantreklike lokaas is. Sowat 23 spesies van vrugte-voedende mot spesies is in die lokvalle versamel, maar daar was net twee vrugte-steek spesies, *Serrodes partita* (Fabricius) (Lepidoptera: Noctuidae) en *Eudocima* sp. Verbasend is *S. partita*, wat vermoedelik die belangrikste plaag is, slegs 6.9% van lokvalvangstes. *Serrodes partita*, is ʼn sporadiese pes, net problematies elke vyf tot 10 jaar na goeie reënval in die Klein Karoo-streek wat ʼn blaargroei oplewing van larvale gasheer, die wilde pruim, *Pappea capensis* (Ecklon & Zeyher), veroorsaak. Gedurende hierdie uitbraak, kan skade aan vrugte van 70-90% wees en dit is veral die geval vir sagteskil sitrus. ʼn Studie van die morfologie van die monddeel bevestig dat slegs twee spesies van vrugte-steek motte teenwoordig was. Lokval vangste oor drie sitrus seisoene is aan vrugte skade in ʼn paar boorde gekoppel. Vrugte-steekmot skade was weereens relatief laag in vergelyking met ander tipes skade soos meganiese en ongedefinieerde skade. Daar was ʼn effense korrelasie tussen *S. partita* lokvalvangste en skade, maar oor die algemeen is skade twee tot drie weke ná ʼn piek in *S. partita* lokvalvangste aangeteken. Klimaatstoestande is ook aangeteken en met weeklikse lokvalvangste van *S. Partita* vergelyk, en terwyl die temperatuur en windrigting geen invloed op mot bevolkings gehad het nie, het reenval in die boorde ʼn effense korrelasie met lokval vangste gehad. Hierdie studie het getoon dat in nie-uitbraak seisoene, die hoof vrugte-steekmot, *S. partita*, vorm net ʼn klein persentasie van die vrugte-voedende motte in sitrusboorde, maar dat produsente nie in staat is om die verskil tussen vrugte-steek spesies en die skadelose vrugte-suig spesies te bepaal nie. Verder bly vars piesang die beste metode vir die aanlokking van vrugte-steekmotte in lokvalle, maar dit is nie koste-effektief nie, en dus bly ʼn kommersieel lewensvatbare protokol vir die monitering van hierdie spesies ontwykend.

Introduction

In 1999, there was a devastating outbreak of the fruit-piercing moth, *Serrodes partita* (F.), in the Eastern Cape, followed by an invasion of the fruit-sucking moth, *Achaea lienardi* (Moore, 2010). Miho Wase Satsuma mandarin orchards were worst affected, suffering substantial pre- and post-harvest losses in Sundays River Valley, Gamtoos River Valley, Kat River Valley and Knysna.

A similar outbreak occurred in the Eastern Cape in March 2009, exactly 10 years later (Moore, 2010). This outbreak affected Sundays River Valley and the Kat River Valley most severely. Although a few reports of the occurrence of *S. partita* were received from individuals within the farming community, it was chiefly just the damage that was observed, and therefore it was not confirmed whether *S. partita* did lead the invasion again.

However, *A. lienardi* was present in Satsuma mandarin orchards in particular, in high numbers for several weeks. Large numbers of fruit dropped as a result and a lot of damaged fruit had to be sorted at harvest – both in the orchard and on delivery to the packhouse.

This supported the assertion by Rust & Myburgh (1986) that epidemic outbreaks in the Eastern Cape and southern part of the Western Cape occur every 5-10 years after heavy summer rains in the Little Karoo and parts of the Great Karoo. Such rains induce the jacket plum, *Pappea capensis*, which is the *S. partita* larval host plant, to flush profusely. On completion of the developmental cycle the moths leave these trees and seek wild or cultivated fruit to feed on. Generally, around two months after these heavy rains, millions of moths can migrate up to 500 km, often finding early ripening mandarins.

No chemical option is available or considered to be effective for control of fruit-feeding moths. These moths are extremely hardy to chemical pesticides (Kriegler, 1962). In addition, fruit-piercing moths do not remain in an orchard for more than one night. Any subsequent infestation is therefore a result of a new wave of migrating moths. Currently, the only way in which the fruit-piercing moth can be controlled is by the erection of a barrier of mercury-blended lamps to deter the moths (Hofmeyr, 2003).

However, it is difficult to predict if and when there is going to be an invasion of a damaging level of fruit-piercing moths into a citrus orchard, as not only are they nocturnally active but there is no existing means of monitoring for the pest. Consequently, a citrus industry funded study was recently conducted (Robinson *et al*, 2012) with the purpose of identifying a bait which is attractive to fruit-feeding moths and can thus be used for monitoring purposes. Banana was identified as the most attractive of a range of baits tested. However, due to the low level of fruit-feeding moths present, it was not possible to establish any reliable relationship between moth catches and fruit damage. It was therefore necessary to expand this study.

Gunn (1929) found that baits using decaying fruit were effective against *A. lienardi* but not against fruit-piercing moths. Moore (2010) recommended mixing decaying fruit with molasses and a non-pungent (non-repellent) toxicant for controlling fruit-sucking moths in a packhouse. Fay (2003) developed and patented a novel bait for control of fruit-piercing moths in Australia, particularly *Eudocima* spp. Reddy *et al* (2007) found that *E. phalonia* was significantly attracted to feed on fruit puree with Agar and Phytogel more than on fruit puree with Agarose. Of the 15 fruit baits tested, moths preferred to feed on banana baits more than on any other (followed by guava and orange), agreeing with the more recent study by Robinson *et al* (2012).

Two general categories of fruit-feeding moths exist. These are fruit-piercing moths and fruit-sucking moths. Jack (1922) originally made this distinction, based on the morphology of the moth's proboscis. Only fruit-piercing moths have the ability to cause primary damage i.e. to damage healthy fruit, as they have a modified proboscis. *Serrodes partita* appears to be the most prolific and most damaging of these (Moore, 2010). Fruit-sucking moths can only feed on fruit which has already been damaged, very often by fruit-piercing moths. Johannsmeier (1998) lists 11 species of fruit-piercing moths in five genera, which have been recorded on citrus in South Africa. Hofmeyr (2003) lists a sixth genus, *Plusiodonta*. All of these species belong to the family Noctuidae, or at least to the superfamily Noctuoidea. Nine genera of moths have been listed as possible fruit-sucking moths on citrus (Johannsmeier, 1998). By far the most common is *A. lienardi* (Boisduval), which like the fruit-piercing moths is also a noctuid.

Due to the sporadic nature of fruit-piercing moth outbreaks, or possibly rather the extended intervals between outbreaks, insufficient work has been conducted on their control and even more so on their monitoring. In addition to the accounts from the Eastern and Western Cape, reports have also been received from Alicedale Farm in Tshipise of fruit-piercing moth damage to grapefruit over the last few years. During the report period we sought to verify this.

This study was initiated in 2013 as a one-year study at BSc Honours level. The project was continued as an MSc study for 2014-2015. For a complete report of the study, the MSc thesis can be consulted (Goddard, 2016).

Objectives

- To confirm the attractiveness of banana, as a trap bait, to fruit-piercing and also fruit-sucking moths.
- To investigate synthetic alternatives to bananas (e.g. isopentyl acetate) for attracting fruit-feeding moths. Or to formulate banana to prolong its field life.

- To determine the level of presence of fruit-feeding moths in citrus orchards (particularly Satsuma Mandarins) during the course of the season, even before fruit are considered to be susceptible.
- To establish a relationship between trap catches of fruit-feeding moths in orchards and the level of damage which they cause to fruit, which will provide an early warning threshold for unacceptable levels of presence requiring some action.
- To identify all possible species of fruit-feeding moths caught in traps and observed in orchards.
- To monitor the relationship between larval infestation of jacket plums in the Karoo and subsequent outbreaks in citrus in the Eastern Cape
- To determine the status of fruit-piercing moth in grapefruit orchards in northern Limpopo Province.

Materials and methods

Study sites

During the 2013 growing season two growing regions were chosen, with both based in the Eastern Cape. The first region was located in the Kat River Valley and the second region was in the Grahamstown area. Within the Kat River Valley growing region, there were two farms, Blinkwater (32°39'34"S, 26°32'35"E) and Riverside (32°45'44"S, 26°36'50"E). Within the Grahamstown area, there was only a single farm, Mosslands (33°23'55"S, 26°25'38"E), which was situated 20km outside of Grahamstown. With the continuation of the study in 2014 an additional growing region, Sundays River Valley, was added to the study sites. The Kat River Valley growing region now consisting of two new sites, Bath Farm (32°49'40"S, 26°40'17"E), and Glinkwater Farm (32°40'33"S, 26°33'46"E). With the addition of the Sundays River Valley growing region, four new sites were chosen, Dunbrody Estates-Enterprise (33°29'03"S, 25°34'30"E) and Riverside (33°28'02"S, 25°33'29"E and 33°28'52"S, 25°34'26"E), Hitgeheim (33°30'15"S, 25°36'27"E) and Halaron Farm (33°29'26"S, 25°40'25"E) and in 2015 Hitgeheim was replaced by a third orchard at Dunbrody Estates (33°27'32"S, 25°33'16"E). A site up in Tshipise, Limpopo (22°37'59"S, 30°08'33"E), was the only site that had a different variety of citrus, namely grapefruit. All the sites in the Eastern Cape that were chosen grew the same variety of citrus, Satsuma Mandarin, as these are the earliest ripening fruit and have been worst affected by *S. partita* in the past.

Baits

In 2013 isopentyl acetate, an artificial flavour for processed banana foods was compared to fresh banana and used to monitor fruit-feeding moths in the Kat River Valley area and Grahamstown growing region. The study continued into 2014 and 2015, where in 2014 fresh banana was compared to frozen banana, four artificial carp baits (which are used for fishing; each bait contained a different artificial banana flavour), a banana weevil (*Cosmopolites sordidus* (Germar, 1824) (Coleoptera: Curculionidae)) lure and a control (no bait). In the 2015 growing season, fresh banana was compared to three different ripening stages of banana, made from a suite of chemicals that represented each of the ripening stages and a control.

Traps and Experimental layout

The traps were placed on trees in the north-eastern side of each orchard (Fig. 3.6.3.1) because these trees are historically the most affected by *S. partita*, as the moths generally come from north of the orchards, as the moths tend to fly into the prevailing wind in the Eastern Cape, which is generally southerly. The traps were placed on the southern side of the selected trees so that they could be shaded from rapid desiccation. Each trap was secured at approximately 2 m high. In order to protect traps from undesirable environmental effects, such as excessive wind and dust, the traps were placed in the third row from the northern edge and four trees in, so they would all have an equal chance of attracting moths. Traps were placed 12 m apart, with traps being evenly spaced in each orchard. This spacing was used (Fig. 3.6.3.1) to reduce the chance of competition between baits but still close enough to expose the baits to the same moth population, making a fair comparison between baits. The baits were placed inside an open plastic cage that attached to the lid of the yellow bucket funnel trap.

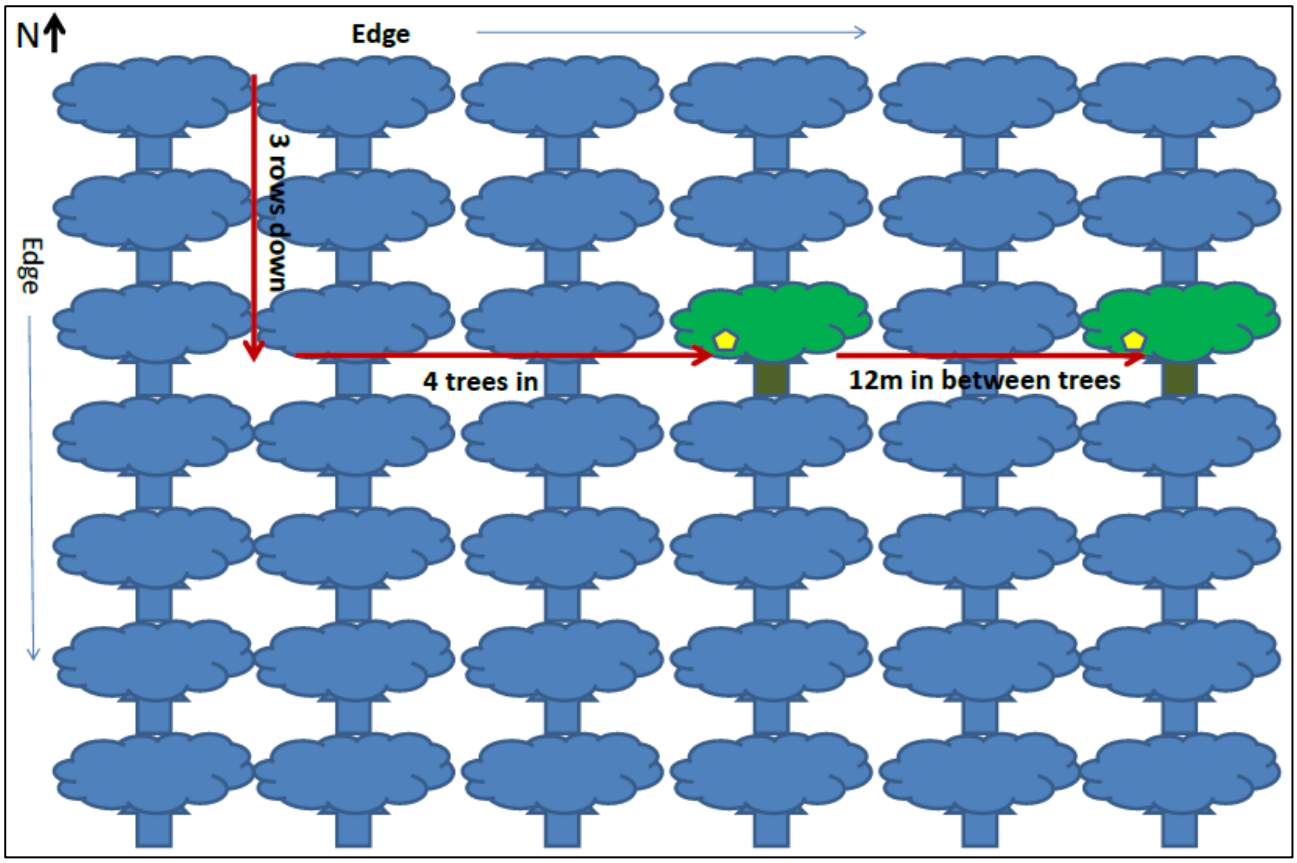

Figure 3.6.2.1. Layout of trap trees in orchards for monitoring of fruit feeding moths.

Damage assessment

Fruit damage on the tree was monitored weekly, for six weeks (Grahamstown and Kat River Valley) in 2013, eight (Sundays River Valley) and 10 (Kat River Valley) weeks in 2014 and four (Sundays River Valley) and five (Kat River Valley) weeks in 2015, after the first signs of ripening until harvest. In the Tshipise growing region, for both 2014 and 2015, fruit were assessed for damage one week before harvest, in the same 10 grapefruit orchards. The fruit damage survey consisted of inspecting 20 fruit on each of 10 trees in five orchards in 2013 and 10 orchards in both 2014 and 2015, which is similar to the method used by Robinson *et al.* (2012) and King and Thompson (1958). Rows and trees were chosen by using a random number generator. Two minutes was spent at each tree, where fruit were sampled from around the whole tree and the fruit were chosen at random.

A damage guide, similar to that used by Robinson *et al.* (2012) was employed (Fig. 3.6.2.2). Damage was recorded and categorised, as being caused by fruit-piercing moth, snails, birds, mechanical (fruit split, damage caused by tractors or by other machinery), diseased fruit (such as citrus scab and misshapen fruit) or other (which was undefined damage, sunburn or other pests, such as red scale or mealybug). However, precise categorisation of damage may not have been 100% accurate, for example, some “snail damage” might have been caused by locusts, and what was defined as diseased fruit might actually have been light chemical burn. However, the identification of fruit-piercing moth damage was accurate, as damage is symptomatically specific to fruit-piercing moths (Fig. 3.6.2.2). Fruit-piercing moth damage in each orchard was then compared with the weekly trap catches of *S. partita*.

Moth identification

Moths sampled weekly during the three growing seasons, in each of the growing regions, Kat River Valley, Sundays River Valley, Grahamstown and Tshipise, were identified to species and if this was not possible, they were identified to the lowest scientific name possible. Pinhey (1975) and Johannsmeier (1998) were used to identify the moths. Once the moths were identified, each species description was given, including any host records. All specimens were lodged in the Rhodes University collection and assigned an AcRh number.

Weather data

Temperature (maximum and minimum), wind direction and rainfall data were obtained from the South African Weather Service. The weather data was used to determine if there was a correlation between the peaks in *S. partita* and *A. lienardi*, to see if weather influenced the flight of the moths.

Weather data (rainfall, temperature (minimum and maximum), wind speed and direction) for the 2013, 2014 and 2015 growing seasons was obtained from the South African Weather Service. Weather data was compared with the activity of weekly trap catches of *S. partita* in each orchard.

Orchard:

Row: Tree:

	Damage Types					
Tree	Snail	fruit-piecring moth	Bird	Mechanical	Diseased	Other
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Figure 3.6.2.2. Illustration of the data sheet used to differentiate between damage types.

Results and discussion

Banana and synthetic bait alternatives

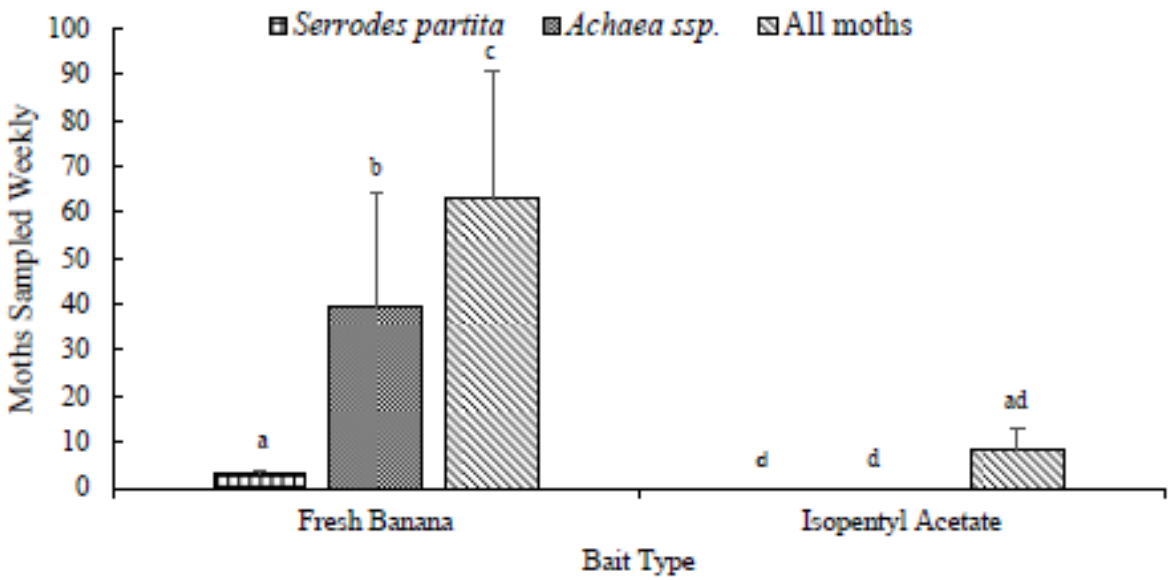

Figure 3.6.2.3. Mean ± SE number of moths (*Serrodes partita, Achaea* spp. and total moths) sampled in 6 traps per bait type (banana and isopentyl acetate), per week over a 12-week sampling period at both Mosslands and Riverside during 2013.

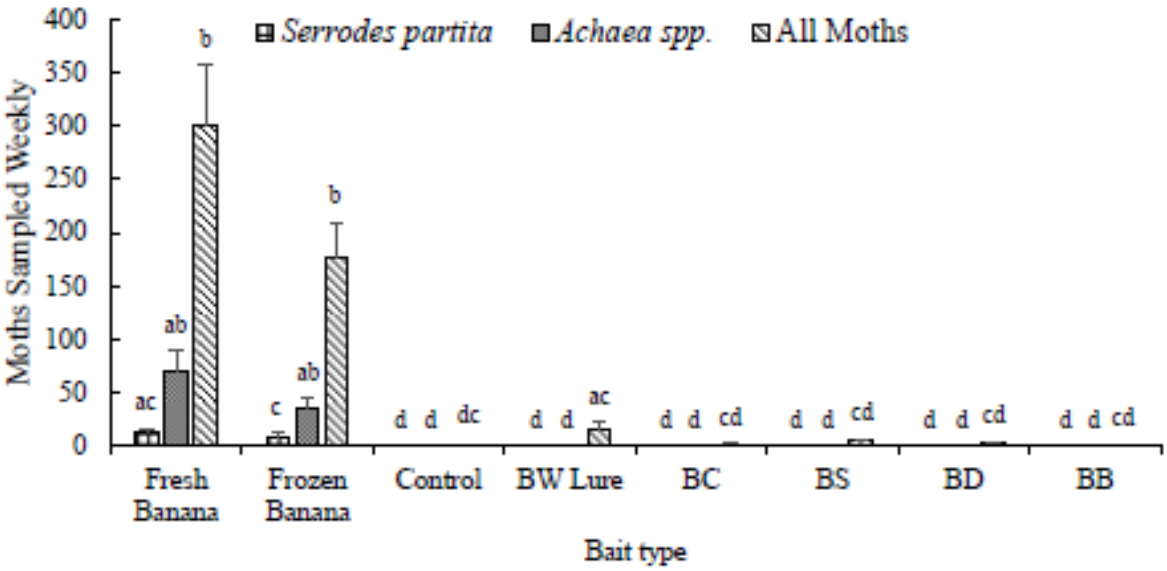

Figure 3.6.2.4. Mean (± SE) number of moths (*Serrodes partita, Achaea* spp. and total moths) sampled in 6 traps per bait type per week over a 12-week sampling period at Blinkwater during 2014. Baits compared were banana (fresh and frozen) and BW Lure, BC, BS, BD, BB and a control.

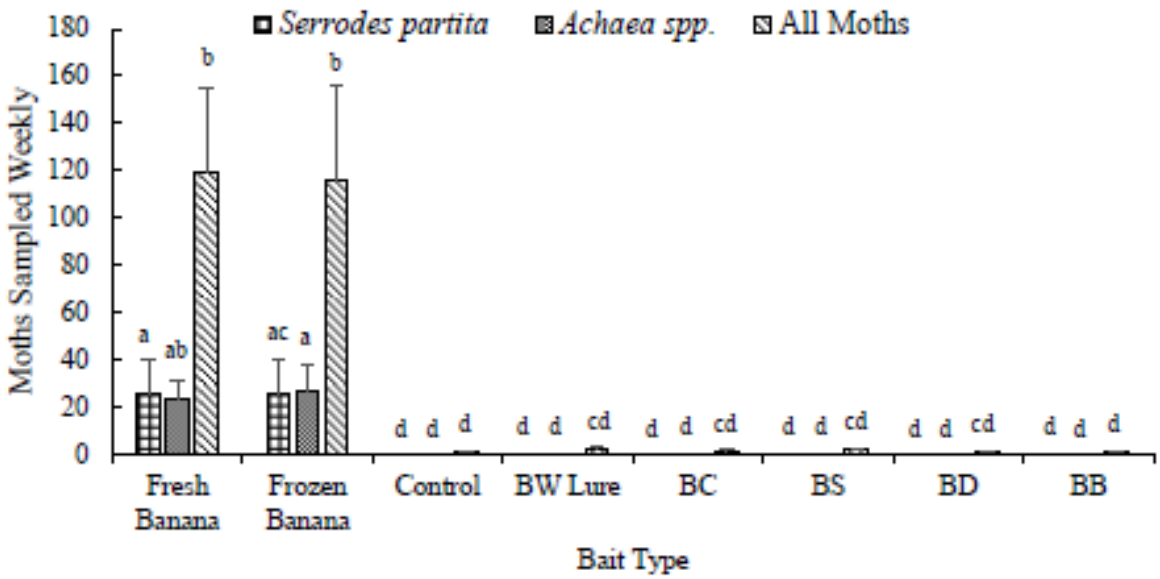

Figure 3.6.2.5. Mean (± SE) number of moths (*Serrodes partita, Achaea* spp. and total moths) sampled in 6 traps per bait type per week over a 12-week sampling period at Dunbrody Estate - Enterprise for 2014. Baits compared were banana (fresh and frozen) and BW Lure, BC, BS, BD, BB and a control.

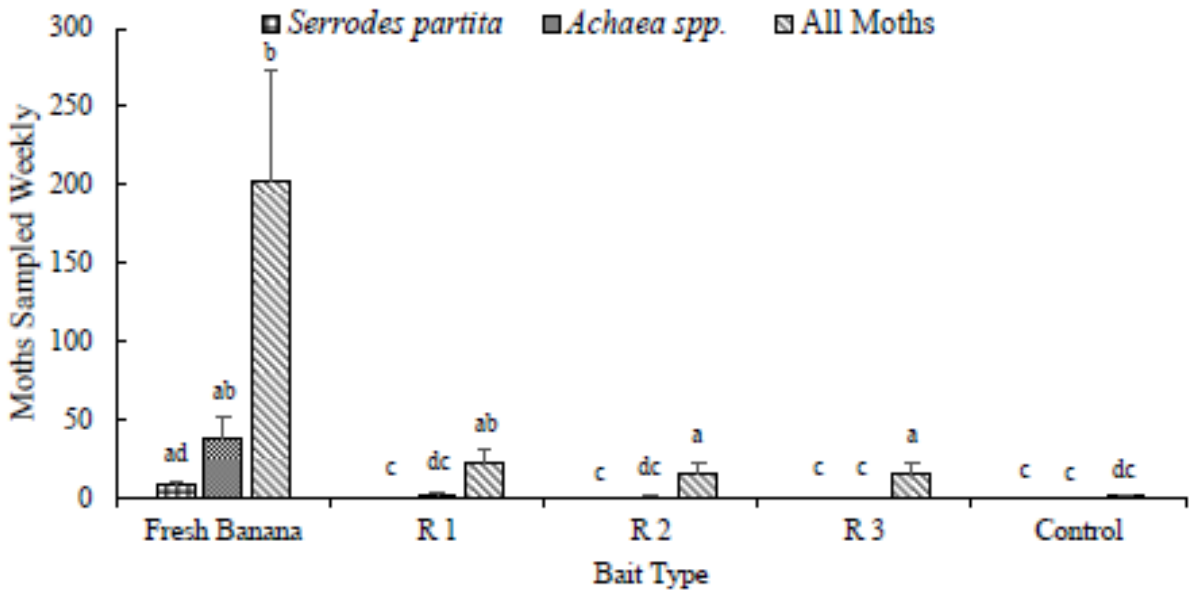

Figure 3.6.2.6. Mean (± SE) number of moths (*Serrodes partita, Achaea* spp. and total moths) sampled in 6 traps per bait type per week over a 5-week sampling period at Blinkwater during 2015. Baits compared were fresh banana, R 1, R 2, R 3 and a control.

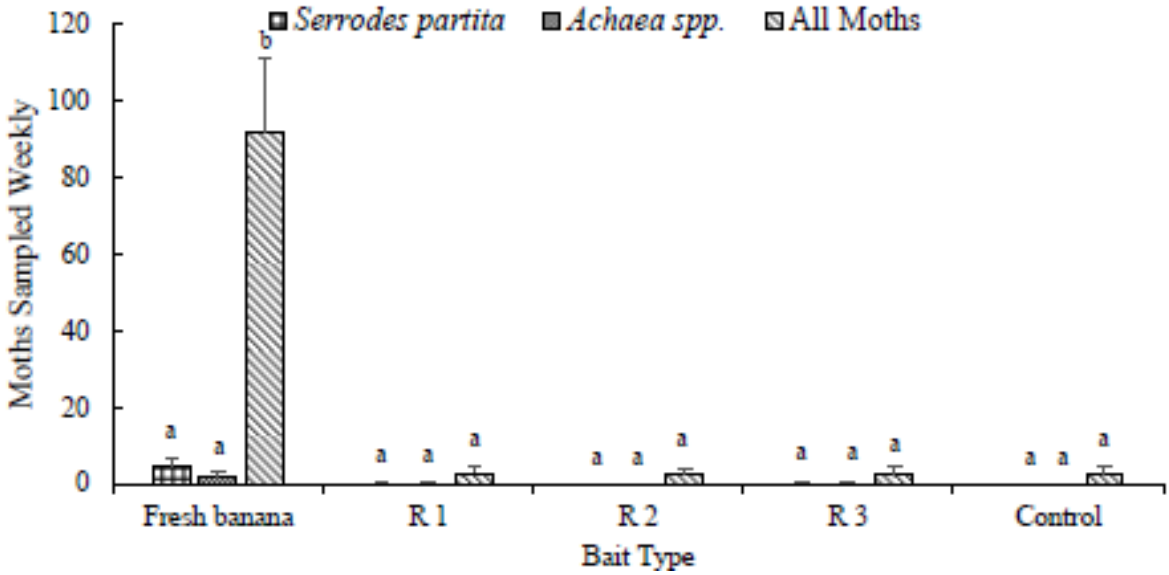

Figure 3.6.2.7. Mean (± SE) number of moths (*Serrodes partita, Achaea* spp. and total moths) sampled in 6 traps per bait type per week over a 5-week sampling period at Dunbrody Estate Enterprise for 2015. Baits compared were fresh banana, R 1, R 2, R 3 and a control.

Moth identification

Table 3.6.2.1. Species of moths from the trap catches over 2013, 2014 and 2015 within Kat River Valley, Grahamstown, Sundays River Valley and Tshipise.

		The species abundance in each growing region (percent and total sampled)							
		Kat River Valley		Grahamstown		Sundays River Valley		Tshipise	
Family	Scientific Name	Total Sampled	Percent Sampled (%)	Total sampled	Percent sampled (%)	Total sampled	Percent sampled (%)	Total sampled	Percent sampled (%)
Noctuidae	*Achaea spp.*	3387	27.65	323	67.86	688	16.33	8	6.50
	Ericeia inangulata	1992	16.25	61	12.82	243	5.77	11	8.94
	Shingomorpha chlorea	905	7.38	5	1.05	32	0.76	38	30.90
	Anomis flava	709	5.78	24	5.04	778	18.46	10	8.13
	Hypanua xylina	555	4.53	12	2.52	1	0.02	--	--
	Hypanua roseitincta	548	4.47	--	--	--	--	--	--
	Serrodes partita	442	3.61	24	5.04	712	16.90	--	--
	Nagia sacerdotis	492	4.01	8	1.68	20	0.47	--	--
	Parallelia algira	184	1.50	15	3.15	13	0.31	3	2.44
	Ophiusa tirhaca	195	1.59	--	--	5	0.12	--	--
	Ulotrichopus catocala	134	1.09	2	0.42	--	--	--	--
	Hydrillodes uliginosalis	123	1.00	--	--	764	18.13	--	--
	Cuneisigna obstans	35	0.29	--	--	--	--	--	--
	Prodotis stolida	30	0.24	--	--	4	0.09	--	--
	Anua diamiris	21	0.17	--	--	6	0.14	--	--
	Rhodogastria bauri	23	0.19	--	--	--	--	--	--
	Aganais speciosa	9	0.07	--	--	7	0.17	--	--
	Digama culta	5	0.04	--	--	21	0.50	--	--
	Cyligramma latona	2	0.02	--	--	--	--	--	--
	Grammodes congenita	--	--	--	--	1	0.02	--	--
	Spodoptera capicola	--	--	--	--	--	--	32	26.02
	Eudocima sp.	--	--	--	--	--	--	3	2.44
Tortricidae		149	1.22	--	--	9	0.21	--	--
Undetermined		2316	18.90	2	0.42	910	21.60	18	14.63
Shannon-Wiener diversity index (H)		2.1930		1.1919		1.8717		1.7733	
Species Evenness (E)		0.7203		0.5176		0.6606		0.8528	

-- Was not collected in the growing region

Damage assessment

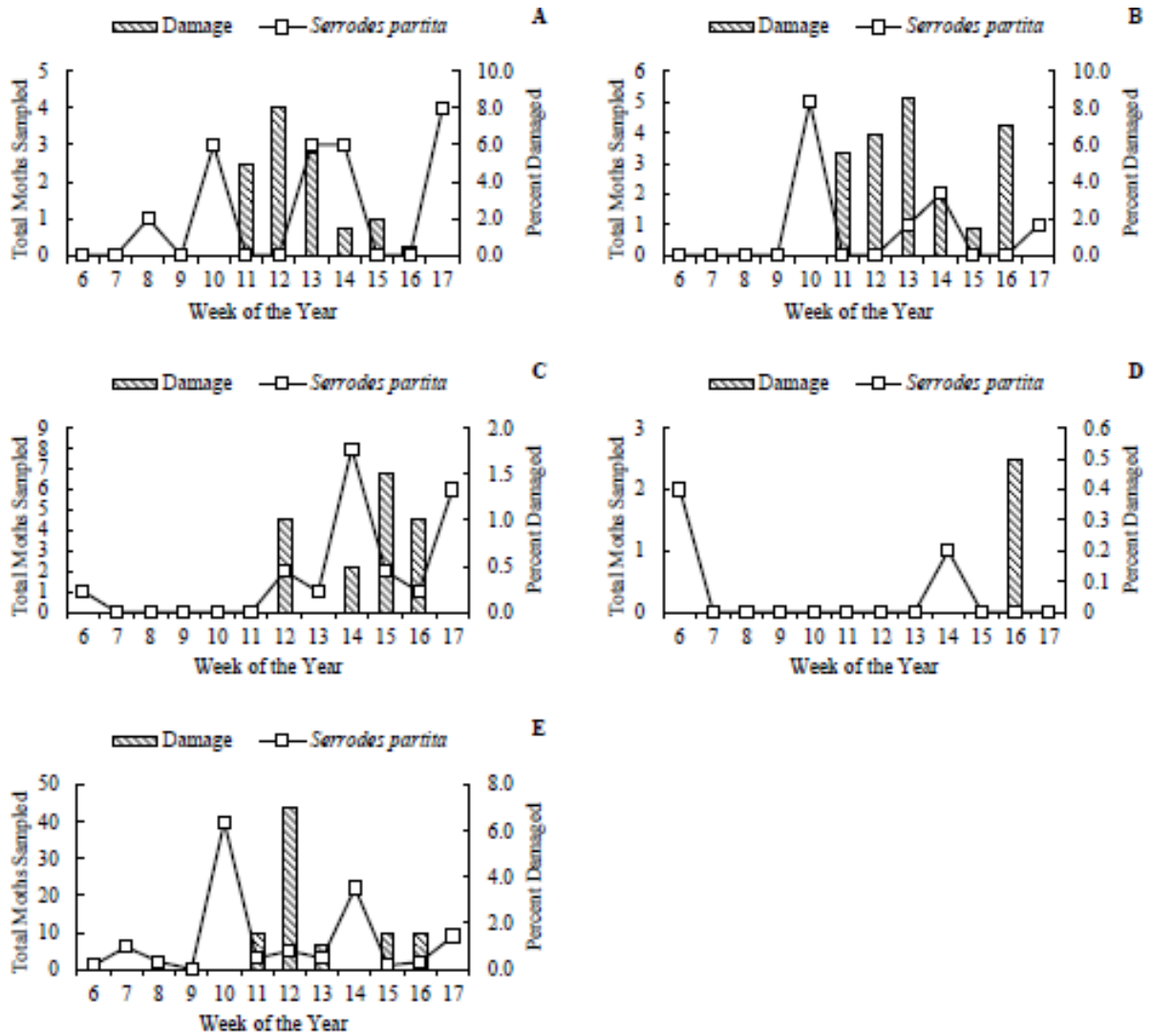

Figure 3.6.2.8. The weekly trap catches of *Serrodes partita* compared to the weekly damage caused by *Serrodes partita* to the fruit over a 12-week period in the orchards in the Kat River Valley and Grahamstown in 2013. **A** – Riverside A; **B** – Riverside B; **C** – Mosslands A; **D** – Mosslands B; **E** – Blinkwater.

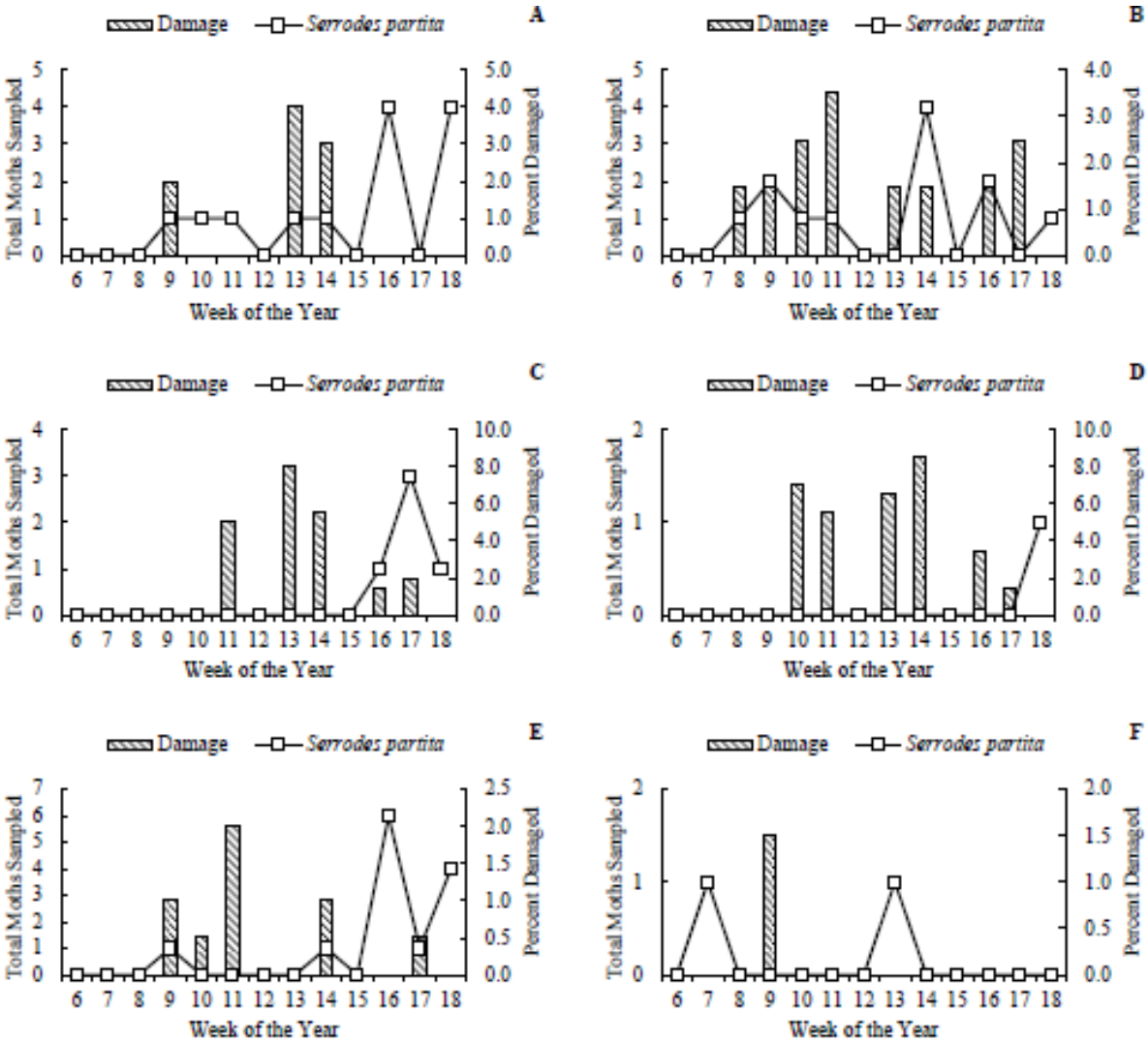

Figure 3.6.2.9. The weekly trap catches of *Serrodes partita* compared to the weekly damage caused by *Serrodes partita* to the fruit over a 13-week period in the orchards in the Kat River Valley in 2014. **A** – Bath Farm A; **B** – Bath Farm B; **C** – Riverside A; **D** – Riverside B; **E** – Bath Farm A; **F** – Bath Farm B.

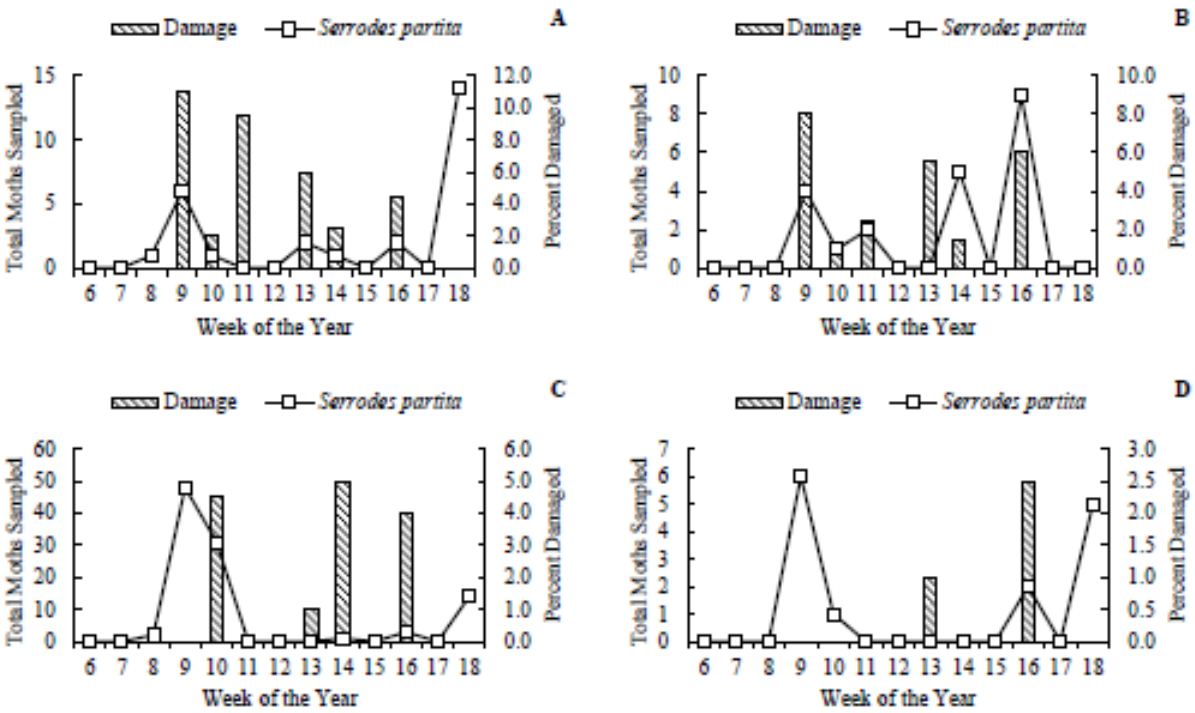

Figure 3.6.2.10. The weekly trap catches of *Serrodes partita* compared to the weekly damage caused by *Serrodes partita* to the fruit over a 13-week period in the orchards in Sundays River Valley in 2014. **A** – Hitgeheim; **B** – Halaron Farm; **C** – Dunbrody Estates - Riverside A; **D** – Dunbrody Estates - Riverside B.

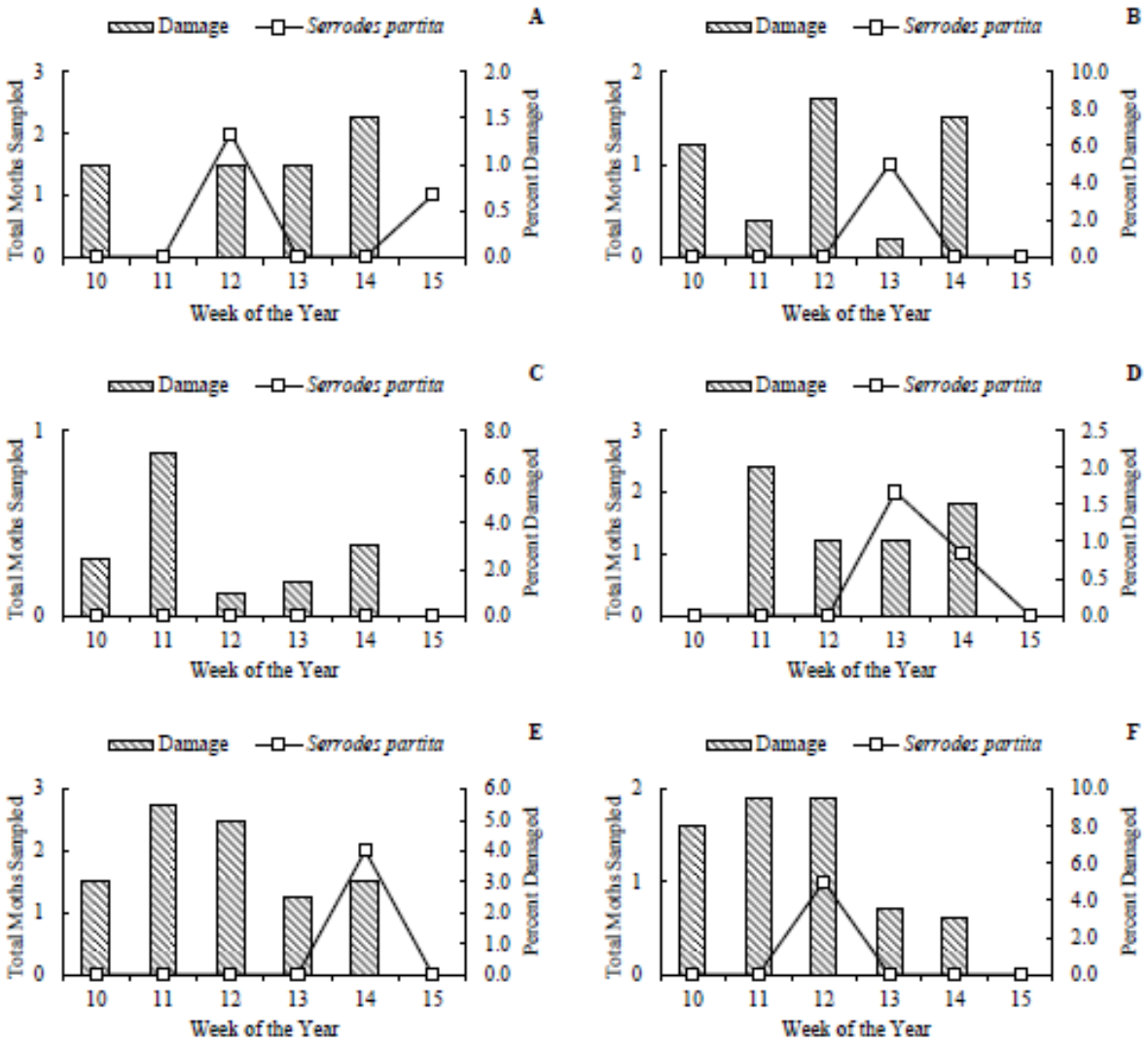

Figure 3.6.2.11. The trap catches of *Serrodes partita* compared to the weekly damage caused by *Serrodes partita* to the fruit over a 6-week period in the orchards in the Kat River Valley in 2015. **A** – Bath Farm A; **B** – Bath Farm B; **C** – Riverside A; **D** – Riverside B; **E** – Bath Farm A; **F** – Bath Farm B.

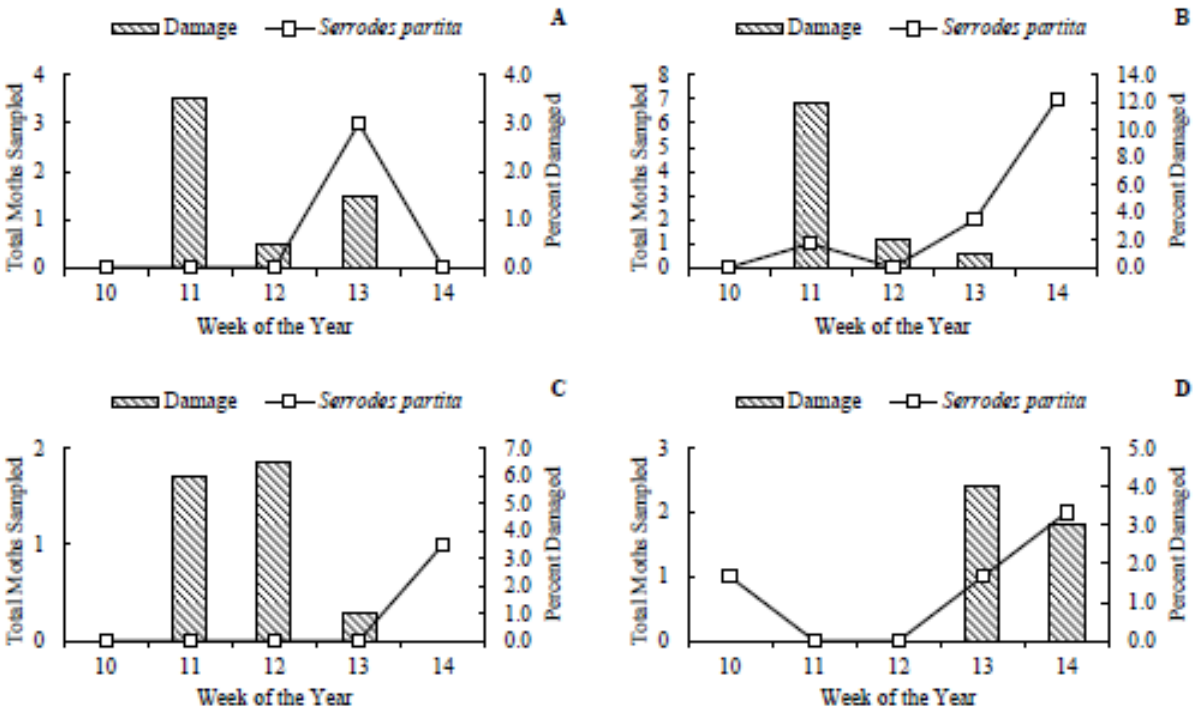

Figure 3.6.2.12. The weekly trap catches of *Serrodes partita* compared to the weekly damage caused by *Serrodes partita* to the fruit over a 5-week period in the orchards in the Sundays River Valley in 2015. **A** – Halaron Farm; **B** – Dunbrody Estates - Riverside A; **C** – Dunbrody Estates - Riverside B; **D** – Dunbrody Estates - Riverside C.

Conclusion

Banana was shown to be the most effective attractant to monitor fruit-feeding moths within Satsuma orchards. However, there were a few *S. partita* sampled with R1 and R3, which were artificially made baits resembling different ripening stages of banana. This indicates potential for finding an alternative synthetic bait to banana. As banana was shown to be a broad attractant, attracting various species of fruit-feeding moths, it isn't ideal, as the majority of fruit-feeding moths present in orchards and thus the majority of moths sampled were fruit-sucking moths, which do not cause primary damage. Even though there was only relatively low activity of *S. partita* within the orchards in this study, there was an average of 4% damage caused to fruit. This could indicate that banana may not be as attractive as initially thought as it might only be catching a small proportion of the actual number of *S. partita* within the orchards, resulting in a poor correlation between weekly trap catches of *S. partita* and damage. However, it does not matter how many or how few are sampled in the traps, as long as it is possible to relate catches to fruit damage.

Future research

Future research could include: (1) looking at possible alternative attractants to fruit for the control of *S. partita,* such as sex pheromones, as there is a lot of by-catch of fruit-sucking moths; (2) or conducting laboratory tests to determine which volatiles or group of volatiles from banana are the most attractive to *S. partita*; (3) developing of a better volatile or bait dispenser for attracting *S. partita* within the orchards; (4) establishing a laboratory culture of *S. partita*, so to (a) better understand the biology of the moth, to prospect for (i) pathogens, and (ii) rear biocontrol agents (e.g. the tachinid fly), and (b) to conducted laboratory trials to test (i) pathogens, (ii) parasitoids, (iii) chemical pesticides and (b) to achieve a higher level of accuracy in comparing the attractiveness of baits to banana because of the exclusion of the multitude of confounding variables found within the field; (5) placing traps at different heights in trees or positions within orchards to determine optimal trap positioning for maximum possible catches; (6) comparing the activity and movement of *S. partita* between orchards, including host range; (7) recording daily or even hourly (instead of weekly) weather conditions in the orchards and the larval host range; this could aid in better predicting future movements of *S. partita*, particularly outbreaks; (8) monitoring fruit-piercing moth damage on fallen fruit and fruit on the trees, which could assist in determining a correlation between activity of *S. partita* and damage; and (9) monitoring activity of fruit-feeding moths throughout the growing season from blossom to harvest could lead to a better understanding of when *S. partita* is active

in the orchards, thus monitoring damage so as to better determine when fruit first becomes susceptible to fruit-piercing moth damage.

Technology transfer

A poster was presented at the Entomological Society of Southern Africa conference (ESSA) in 2013 and an oral paper was presented at the Citrus Symposium 2014 and ESSA 2015.

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

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

Summary

Although woolly whitefly (WWF) was successfully reared on citrus seedlings under net in 2014 and there was adequate host material for the imported parasitoid *Cales noacki*, the WWF culture started declining in 2015 and eventually collapsed in winter. We had released parasitoids at Mooinooi in the North-West Province, at several sites around Nelspruit and at two sites in the Eastern Cape. However, attempts to recover *C. noacki* from those release sites have only resulted in 10 parasitoids from Mooinooi, three from Nelspruit and none from the Eastern Cape. It has therefore not been possible to release large numbers of parasitoids in the Eastern and Western Cape from orchards in the north where it had become established. Further attempts to recover the parasitoid will be made but if these fail we may need to import some more. However, the reason for the collapse of the WWF culture must first be rectified so that we can rear the host material in abundance before more parasitoids are introduced.

Opsomming

Hoewel wollerige witvlieg (WWF) op sitrus saailinge onder nette in 2014 suksesvol geteel is en daar voldoende gasheer materiaal vir die ingevoerde parasitoïed *Cales noacki* was, het die WWF kultuur in 2015 begin daal en uiteindelik in die winter in duie gestort. Ons het parasitoïde by Mooinooi in die Noord-Wes Provinsie, op verskeie plekke rondom Nelspruit en op twee plekke in die Oos-Kaap vrygelaat. Pogings om *C. noacki* van die vrystellings persele te herkry het net 10 parasitoïde van Mooinooi, drie van Nelspruit

en geen van die Oos-Kaap opgelewer. Dit was dus nie moontlik om groot getalle parasitoïde in die Oos- en Wes-Kaap vry te laat uit boorde in die noorde waar dit gevestig het. Verdere pogings om die parasiet te herkry sal gemaak word, maar indien hierdie misluk sal dit dalk nodig wees om nog in te voer. Die rede vir die ineenstorting van die WWF kultuur moet egter eers reggestel word dat dit moontlik is om groot getalle van die gasheer te teel voor nog parasitoïde ingevoer word.

PORTFOLIO: DISEASE MANAGEMENT

4.1 PORTFOLIO SUMMARY

By Paul H Fourie (Portfolio Manager: Disease Management, CRI)

The Disease Management portfolio is continuing to serve the southern African citrus industry. Most grower priorities are addressed in projects designed to meet certain short-, medium- and long-term strategic objectives. These service and research objectives/strategies and highlights from the various programmes are briefly summarised below. Progress of the 2015-16 reporting period is summarised in the programme summaries.

Service objectives in Graft Transmissible Diseases programme are to provide diagnostic services for the Citrus Improvement Scheme (CIS) through re-indexing of mother block trees, pathogen elimination and pre-immunisation of new entries. These essential diagnostic services are ongoing and are continually reviewed in order to improve wherever possible (see CIS report). Since the performance of certain 'cleaned-up' CIS material has been criticised, a project was started to evaluate the horticultural performance of old-clone material with CIS material (1074); infected and CIS trees were planted out but researchers are finding it difficult to find trials sites to plant the infected treatments. Research objectives are largely focussed on sustainable control of *Citrus tristeza virus* (CTV), which is based on cross-protection. On a more fundamental level, the mechanisms involved in mild strain cross protection is research (885B, 1100), while applied research projects evaluate the suitability of candidate cross-protection sources for various climate regions, citrus types and cultivars (738, 739, 742, 789, 968). The epidemiology of African Citrus Greening, and specifically the alternative hosts of the bacterium, is studied. Importantly, this project also collaborates with international research on the feared Asiatic form of this disease (886B). Outcomes from this project proved invaluable to elucidate the false positive detection of Asian Citrus Greening (HLB) in central Africa. The embryo-rescue clones from chimera fruit on greening infected plants initially proved to be greening tolerant, but developed low levels of greening symptoms (815). The project was finalized, but the lines should be evaluated further in future projects.

The Soilborne Diseases programme researches sustainable options (alternatives to harsh chemicals) for root rot and citrus nematode control, and certain promising options have been identified (762, 1030). The etiology and control of Armillaria root rot (1068) are being investigated and it was clear that two distinct complexes of pathogens, and not Armillaria, caused decline in northern and southern regions. Early diagnosis of citrus tree decline is essential to improve the chances of remedial actions. Multiple parameters have been evaluated as potential decline indicators prior to severe (visual) symptom development and the most suitable parameters are being studied further in ongoing research (1092). Preventative and curative management of soilborne diseases in citrus nurseries is studied in a project co-funded by CRI and the Department of Science and Technology (DST)'s Research on Citrus Exports (RCE) funding (1101 / RCE-5), which also supports the CIS's goal of supply of disease free citrus trees to growers in southern Africa.

In the Citrus Black Spot programme, a considerable amount of time is spent on technical market access support. A collaborative project with USA, Brazilian and Argentinian researchers to develop a probabilistic model to quantitatively predict the risk of fruit as a pathway for CBS is in the final stages of conclusion and a scientific article is being prepared (1026). The global population structure of the CBS pathogen is studied by means of genetic markers and indicated the genetic differences between populations in China, Australia, South Africa, Brazil and the USA, with the estimated evolutionary age of the populations declining in that order (977). Spray programmes are continuously studied to improve our understanding of CBS control, our ability to cost-effectively control CBS, to manage fungicide resistance, improve formulations, and to register new active ingredients (970, 1012). Highlights from these trials are the imminent registration of novel compounds and combinations that should save growers up to two spray rounds. Six new projects were initiated on CBS. Three projects will study knowledge gaps in CBS epidemiology: the projects at CRI (RCE-6) and QMS Laboratories (RCE-8) (both co-funded by RCE funding) are making good initial progress, while the third project at an external service provider was terminated due to insufficient progress. The potential of reduced volume fungicide application to control CBS are investigated in a semi-commercial trial (1132). A web-based information system is being developed to improve CBS management in a RCE

co-funded project (RCE-7); this system aims to package weather and CBS epidemiological model output to improve general understanding of CBS epidemiology and to enable better informed CBS management.

In the Fruit and Foliar Diseases programme, new control options for Alternaria brown spot (ABS) are being studied (750), but lack of trial sites hampered progress. Radar technology is used to characterize citrus tree canopies, with the final aim to improve sprayer calibration (1089). A PhD study on the use of adjuvants to improve fungicide foliar spray deposition and control of Alternaria brown spot are being concluded (1096).

The Postharvest Diseases programme remains a very high priority and several projects were directly aimed at improving postharvest disease management in packhouses. Potential alternative fungicides and sanitisers are continuously screened in pilot trials (123), before further trials are recommended. Imazalil, thiabendazole and pyrimethanil drench and in-line (fungicide bath or JBT heated flooder application) application and subsequent control of green mould were studied, giving valuable insight into the optimal use of these postharvest fungicides (1103, 1104, 1126). The effects of postharvest treatments on the viability and reproduction potential of CBS fruit lesions (1127) are studied in a project co-funded by CRI and the DST's Postharvest Innovation Programme, and clearly supports the argument that harvested fruit is not a viable pathway for CBS spread.

The Diagnostic Centre (DC) continues to perform a sterling service to the Citrus Improvement Scheme through routine soil and water analyses for *Phytophthora* and nematodes, as well as through these analyses in research experiments in the Soilborne Diseases programme. In total, 11877 samples were analysed by one diagnostician, a technician and two general assistants; this is 42% more samples than in the previous report period.

In general, good progress was made in Disease Management. Apart from excellent 'non-research' support, such as for biosecurity, improvement scheme, market access, and formal and *ad hoc* extension activities, the quality and quantity of tangible research outputs are maintained at high standard through consolidated and focused research.

PORTEFEULJE-OPSOMMING

Die siektebestuurportefeulje gaan voort om Suidelike Afrika se sitrus-industrie te dien. Die meeste produsente-prioriteite word aangespreek in projekte wat ontwerp word om sekere kort-, medium- en langtermyn strategiese doelwitte te bereik. Hierdie diens- en navorsingsdoelwitte/-strategieë en hoogtepunte van die verskeie programme word kortliks hieronder opgesom. Die vordering vir die 2015-16 verslagperiode word in die program-opsommings opgesom.

Diensdoelwitte in die ent-oordraagbare siekte program is om diagnostiese dienste aan die Sitrusverbeteringskema (SVS) te verskaf, deur her-indeksering van moederblokbome, groeipunt-enting en preïmmunisasie van nuwe kultivars. Hierdie noodsaaklike diagnostiese dienste is deurlopend en word deurgaans geëvalueer ten einde te verbeter waar nodig (sien SVS verslag). Aangesien die prestasie van sekere "skoongemaakte" SVS materiaal gekritiseer is, is 'n projek begin om die hortologiese prestasie van voor-Skema materiaal, met SVS materiaal, te evalueer (1074); geïnfekteerde en SVS bome is uitgeplant, maar navorsers vind dit moeilik om proefpersele te vind om die geïnfekteerde behandelings te plant. Navorsingsdoelwitte fokus grootliks op volhoubare beheer van *Sitrus Tristeza virus* (CTV), wat op kruisbeskerming gebaseer is. Op 'n meer basiese vlak, word die meganismes betrokke in kruisbeskerming nagevors (885B, 1100), terwyl toegepaste navorsingsprojekte die volhoubaarheid van potensiële kruisbeskermingsbronne vir verskeie klimaatstreke, sitrustipes en kultivars evalueer (738, 739, 742, 789, 968). Die epidemiologie van Afrika Sitrusvergroening, en veral die alternatiewe gashere van die bakterie, word bestudeer. Van belang is dat hierdie projek ook in samewerking met internasionale navorsing op die gevreesde Asiatiese vorm van hierdie siekte plaasvind (886B). Uitkomste van hierdie projek is van onskatbare waarde ten einde die vals positiewe waarneming van Asiatiese Sitrusvergroening (HLB) in sentraal-Afrika te verduidelik. Die kultivars vanaf chimera vrugte op vergroening geïnfekteerde plante, het aanvanklik belofte getoon om vergroeningsbestand te wees, maar het lae vlakke van vergroeningsimptome ontwikkel (815). Die projek is gefinaliseer, maar die lyne moet verder in toekomstige projekte geëvalueer word.

Die grondgedraagde siekte program doen navorsing op volhoubare opsies (alternatiewe vir harde chemikalieë) vir beheer van wortelvrot en die sitrusnematode, en sekere belowende opsies is geïdentifiseer (762, 1030). Die etiologie en beheer van Armillaria wortelvrot (1068) is ondersoek en dit is duidelik dat twee afsonderlike komplekse van patogene, en nie Armillaria, agteruitgang in noordelike en

suidelike areas veroorsaak het. Vroeë diagnose van sitrusboom-agteruitgang is noodsaaklik om die kanse vir herstellende aksies te verbeter. Veelvuldige parameters is as potensiële agteruitgang-indikators, vóór ernstige (sigbare) simptoom-ontwikkeling, geëvalueer, en die mees geskikte parameters word verder in voortgaande navorsing bestudeer (1092). Voorkomende en genesende bestuur van grondgedraagde siektes in sitruskwekerye word bestudeer in ’n projek wat gesamentlik deur CRI en die Departement van Wetenskap en Tegnologie (DST) se Navorsing op Sitrus-uitvoere (RCE) befonds word (1101 / RCE-5), wat ook die SVS se doelwit ondersteun om siektevrye sitrusbome aan produsente in suidelike Afrika te verskaf.

In die Sitrus Swartvlek (SSV) program word ’n aansienlike hoeveelheid tyd op tegniese marktoegang-ondersteuning spandeer. ’n Gesamentlike projek met die VSA, Brasiliaanse en Argentynse navorsers, ten einde ’n waarskynlikheidsmodel te ontwikkel om kwantitatief die risiko van vrugte as ’n verspreidingsweg vir SSV te voorspel, is in die finale fases van afsluiting en ’n wetenskaplike artikel word voorberei (1026). Die globale populasie-struktuur van die SSV-patogeen is deur middel van genetiese merkers bestudeer en het die genetiese verskille tussen populasies in China, Australië, Suid-Afrika, Brasilië en die V.S.A. aangedui, met die geskatte ouderdom van die populasies wat in daardie volgorde afneem (977). Spuitprogramme word voortdurend bestudeer ten einde ons kennis van SSV-beheer en ons vermoë om SSV koste-effektief te beheer te verbeter, om fungisiedweerstand te bestuur, formulasies te verbeter, en om nuwe aktiewe bestanddele te registreer (970, 1012). Hoogtepunte vanuit hierdie proewe, is die naderende registrasie van nuwe verbindings en kombinasies wat produsente tot soveel as twee spuitrondtes behoort te spaar. Ses nuwe projekte is op CBS geïnisieer. Drie projekte sal kennisgapings in CBS epidemiologie bestudeer: die projekte by CRI (RCE-6) en QMS Laboratories (RCE-8) (beide word befonds deur RCE) maak goeie aanvanklike vordering, terwyl die derde projek by ’n eksterne diensverskaffer weens onvoldoende vordering, beëindig is. Die potensiaal van verminderde volume fungisiedtoediening ten einde CBS te beheer word in ‘n semi-kommersiële proef ondersoek (1132). ’n Web-gebaseerde inligtingsisteem word in ’n RCE mede-befondste projek (RCE-7) ontwikkel om CBS bestuur te verbeter; hierdie sisteem het ten doel om weer- en CBS epidemiologiese model-uitsette te verpak, om die algemene kennis van CBS epidemiologie te verbeter, en om beter ingeligte CBS bestuur moontlik te maak.

In die vrug- en blaarsiekte program, word nuwe beheer-opsies vir Alternaria bruinvlek (ABV) bestudeer (750), maar tekort aan proefpersele het vordering belemmer. Radartegnologie word gebruik om sitrusboom lowerdakke te karakteriseer met die uiteindelike doel om spuitkalibrasie te verbeter (1089). ’n PhD-studie op die gebruik van benatters om swamdoder toediening en beheer van Alternaria bruinvlek te verbeter, word saamgevat (1096).

Die na-oes siekte program bly ’n baie hoë prioriteit en verskeie projekte is direk gerig op die verbetering van na-oes siektebestuur in pakhuise. Moontlike alternatiewe fungisiedes en saniteerders word deurlopend in loodsproewe geëvalueer (123) voordat verdere proewe aanbeveel word. Imazalil, thiabendazole en pyrimethanil stort (‘drench’) en in-lyn (fungisiedbad of JBT verhitte vloedtoediening) toediening, en gevolglike beheer van groenskimmel, is bestudeer, en het waardevolle insig in die optimale gebruik van hierdie na-oes fungisiede gegee (1103, 1104, 1126). Die effekte van na-oes behandelings op die lewensvatbaarheid en voortplantingspotensiaal van CBS vrugletsels (1127), word in ’n projek wat gesamentlik deur CRI en die DST se Na-oes Innovasie Program befonds word, bestudeer, en ondersteun duidelik die argument dat geoeste vrugte nie ’n lewensvatbare pad vir CBS verspreiding is nie.

Die Diagnostiese Sentrum (DS) lewer steeds ’n uitstekende diens aan die Sitrusverbeteringskema deur roetine grond- en waterontledings vir *Phytophthora* en aalwurms, asook deur ontledings vir navorsers in die Grondgedraagde Siekte program. In totaal het die DS 11877 monsters geanaliseer deur een diagnostikus, een tegnikus en twee algemene assistente; dit is 42% meer monsters in vergelyking met die vorige verslagperiode.

Goeie vordering is oor die algemeen in Siektebestuur gemaak. Afgesien van uitstekende ‘nie-navorsing’ ondersteuning soos vir biosekuriteit, verbeteringskema, marktoegang, en formele en *ad hoc* voorligtingsaktiwiteite, het die kwaliteit en kwantiteit van tasbare navorsingsuitsette deur gekonsolideerde en gefokusde navorsing voortgesit.

4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: G. Cook (CRI)

4.2.1 Programme summary

Vegetative propagation methods such as grafting are shadowed by the potential to disseminate pathogens that are present in budwood and include bacteria, viruses and viroids. The first line of defence is to ensure supply of pathogen-free propagation material. This provision is reliant on good diagnostic capabilities to screen for known pathogens. In instances where pathogens are vectored by insects which occur endemically, further control strategies are required. These interventions can include vector control, cross-protection as applied for *Citrus tristeza virus* (CTV) disease control, inoculum removal as done for African greening disease control and also the preferential use of varieties and rootstocks that exhibit tolerance or resistance to the pathogens. A strong research emphasis is placed on the CTV cross-protection programme to mitigate the effects of this virus especially in grapefruit. This virus is extremely complex and consists of a number of strains and variants which naturally occur as strain mixtures. Fortunately, diagnostics to identify these strains have progressed significantly, enabling studies to identify which strains are involved in disease expression and which are required to mitigate the disease in cross-protection strategies (4.2.4 and 4.2.8). The most appropriate approach to study the disease is to identify single-strain sources and to apply them in glasshouse and field trials to evaluate their impact on the host. Two glasshouse trials are in progress to investigate the symptom expression of single CTV strains and strain mixtures in grapefruit and also to challenge single strains with severe sources to assess their cross-protection abilities (4.2.8). Field trials to assess the field performance of promising CTV sources in grapefruit, sweet orange and soft citrus are reported on in projects 738, 739, 742, 789 and 968. Adding to diagnostic capabilities, a bio-informatic pipeline for virus diagnostics based on NGS data was optimized and packaged in a user-friendly interface named Truffle (http://truffle.sourceforge.net). This tool has the potential to fast track cultivar introductions from other citrus repositories by confirming pathogen-free status of known pathogens and replacing the need for certain biological processes (4.2.8). A comparative trial to test the horticultural performance of pre-scheme material containing viroids and CTV compared to material supplied by the Citrus Improvement Scheme (CIS) was established at Burgersfort this season (4.2.11). This is a long term assessment. Research into African Greening was conducted in two projects. Field evaluation of sweet orange, embryo-rescued clones, selected for resistance/tolerance to greening was finalised and although the clones were not shown to be resistant, indications for possible tolerance was noted. Continued evaluation of symptoms on these clones is advised as their horticultural performance was good compared to commercial controls (4.2.3). The epidemiology of African Greening was further investigated in Project 886B. Indigenous plants of the citrus family (Rutaceae) were evaluated for their ability to host "*Candidatus* Liberibacter africanus" (Laf), the African Greening pathogen. Laf has not been detected in any of the indigenous Rutaceae plants; however, some Rutaceous genera were shown to harbour Liberibacter subspecies of Laf and include *Zanthoxylum*, *Vepris*, *Clausena, Teclea, Oricia* and *Calodendrum*. Liberibacter subspecies-specific tests were developed to LafC, LafCl, LafT, LafV and LafZ, and are useful for further diagnostic purposes.

4.2.1 Programopsomming

Vegetatiewe vermeerderingmetodes soos enting het die potensiaal om patogene, soos bakterieë, virusse en viroïede, teenwoordig in die okuleerhout, te versprei. Die eerste vereiste is dus om gesonde voortplantingsmateriaal te verskaf. Hierdie vereiste is afhanklik van goeie diagnostiese vermoëns om vir bekende patogene te kan toets. In gevalle waar patogene versprei word deur insekte wat endemies voorkom, word verdere beheerstrategieë vereis. Hierdie ingrypings kan vektor beheer insluit asook kruisbeskerming soos toegepas vir *Citrus tristeza virus* (CTV) siektebeheer, inokulum verwydering soos vir Afrika Vergroening beheer toegepas word asook die gebruik van variëteite en onderstamme wat verdraagsaamheid of weerstand toon teen die patogene. 'n Navorsingsklem is op CTV kruis-beskerming geplaas om die gevolge van die virus te beperk, veral in pomelos. Hierdie virus is uiters kompleks en bestaan uit 'n aantal rasse en variante wat in die natuur gewoonlik as mengsels voorkom. Diagnostiese identifikasie metodes om hierdie rasse en variante te kan onderskei, het oor die laaste paar jaar aansienlik gevorder en die studie van rasse betrokke by siekte uitdrukking, asook die wat betrokke is by die beperking van siekte uitdrukking, moontlik gemaak (4.2.4 en 4.2.8). Die mees geskikte benadering om die siekte te ondersoek is om enkel-ras bronne te identifiseer en in glashuis en veld proewe te evalueer. Twee glashuisproewe ondersoek die simptoomuitdrukking van enkele CTV rasse en ras mengsels in pomelo wat later aan strawwe CTV bronne blootgestel sal word om hulle kruis-beskerming vermoëns te bepaal (4.2.8). Veldproewe word deurlopend geëvalueer om te bepaal watter CTV preïmmuniseringsbronne die beste beskerming bied en beste produksie lewer in pomelos, soetlemoen en sagte sitrus (Projekte 738, 739, 742, 789 & 968). ʼn Belangrike toevoeging tot diagnostiese vermoëns is die ontwikkeling van ʼn bio-

informatiese pyplyn vir virusdiagnose gebaseer op die NGS data wat geoptimiseer en verpak is in 'n gebruikersvriendelike program genoem Truffle (http://truffle.sourceforge.net). Die tegnologie het die potensiaal om vinniger vrystelling van nuwe sitrusmateriaal te bewerkstellig deur sekere biologiese prosesse te vervang (4.2.8). 'n Veldproef om die tuinboukundige prestasie van pre-skema materiaal, wat viroïede en CTV bevat, met materiaal voorsien deur die Sitrus Verbetering Skema (GOS) te vergelyk, is hierdie seisoen gevestig by Burgersfort (4.2.11). Hierdie is 'n langtermyn ondersoek. Twee projekte het op Vergroening gefokus. Veldevaluasie van embrio-herwinningsklone vanaf soetlemoen wat potensiële weerstand / toleransie teen Vergroening getoon het, is voltooi (4.2.3). Alhoewel die klone nie bestandheid gewys het nie, is daar aanduidings vir moontlike verdraagsaamheid en voortgesette evaluering van simptome van hierdie klone word aanbeveel. Die tuinboukundige prestasie van sommige van hierdie klone het goed vergelyk met die kommersiële kontrole (4.2.3). Die epidemiologie van Afrika Vergroening is verder ondersoek in projek 886B. Inheemse plante van die sitrus familie (Rutaceae) word geëvalueer vir hul vermoë om as alternatiewe gashere vir "Candidatus Liberibacter africanus" (Laf), die Afrika-vergroening patogeen, te dien. Laf is nog nie in enige inheemse Rutaceae plante gevind nie. Sommige genera in die Rutaceae familie, *Zanthoxylum*, *Vepris*, *Clausena, Teclea, Oricia* en *Calodendrum*, het wel soortgelyke Liberibacters wat van Laf verskil, gehad. Liberibacter subspesie-spesifieke toetse is ontwikkel om LafC, LafCl, LafT, LafV en LafZ, en word benodig vir verdere diagnostiese doeleindes.

4.2.2 **FINAL REPORT:** *Citrus tristeza virus* cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley
Project 738 (2004 - 2016) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

Indications of a severe *Citrus tristeza virus* component in the Nartia (GFMS12) cross-protecting source necessitated the separation of the strain populations into sub-isolates by single aphid transmissions (SAT). These sub-isolates were derived from two Nartia sources (A = GFMS12, C = GFMS14) and a Mouton source derived from sweet orange. The GFMS14 and Mouton SAT sub-isolates were done at Beltsville, USA, and imported back to South Africa. After biological indexing, four sub-isolates showed potential for further evaluation (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). Two sub-isolates from the ARC-ITSC of GFMS12 (GFMS12-7, GFMS12-9) were included in the trial as well as GFMS12 (previous standard cross-protection source for white grapefruit) and GFMS35 (current cross-protection source for all grapefruit). Virus-free Star Ruby trees were prepared in a glasshouse and were inoculated with the various CTV sources. A virus-free treatment was included as a control and field-infection indicator. Trees were planted in the Kakamas area in the Northern Cape province in 2004. This trial was a duplicate experiment of Project 679, planted in 2003 in Swaziland, and the two experiments were aimed at assessing the effect of the CTV sources in different climatic regions. Similar trials were also planted in 2007 in the Malelane and Letsitele areas (Project 742). This was the 11th year of the trial and the final evaluation. Tree canopy volumes were determined and fruit harvested and graded into export sizes. Trees grew slower than would be the case in more favourable grapefruit production areas due to the cooler winter climate. The trees also took longer to come into production. Significant differences in tree sizes were found between treatments. Trees inoculated with GFMS12 were the largest and trees inoculated with GFMS35 and B389-1 the smallest. Results with the GFMS12 treatment are in contrast with results obtained in the Letsitele trial (Project 742), where a significant reduction in tree size was associated with GFMS12 due to severe stem-pitting. This was the fifth harvest of the trial and no significant differences were found in cumulative yields between treatments over 5 years and no treatment was consistently associated with a high percentage of small fruit. Therefore, crop production did not differ significantly between CTV treatments. The control trees remained symptomless after 11 years in the field, indicating low pressure of aphid and/or severe CTV at the trial. However, trees inoculated with sub-isolate B389-1 developed unacceptable stem-pitting, whilst trees inoculated with GFMS12 developed very little stem pitting. These results are again in contrast to stem-pitting results obtained in trials planted in different climatic regions; Letsitele (Project 742) and Swaziland (Project 679) for these two CTV sources and suggest that CTV symptom expression is influenced by the climate in which the citrus host is found. The horticultural assessment of this trial is complete, but further analysis regarding the present CTV strain profiles of the trial trees will continue in Project 1100.

Opsomming

Weens aanduidings van ʼn strawwe *Citrus tristeza virus (*CTV) komponent in die Nartia (GFMS12) kruisbeskermingsbron was dit nodig om die virus populasie in sub-isolate deur middel van enkel plantluis oordragings te skei. Sub-isolate is vanaf twee Nartia bronne (A=GFMS 12, C=GFMS 14) en ʼn Mouton bron verkry. Die GFMS 14 en Mouton sub-isolate is by die kwarantyn fasiliteit in Beltsville, VSA, voorberei en

terug na Suid Afrika ingevoer. Nadat die sub-isolate deur biologiese indeksering geëvalueer is, is gevind dat slegs vier potensiaal toon vir verdere evaluasie (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is by die proef ingesluit. GFMS 12 (vorige kruisbeskermingsbron) en GFMS 35 (huidige kruisbeskermingsbron) is as kontrole verwysings gebruik. Virusvrye Star Ruby boompies is in ŉ glashuis voorberei en met die verskeie bronne gepreïmmuniseer. ŉ Virusvrye behandeling is as kontrole ingesluit wat natuurlike besmettings sal aandui. Hierdie proef is ŉ herhaling van Projek 679 wat in Swaziland aangeplant is, asook gedeeltelike herhaling van proewe aangeplant in Malelane en Letsitele (Projek 742). Die verskeie proewe dien om CTV in die verskillende sitrus produserende streke te evalueer. Nadat preïmmunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing in die Noord Kaap provinsie gedurende 2004 uitgeplant en jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte geëvalueer. Die proef was in sy 11de jaar en die finale evaluasie is afgehandel. Boom volumes is bepaal en vrugte geoes en gegradeer volgens uitvoer vruggroottes. Die bome het heelwat stadiger gegroei as bome in gunstiger pomelo produksiegebiede as gevolg van die koeler winter klimaat. Die bome het ook langer geneem om in produksie te kom. Beduidende verskille in boom groottes is gevind tussen behandelings. Bome geënt met GFMS12 was die grootste en bome geënt met GFMS35 en B389-1 die kleinste. Resultate met die GFMS12 behandeling is in teenstelling met die resultate verkry in die Letsitele proef (Projek 742), waar 'n aansienlike vermindering in boomgrootte gevind is as gevolg van ernstige stamgleuf. Dit was die vyfde oes van die proef en geen beduidende verskille is gevind in die 5-jaar kumulatiewe opbrengste tussen behandelings nie en geen behandeling het gereeld hoë persentasie van klein vrugte gelewer nie. Produksie was dus nie beïnvloed deur die CTV behandelings beduidend nie. Die bome wat virus-vry geplant is, het simptoomloos gebly ná 11 jaar, wat op 'n lae plantluis en / of ernstige CTV druk dui. Bome geënt met sub-isolaat B389-1 het onaanvaarbare stamgleuf ontwikkel, terwyl bome geënt met GFMS12 baie min stamgleuf ontwikkel het. Hierdie resultate is in teenstelling met stamgleuf resultate vir hierdie twee CTV bronne verkry in proewe geplant in ander klimaatstreke [Letsitele (Projek 742) en Swaziland (Projek 679)] en dui daarop dat CTV simptoom uitdrukking beïnvloed word deur die klimaat waarin die sitrus gasheer geplant is. Die hortologiese evaluasies van hierdie proef is afgehandel, maar verdere analise met betrekking tot die huidige CTV ras-profiele van die proef-bome sal voortgaan in Projek 1100.

Introduction

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

Objectives

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

Performance evaluation is based on:

- Presence/severity of stem pitting
- Tree canopy volume
- Yield
- Fruit size

Materials and methods

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

Tree canopy volumes were determined using the formula $V=S2(\pi h -1.046S)$, where S is canopy radius and h is the height of the fruit bearing canopy.

Fruit yield (kg/tree) and fruit size distribution was determined at harvest according to export size categories.

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

Results and discussion

Tree size: Average tree canopy volumes (m^3) per treatment are presented in Table 4.2.2.1. Significant differences in tree size were observed between the different CTV treatments. Trees inoculated with GFMS12, GFMS12-7 and B390-3 were the largest and trees inoculated with GFMS35 and B389-1 and B389-4 the smallest. Results with the GFMS12 treatment are in contrast with results obtained in the Letsitele trial where a significant reduction in tree size was associated with GFMS12 due to severe stem-pitting.

Tree health: Average stem-pitting ratings per treatment are presented in Table 4.2.2.1. Trees containing sub-isolate B389-1 developed significantly more stem-pitting than other treatments. Although the average rating was mild (rating between 1 - 2), half of the trees with this treatment showed severe stem-pitting. This is in contrast to results obtained in Letsitele and Swaziland where treatments with B389-1 were not associated with any stem pitting. GFMS12 also induced no visible stem pitting in this trial and this is again in contrast to results obtained in Letsitele and Swaziland where this source induced severe stem-pitting. Trees planted virus-free remained symptomless after 11 years, indicating a low level of severe CTV challenge in the area.

Production: This was the fifth harvest of the trial. The average yield per tree for each treatment for the 2015 harvest as well as the cumulative yield over 5 years is presented in Table 4.2.2.2. No significant differences were found in cumulative yields between treatments. No treatment was consistently associated with a high percentage of small fruit over the trial evaluation. Therefore, crop production did not differ significantly between CTV treatments.

Table 4.2.2.1. Average canopy volumes of Star Ruby trees inoculated with various CTV sources and sub-isolates, 11 years after planting in the Orange River Valley

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

* Figures in each column followed by the same letter do not differ significantly at 5% level (Fisher's LSD).
** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Table 4.2.2.2. Average yield (kg/tree) and relative fruit size distribution of Star Ruby grapefruit trees pre-immunised with different CTV sources and sub-isolates, 11 years after planting in the Orange River Valley.

Treatment	Fruit size (kg)						Total kg/tree	Cumulative yield: 2011-15
	27	32	36	40	48	64		
B389/1	3.3 a	3.0 a	11.8 a	20.6 ab	35.1 ab	6.7 a	80.7 a	412.9 a
B389/4	4.9 a	3.9 a	18.4 abcd	23.7 abc	33.8 a	8.2 a	93.3 ab	441.4 a
B390/3	4.0 a	5.6 ab	19.0 abcd	34.2 c	51.7 b	13.6 a	128.3 b	489.5 a
B390/5	9.3 ab	7.7 abc	20.9 bcd	27.0 abc	32.5 a	10.8 a	108.3 ab	471.2 a
GFMS 12/7	15.2 b	12.1 bc	26.8 d	26.9 abc	28.4 a	8.8 a	118.3 a	470.1 a
GFMS 12/9	8.9 ab	5.7 ab	16.4 abc	14.9 a	23.6 a	7.8 a	77.3 a	420.9 a
GFMS 12	9.9 a	13.5 c	24.6 cd	24.1 abc	32.8 a	11.6 a	116.6 ab	443.7 a
GFMS 35	3.4 a	2.6 a	13.3 ab	20.3 ab	32.3 a	8.1 a	80.1 a	443.6 a
Virus-free	6.8 a	7.7 abc	22.1 cd	30.2 bc	38.2 ab	12.1 a	117.2 a	452.5 a

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

Conclusion

Tree growth was slower at this trial site, compared to other grapefruit production areas as the cooler winter climate suppressed growth compared to the more favourable grapefruit production areas. Trees inoculated with sub-isolate B389-1 developed unacceptable stem-pitting, whilst trees with GFMS12 developed very little stem pitting. These results are in contrast to the findings in the Letsitele and Swaziland regions. The control trees remain symptomless after 11 years in the field, indicating low pressure of aphid and/or severe CTV at the trial. These findings suggest that CTV symptom expression is influenced by the climatic conditions in which the citrus host is found. The CTV strain profiles of each trial tree will be determined in Project 1100 and compared to the strain profiles of the same treatments in the other climatic regions to establish whether the original treatment populations have changed by introductions of other strains by aphid transmission.

Technology transfer

None to date.

Oral Presentations at Scientific conference

Breytenbach, J.H.J., Cook, G. & van Vuuren, S.P. Field performance of various *Citrus tristeza virus* cross-protection sources trialled in grapefruit in different climatic regions. 20th IOCV Conference, Chongqing, China, 10-15 April 2016.

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4.2.3 FINAL REPORT: Evaluation of citrus material for greening resistance

Project 815 (2006 - 2016) by S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

Summary

Attempts were made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. In 2006, two embryo rescue clones, GTC-E2 and GTC-T2 were identified as symptomless after exposure to the citrus greening vector. PCR confirmed that they were free of the greening organism. A third clone, GTC-14, showed possible tolerance. These three clones have been multiplied on virus-free rootstocks and separately pre-immunised with two *Citrus tristeza virus* sources where after they were planted during 2007 in an orchard for field evaluations. Greening symptoms were observed on three trees of two clones after 7 years and the presence of the Liberibacter in these trees was confirmed by PCR. All the other trees were symptomless and tested negative by PCR. Trees of clone GTC-T2 were all free of greening. The following season one of the three positive trees was free of greening symptoms and tested free from Liberibacter by PCR. This may be an indication of resistance where the greening organism was prevented from multiplication and spreading. No other infections were found. However, in a separate bush trial where triozids are in abundance at times, none of the clones were completely free from greening. Development of symptoms on these trees should be monitored. The fifth crop was harvested from the orchard trial and the external fruit quality compared favourably with the Midknight Valencia control. Clone GTC-E2 had the best production and was significantly better than the Midknight Valencia. The cumulative yield for five years of clone GTC-E2 was also significantly better than that of Midknight Valencia. The production of large fruit was less for the GTC-14 clone trees.

Opsomming

Daar is gepoog om vergroening weerstandbiedendheid te verkry deur embrio's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Twee embrio-herwinningsklone, GTC-E2 en GTC-T2, is in 2006 as simptoomloos geïdentifiseer na blootstelling aan besmette vektore van sitrusvergroening. PKR het getoon dat hulle vry van die organisme is. ʼn Derde kloon, GTC-14, het moontlike toleransie getoon. Die drie klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* bronne gepreïmmuniseer en gedurende 2007 in ʼn boord vir verdere evaluasie uitgeplant. Na 7 jaar is vergroeningsimptome vir die eerste keer op drie bome van twee klone waargeneem. Die teenwoordigheid van die Liberibacter is deur PKR bevestig. Al die ander bome was simptoomloos en het negatief getoets met PKR. Al die bome van kloon GTC-T2 was vry van vergroening. Die volgende seisoen was een van die drie positiewe bome vry van vergroening simptome en vry van Liberibacter met PKR getoets. Hierdie mag ʼn aanduiding wees van weerstand waar die organisme verhoed word om te vermeerder of om te versprei. Geen verdere besmettings het voorgekom nie. Nietemin, in ʼn aparte bosproef waar die vektor soms in groot getalle voorkom, was nie een van die klone heeltemal vry van vergroening nie. Die ontwikkeling van simptome op hierdie boompies moet verder gemoniteer word. Die vyfde oes is van die boordproef verkry en eksterne vruggehalte vergelyk goed met die Midknight Valencia

kontrole. Kloon GTC-E2 het die beste produksie gelewer en was betekenisvol beter as Midknight Valencia. Die kumulatiewe produksie oor vyf jaar van kloon GTC-E2 was ook betekenisvol beter as Midknight Valencia. Die produksie van groot vrugte was minder by die GTC-14 kloon bome.

Introduction

Greening disease in South Africa, caused by the gram negative bacterium "*Candidatus* Liberibacter africanus" (Garnier *et al.*, 2000*)*, remains the most destructive disease in the cooler production areas of South Africa. The disease was first reported in 1928 (Oberholzer *et al.*, 1965) and was thought to be caused by a heavy metal toxicity (van der Merwe & Andersen, 1937). In 1973 it was demonstrated that greening in South Africa is caused by a gram negative bacterium (Moll & Martin, 1973). Crop losses of 30 to 100% have been recorded in some areas due to the unmarketability of infected fruit as well as premature fruit drop (Schwarz, 1968). Greening was not observed in the Eastern Cape Province despite the presence of the triozid insect vector, *Trioza erytreae* (Del G) but only occurred sporadically in the Western Cape Province (Oberholzer *et al.*, 1965; Schwarz, 1968). In the Western Cape the origin of infected trees was traced to nurseries in infected areas in the Northern Provinces (Schwarz, 1968). Subsequently the movement of citrus material from infected areas to uninfected areas in South Africa was prohibited. Despite this, and the present control measures of using certified healthy planting material, insect control by systemic insecticides and the eradication of infected plant material (Buitendag & von Broembson, 1993), the disease was again detected in the Western Cape Province in 1995 (Garnier *et al.*, 2000). Currently the disease is still invading formerly greening-free areas (Pretorius *et al.*, 2006). The ultimate control measure will be the use of resistant plant material.

Attempts in South Africa to obtain greening resistant citrus cultivars by conventional breeding have been unsuccessful (de Lange *et al.*, 1985). The main obstacle has been the absence of proven sweet orange (*Citrus sinensis* (L.) Osb.), mandarin (*C. reticulata*), grapefruit (*C. paradisi* Macf.) and lemon (*C. limon* (L.) Burm.f.) cultivars resistant to greening. Thousands of seedlings with pollen parents of Palestine sweet lime (*C. aurantifolia* (Chrism.) Swing, citron (*C. medica* L.), shaddock (*C. grandis* (L.) Osb.) and sour orange (*C. aurantium* L.) were planted among greening infected trees and none have shown any resistance or tolerance. In another attempt, clones were collected by Dr. C.H. Buitendag from "healthy" twigs growing from infected branches in heavily infected orchards. None of these have shown tolerance or resistance in subsequent field evaluations (Unpublished data, Citrus Research International).

Chimera development on citrus fruit is a genetic disorder and occurs frequently and some cultivars are more prone than others. Greening affected fruit with chimeras are observed on a regular basis on diseased branches and these fruit often display "healthy looking" sectors in contrast to the affected part of the fruit. Seeds removed aseptically from these sectors and regenerated on artificial medium may possibly result in resistant plants using embryo rescue. Regenerated plants can be artificially challenged by the greening bacterium by means of the triozid insect vector *T. erytreae*. Using molecular techniques (Hocquellet *et al.*, 1999; Irey *et al.*, 2006; Li *et al.*, 2007) for evaluation after challenges may prove to be a rapid approach in identifying truly resistant or tolerant clones.

Citrus greening remains the most destructive disease in the cooler production areas. Despite the present control measures, the disease still invades plantings. The ultimate control measure will be the use of resistant plant material. Several new laboratory techniques became available allowing the detection and classification of the bacterium in plants and the triozid vector (Hocquellet *et al.*, 1999; Planet, *et al.,* 1995*;* Villachanoux, *et al.*, 1992). These techniques will assist in the development of plants resistant to the bacterium.

Objectives

The aim of this study was to screen citrus material, recovered through embryo rescue and tissue culture, for genetic resistance against greening disease by biological and molecular methods.

Materials and Methods

Field trial

Embryo rescue clones that showed potential resistance (GTC-ER, GTC-T2) or tolerance (GTC-14) to greening Liberibacter (van Vuuren & Manicom, 2009) were multiplied on virus-free rough lemon rootstocks in replicates of 10. GTC-CV, a Croc Valley selection was also included in the trial. This selection showed potential as a tolerant mutation in the field but was susceptible in glasshouse tests. As a control, virus-free 'Midknight' Valencia was used as the standard commercial cultivar. Five replicates of the virus-free

clones/selection as well as the control were pre-immunised with the standard LMS 6 *Citrus tristeza virus* (CTV) source (Van Vuuren *et al.*, 2000a) and the other five with CTV source SM 49 [GXI] (Van Vuuren *et al.*, 2000b) previously reported to give some protection against greening. ELISA was done on all the plants before they were planted in the field in a greening area according to a randomised block design during November 2007. The experimental block was surrounded by greening infected trees. Normal orchard practices were followed to control triozids and greening infection was visually monitored on a regular basis and confirmed by PCR.

The following data was collected from the field trial:

a) The trees were inspected each year during the winter months for greening leaf and fruit symptoms. Leaf samples were taken from each tree and subjected to PCR tests. Leaf samples were also taken from adjacent old trees and subjected to PCR testing to determine the presence of the greening pathogen.
b) Fruit were harvested during July/August of each year and graded in export sizes.

PCR

DNA isolation and detection of the greening organism in plants: Total DNA was extracted from trial trees using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle & Doyle, 1990). Extraction from citrus leaves were made from midribs of 4 leaves per plant which were macerated in a maceration bag with 4 ml CTAB buffer containing 1% mercapto-ethanol. An equal volume of chloroform was added, vortexed and centrifuged at 13 000 rpm. Nucleic acids in the supernatant were precipitated with ethanol. After centrifugation the pellets were suspended in 100 µl distilled water.

A PCR using the A2/J5 primer set, which targets the bacterial β-operon coding for ribosomal proteins of Liberibacter species, was used for detection of Laf (Hocquellet *et al*, 1999). PCR reactions were done in 20 µl reaction volumes using GoTaq® Hot Start Green Master Mix (Promega, Madison, USA) and 2 µl of DNA extract. Thermo-cycling parameters consisted of one cycle of 94 °C for 3 min followed by 35 cycles of 94 ˚C for 20 s, 57 ˚C for 30 s, 72 ˚C for 20 s and a final extension at 72 °C for 5 min.

Bush trial

No greening symptoms were observed after 6 years in the original field trial and the absence of the Liberibacter in the trial trees was confirmed by PCR. This made it necessary for further evaluations at a different site where triozids are abundant and uncontrolled.

Carrizo citrange rootstocks were obtained from Esselen nursery in Malelane. Budwood was cut from each treatment in the field trial. Olinda Valencia trees were made as an additional control since the ER clones originated from Olinda Valencia. Olinda Valencia is currently not cultivated commercially and was the reason why it was not used as a control in the commercial trial. No virus-free bud wood of Olinda Valencia was available from the Nucleus Blok at CRI and therefore healthy looking bud wood was cut from field trees at Crocodile Valley (Pty) Ltd. The newly made sweet orange trees were self-indexing for the greening Liberibacter and displayed no greening symptoms. The trees tested negative for the presence of the Liberibacter with PCR before planting. Trees were planted randomly between two rows of young trees at CRI premises that are infected with the greening Liberibacter. The presence of adult triozids, eggs and nymphs were monitored after planting.

Results and Discussion

Greening infection

Field trial:

Trees made from three potential greening tolerant ER clones (GTC) (GTC-E2, GTC-T2, GTC-14), as well as a field clone (GTC-CV), were planted during 2007 at Crocodile Valley (Pty) Ltd. The trees were inspected regularly and no adult triozids or signs that triozid insects were present (eggs, nymphs or triozid nymph-marks on leaves) were observed.

During August 2014 three trees were found displaying greening leaf symptoms on twigs or branches (Table 4.2.3.1). The trees were number 4 (GTC-14 with CTV SM49), number 5 (GTC-E2 with CTV SM49) and number 25 (GTC E2 with CTV LMS6). Leaves from the symptomatic twigs or branches were sampled and submitted for PCR tests. They all tested positive. The trees were re-sampled in sectors of the main directions and again submitted for PCR tests. Samples taken in the positive sectors contained leaves with and without greening symptoms. Each sample from positive sectors of trees number 4 and 5 contained one symptomatic leaf while the sample of tree number 25 contained two symptomatic leaves. The sample

of tree number 4 taken from the symptomatic sector tested negative and that of tree number 5 weak positive indicating a dilution effect when asymptomatic leaves are included in a sample. The sample of tree 25, with two symptomatic leaves, tested positive again.

None of the other trees, including the controls, showed any greening foliar symptoms. PCR confirmed the absence of greening infection in these trees.

During the 2015 inspection, leaf symptoms were again found on tree number 4 (GTC-14 with CTV SM49) and tree number 25 (GTC E2 with CTV LMS6). No leaf symptoms could be found on tree number 5 (GTC-E2 with CTV SM49). Leaf samples were taken around the canopy from all the trees for PCR analysis. Two samples were taken from trees displaying symptoms or possible infection. The (a) sample targeted the actual symptoms or potential symptoms while the (b) sample was taken around the canopy as for the other trees. Only two trees that tested positive during 2014 tested positive. The other trees, including tree number 5 that tested positive during 2014, tested negative (Table 4.2.3.1).

Six samples were taken from trees with symptomatic branches that were adjacent to the experimental trees. These samples were tested for the presence of greening with PCR. All six samples tested positive. This confirmed the presence of the Liberibacter in the area.

Apart from the two greening positive trees, greening-like fruit symptoms were also found on other trees (Table 4.2.3.1 and 4.2.3.2). The question arises whether these symptoms were greening or possibly due to CTV infection since the trees tested negative for greening by PCR. In one tree, fruit size was greatly reduced, and fruit were lopsided and green on one side (Fig. 4.2.3.1 and Fig. 4.2.3.2). This tree also tested negative for greening by PCR. CTV genotyping revealed the presence of several CTV genotypes similar to that occurring in the neighbouring Emperor mandarin trees.

Table 4.2.3.1. PCR results of double samples from trees with potential greening infection.

Tree number/sample	**Cultivar or Clone (CTV)**	**Greening-like fruit symptoms**	**PCR result**	
			2014	**2015**
4a: greening-like	GTC-14 (SM 49)	NA[a]	Positive	Positive
4b: around canopy	GTC-14 (SM 49)	Yes	Negative	Positive
5a: greening-like	GTC-E2 (SM 49)	NA	Positive	Negative
5b: around canopy	GTC-E2 (SM 49)	No	Positive	Negative
10a: greening-like	Midknight (LMS 6)	NA	NT[b]	Negative
10b: around canopy	Midknight (LMS 6)	No	Negative	Negative
25a: greening-like	GTC-E2 (LMS 6)	Yes	Positive	Positive
25b: around canopy	GTC-E2 (LMS 6)	Yes	Positive	Positive
33a: greening-like	GTC-T2 (SM 49)	NA	NT	Negative
33b: around canopy	GTC-T2 (SM 49)	No	Negative	Negative
35a: greening-like	GTC-14 (SM 49)	NA	NT	Negative
35b: around canopy	GTC-14 (SM 49)	No	Negative	Negative

[a] NA = Not applicable (no fruit)
[b] NT = Not tested

Table 4.2.3.2. The occurrence of fruit with greening-like symptoms*.

Cultivar or Clone	**Pre-immunising CTV source**	**Mean number of fruit per tree with Greening-like symptoms****	**Percentage**
Midknight	LMS 6 (standard)	0.2 NS	0.03
Midknight	SM 49	0.2	0.04
GTC-CV	Original CTV source	0.0	0.00
GTC-E2	LMS 6	3.6	0.32
GTC-E2	SM 49	0.4	0.04
GTC-T2	LMS 6	1.8	0.18
GTC-T2	SM 49	0.0	0.00
GTC-14	SM 49	20.4	1.90

- NS = Values in each column did not differ statistically significantly at the 5% level (Fisher's LSD)
- ** Symptoms could also have been induced by CTV infection (Fig. 1).

Figure 4.2.3.1. Small fruit together with normal sized fruit on the same branch.

Figure 4.2.3.2. Lopsided fruit showing poor colouring. The green side of the fruit is not typical olive green and did not display the lead colour when pressure was applied.

Bush trial:
Adult triozids were observed on all the trees after planting. Eggs and nymphs were only observed on trees with new flush. No greening symptoms were observed. Leaf samples were taken and submitted for PCR tests. The results are presented in Table 4.2.3.3.

No trend can be observed from the results. One control had a high infection and the other low. With the ER clones it was the same, one high and two low. No greening symptoms could be observed on any of the plants, including the controls.

Table 4.2.3.3. Number of greening infected trees of different cultivars and clones planted near natural bush with no insect control measures applied.

Cultivar or Clone	PCR result (number positive/6)
Midknight Valencia (Control)	5
Olinda Valencia (control)	2
GTC-E2	2
GTC-T2	6
GTC-14	2

Yield

The average yield (kg/tree) of each treatment for 2015 is presented in Table 4.2.3.4 together with that of previous years; 2010 to 2012, 2013 and 2014, as well as the cumulative yield for six years. All the clones were high yielding. The highest annual and cumulative yield was achieved where GTC-E2 were pre-immunised with SM49, on average 196 and 622 kg per tree, respectively. This was significantly better than the Midknight Valencia control trees with either CTV sources. Production of the other clones was also better than the Midknight control but not significantly.

Table 4.2.3.4. Average production per tree of 8-year-old ER clones in comparison with Midknight Valencia.

Cultivar or Clone	Pre-immunising CTV source	Yield (kg)									
		2010 to 2012	2013		2014		2015		Cumulative		
Midknight	LMS 6 (control)	39	44	b	72	c	138	bc	312	b
Midknight	SM 49	39	51	ab	60	c	137	bc	304	b
GTC-CV	Orig. CTV	46	59	ab	70	c	118	c	355	b
GTC-E2	LMS 6	59	84	a	93	bc	145	bc	472	ab
GTC-E2	SM 49	93	80	a	136	a	196	a	622	a
GTC-T2	LMS 6	67	78	ab	90	bc	174	ab	472	ab
GTC-T2	SM 49	53	59	ab	84	bc	125	c	421	b
GTC-14	SM 49	70	54	ab	112	b	155	abc	473	ab

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

External fruit quality

External fruit quality (fruit size) of the three ER clones was compared with Midknight Valencia in Table 5. Fruit of counts 48–88 were lower during 2012 than in 2011 (decreased from 92% to 37%) for all the treatments and did not differ between treatments (Table 4.2.3.5). The decrease of large fruit was attributed to the higher yield, however, with a higher yield during 2013, fruit size in the counts 48-88 were better, indicating that the small fruit of the previous year was not due to crop size. Since 2013 fruit size was consistently high except that of clone GTC-14 with only 56% of fruit in the categories of counts 48 to 88.

Table 4.2.3.5. Fruit size distribution of ER clones in comparison with Midknight Valencia at harvest.

Cultivar or Clone	Pre-immunising CTV source	Weight of fruit counts 48 - 88 (kg)		Percentage of fruit counts 48 – 88 (%)			
		2015		2012	2013	2014	2015
Midknight	LMS 6 (standard)	130	ab	50	98	99	94
Midknight	SM 49	132	ab	45	96	99	97
GTC-CV	Original CTV source	95	bc	45	78	80	81
GTC-E2	LMS 6	106	bc	43	77	75	72
GTC-E2	SM 49	148	a	29	81	75	77
GTC-T2	LMS 6	128	ab	42	84	83	74
GTC-T2	SM 49	99	bc	25	79	63	82
GTC-14	SM 49	84	c	22	59	54	56

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

Conclusion

Three ER clones that showed promise of resistance/tolerance in laboratory tests were planted in the field during 2007. Greening symptoms were observed on three trees of two clones after 7 years and the presence of the Liberibacter in these trees was confirmed by PCR. All the other trees were symptomless and tested negative by PCR. Trees of clone GTC-T2 were all free of greening. During the following year, no additional infections were found; on the contrary, symptoms on GTC-E2, one of the three positive trees, were absent and the absence of Liberibacter was confirmed by PCR. This may be due to resistance where the organism was eliminated or overgrown.

At the bush site where triozids were uncontrolled, PCR results showed infection in all three clones, however, no symptoms could be observed. Although this project was terminated, it would be worthwhile to monitor these plants for symptom expression during winter.

It is recommended that these clones be included in cultivar evaluation trials.

Technology transfer

Scientific publication

van Vuuren, S.P. & Manicom, B.Q. 2009. Attempts to obtain Huanglongbing resistant or tolerant sweet orange by embryo rescue from healthy chimeras of diseased citrus fruit. S. Afr. J. Plant Soil, 26(4): 220-224.

Oral presentation

van Vuuren, S.P. & Manicom, B.Q. 2007. Attempts to obtain Huanglongbing resistant or tolerant sweet orange by embryo rescue from healthy chimeras of diseased citrus fruit. XVII Conference of the international Organization of Citrus Virologists, Adana, Turkey, 22-26 October 2007.

Posters

van Vuuren, SP, Cook, G & Breytenbach, JHJ. 2012. Evaluating sweet orange clones for greening resistance. 7th Citrus Research Symposium. Central Drakensberg

van Vuuren, S.P., Cook, G. & Breytenbach. JHJ. 2013. Evaluating sweet orange clones for greening resistance. 19th Conf. IOCV, KNP, South Africa.

van Vuuren, SP, Cook, G & Breytenbach, JHJ. 2014. Evaluating sweet orange clones for greening resistance. 8th Citrus Research Symposium. Central Drakensberg

Further objectives and work plan

The project was terminated, but it would be worthwhile to monitor the bush trial site's plants for symptom expression during winter. It is also recommended that these clones be included in cultivar evaluation trials.

Acknowledgements

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4.2.4 FINAL REPORT: Dynamics of *Citrus tristeza virus* mild and severe strains in mild strain cross-protection strategies

Project 885B (2013 - 2016) by G. Pietersen, D. Read, J. Lubbe and K. Snyders (ARC-PPRI and UP)

Summary

During the course of this project techniques for the identification of *Citrus tristeza virus* strains and their composition in mixed populations were developed and evaluated. These were used to do a survey in Star Ruby orchards in the main grapefruit production areas of Southern Africa. The dominant strain was shown to be the resistance breaking (RB) strain. The techniques were further employed to evaluate the composition of CTV strains in candidate mild strain cross protecting sources and the homogeneity of these determined. Various virus isolation methods were evaluated and attempts made to isolate pure sources of CTV strains. The project formed the bulk of a PhD., two MSc., and three BSc(Hons) projects.

Opsomming

Tydens die uitvoering van hierdie projek is verskeie tegnieke vir die evaluering van die ras samestelling en suiwerheid van *Citrus tristeza virus* (CTV) populasies ontwikkel en getoets. Hierdie is dan gebruik in 'n grootskaalse opname om die CTV rasse in die Suider-Afrikaanse pomelo produksie areas op Star Ruby te bepaal. Die RB, oftewel "resistance breaking", CTV ras is bevind om wydverspreid en meestal die dominante ras in populasies te wees. Die tegnieke is verder gebruik om die ras samestelling van kandidaat

kruisbeskermings bronne te evalueer. Verskeie virus isoleringstegnieke is geëvalueer om suiwer bronne van CTV rasse te verkry. Die projek was deel ŉ PhD, 2 MSc., en drie BSc.(Hons) studies.

Introduction

Citrus tristeza virus (CTV) is an aphid-transmitted closterovirus endemic in South Africa. Stem pitting and decline symptoms of CTV have been major factors limiting productivity of grapefruit locally. Without CTV cross-protection, grapefruit production would be uneconomical in South Africa (van Vuuren *et al*., 1993). In 1973, the Southern African Citrus Improvement Scheme (CIS) was initiated. Marsh grapefruit trees planted in the 1920's and infected with CTV but still producing excellent fruit in the 1970s served as sources of mild sources in the certification scheme to protect grapefruit trees. These sources, Grapefruit mild strain GFMS12, GFMS35 and others were initially evaluated for severity in greenhouses (van Vuuren & Moll, 1987) and then in field trials (van Vuuren *et al*., 1993). Star Ruby grapefruit, pre-immunized with the GFMS12 source however later showed varying degrees of stem-pitting and variable fruit size in field trials and on budwood source trees (van Vuuren & Manicom, 2005; van Vuuren & van der Vyver, 2000). The CTV heterogeneity of the mother GFMS12 source was demonstrated and, based on biological indexing, severe CTV strains were associated with this source (van Vuuren et al., 2000). CTV strains of GFMS12 were separated by single *Toxoptera citricida* transmissions and sub-isolates GFMS12-1 -9 were established (van Vuuren et al., 2000). Two sub-isolates were shown to be less virulent while one sub-isolate was more virulent than the original GFMS12 source (van Vuuren *et al*., 2000). However, the presence of seedling yellows (CTV-SY) symptoms, not found in GFMS12 plants maintained in greenhouses, but in some plants in the field, also suggested that at least some GFMS12 pre-immunized plants had acquired additional CTV strains, probably introduced by aphids (van der Vyver *et al*., 2002). Altered single strand conformational polymorphic (SSCP) patterns, not corresponding to the SSCP profile of the original GFMS12 source supported this (van der Vyver *et al*., 2002). Scott *et al.* (2012) demonstrated that GFMS12 consisted of at least four strains. Zablocki & Pietersen (2014) showed that CT-ZA-3, a variant of the T68 strain was present in pure form in three sub-isolates of GFMS-12

Folimonova *et al*. (2010) demonstrated that hyper- exclusion of one strain of CTV from any others in citrus plants is very specific between strains, i.e. a given strain in a tree only prevents infection of a similar strain in the tree. The exact gene(s) involved and mechanism of cross protection is still not clear but Foliminova, (2012) has demonstrated that the functional p33 protein, rather than the p33 gene is required for super-infection exclusion, and that it functions in a homology dependent manner with a cognate protein from a heterologous strain failing to confer super-infection exclusion. More recently, Bergua *et al.,* (2014) have proposed that super-infection exclusion may be functioning on cellular and whole-organism level by two independent mechanisms.

In view of the strain specific nature of CTV cross protection and the role of p33 in determining this, efficient CTV mild strain cross protection requires; 1) determining the variation of p33 genes of CTV circulating in the Southern African citrus industry, primarily in grapefruit , 2) isolation of strains, 3) characterization and confirmation of homogeneity, 4) pathogenicity testing on a range of citrus hosts, 5) testing the cross-protection ability of the sources, 6) preparation of artificially mixed populations of mild strains to serve as pre-immunizing sources, and 7) to assess the performance of these populations as a pre-immunizing source. In this study we addressed these various aspects.

Objectives and summaries

In view of the diverse objectives of this project and the variation in methods and materials applied, each objective is presented as a separate abstract. A detailed report for each section is available as an annexure.

1. **Assessment of viral amplification and next generation sequencing protocols to help achieve the objectives of this project including:**

   1.1 Finalize assessment of various templates for next generation sequencing and develop a workflow for these. The templates were overlapping small amplicon templates, overlapping large amplicon templates and microRNA templates and were compared with results obtained against dsRNA extracts, total RNA extracts and viral particle enrichment by immunocapture. Develop a workflow for the detection and identification of CTV strains in mixed infections from data generated by NGS on the Illumina platform.

The use of immuno-capture enriched genomic RNA as template did not yield high quality results and a small component of total reads obtained were specific reads to CTV. This method of enrichment is therefore not recommended for future use. PCR amplicons as templates for Illumina sequencing produced more reads mapping to CTV than the immuno-capture particle enrichment template. Unfortunately, amplicon templates limit the information obtained to the specific gene fragment amplified and are not necessarily representative of the entire genome.

1.2 PCR bias associated with conserved primers designed to determine strain diversity within *Citrus tristeza virus* populations

A number of gene regions have previously been used to characterise CTV populations. These include the A-region and the F-region, the p23 gene, the p27 gene and the coat protein gene (CP). In this study, the potential primer-directed bias of primer sets targeting four different genomic regions of various CTV strains previously used in our laboratory was evaluated. These primer sets include those targeting the A and F regions in the variable 5' end of the genome, the p23 gene toward the conserved 3' end of the genome and the p33 gene near the mid-point of the genome. A set of strain-specific primers were designed for each gene region and then used to amplify plasmid CTV inserts of known identity. Mixtures containing equimolar concentrations of each strain-true template were produced and then re-amplified, using the polyspecific consensus primers. The resulting amplicons were then subjected to both Sanger sequencing of multiple clones and Illumina MiSeq sequencing. The MiSeq reads and clone sequences were mapped back or aligned with their corresponding reference sequences. The results of this study confirms that an increased number of nucleotide mismatches between a primer binding site and its conspecific primer leads to decreased ability of primers to amplify the template. While the primer pair targeting the p23 gene appears to have less associated bias than any of the others, the p23 genes location within the 3' half of the genome means that it has inherently less capacity for resolving strains when compared with regions located within the variable 5' half of the genome. The primer pair targeting the p33 gene, proposed for routine use in this study, has significantly reduced associated bias, when compared with primers targeting regions closer to the 5' end and the ability to resolve the major CTV strains at a phylogenetic level, for use in the reliable assessment of a CTV population. This study should also bring the potential effects that unverified primer pairs can have on population datasets, to the attention of plant virologists and to the field of virology as a whole.

2. Determine CTV strains circulating within the citrus industry (mild and severe strains), primarily in grapefruit.

2.1 In healthy-looking or mild symptomatic CTV infected trees in commercial groves and in pre-immunized groves with trees showing severe symptoms and from feral or unpre-immunised (pre-certification scheme) trees.

The diversity of *Citrus tristeza virus* p33 gene populations in commercial Star Ruby orchards was investigated. A total of 192 samples were collected from pre-immunised Star Ruby trees in the production areas of Hoedspruit, Malelane, Swaziland, Northern Cape, Sundays River Valley and Nkwalini Valley, which were all pre-immunised with the GFMS12 cross-protecting source. In addition to these, six samples were collected from non-pre-immunised Star Ruby trees in the Letsitele grapefruit production area, only three of which tested positive for the presence of CTV by PCR. Due to the association of the p33 gene and a proposed mechanism of cross-protection, this gene region was chosen for the analysis of variation in population composition of the collected samples. All of the resulting PCR amplicons from the non-pre-immunised trees were subjected to direct Sanger sequencing. A subset of 92 of these was randomly selected for Illumina MiSeq sequencing.

In establishing the Illumina-based p33 population assay, a concurrent study was carried out to determine the primer-associated PCR amplification bias associated with a number of previously published primer sequences, including those targeting the p33 gene used in this survey. While a significant degree of bias was found to be associated with the primers targeting the p33 gene, particularly against members of the RB clade, this clade was the most prevalent obtained during the survey. It can be assumed that the bias did not materially affect the results of the survey, possibly only underestimating the level of dominance of the RB clade.

Illumina sequence reads mapping to the p33 gene of reference strains within the RB clade were present in all of the populations analysed, being the dominant strain in 54 samples. This supports the results obtained with direct sequencing and confirms the RB strain as a component of the original GFMS12 pre-immunising population. Reads mapping to reference sequences within the Kpg3/SP/T3 p33 gene clade

were also present in most (76%) of the CTV p33 gene populations from pre-immunised trees, and were the dominant p33 gene variant in 30% of the CTV population analysed. Reads mapping to the p33 gene of the HA16-5 reference sequence were next most prevalent within the CTV populations analysed, with 58 out of the 92 pre-immunised trees being positive for this strain. Gene members from the VT clade were represented in just over a third of the populations that were analysed, and occurred sporadically in all but one of the sample sites. Generally, VT associated p33 gene reads were mostly represented at low levels in each dataset (<5%).

Objective 3. Isolate and characterise homogeneous CTV sources

3.1 Amongst previous pre-immunizing source GFMS12
Confirm homogeneity of GFMS12 sub-isolates (12-7, 12-8 and 12-9) using next generation sequencing on an overlapping small amplicon template.

The aim of this study was to characterize the 12-7, 12-8 and 12-9 GFMS 12 sub-isolates as well as CTV source B390-3, using full genome sequencing and the Illumina platform. These sources, apart from B390-3, were previously characterized with the Illumina sequencing technology, but used different template preparations including total RNA extraction, dsRNA extraction and immune-capture followed by a random RT-PCR. This study used total RNA extraction followed by amplification of the complete genome using overlapping amplicons as template for sequencing. Although results obtained support previous findings, this method of template preparation yielded poorer results. We were unable to amplify the complete genomes and the amount of CTV-specific reads obtained was lower than that obtained using dsRNA as template.

All the sources characterized in this study seem to have a predominant CTV strain present with minor components of other strains, based on the 3' half of the genome only as the 5' half could not be amplified and sequenced. The GFMS12-7, 12-8 and 12-9 sources were shown to predominantly contain the CTV strain CT-ZA3 (KC333868.1) whereas B390-3 was shown to contain a RB strain with closest homology to Taiwan-Pum/SP/T1 (JX266712.1).

3.2 Amongst candidate pre-immunizing source New Venture 41-2
Isolate CTV by single aphid transmissions of New Venture 41-2 CTV source.

The New Venture 41-2 source was identified as a possible mild candidate for cross-protection and included in a field trial evaluation. As part of an earlier study the variability of the 1a gene sequence of the New Venture 41-2 source was characterized. Based on the gene fragment characterized in that study it was found the source contained the VT strain. As the source was associated with mild symptoms in field trials, it was decided to use this source for SATs and mechanical transmissions in an attempt to obtain a single-strain VT source.

Single aphid transmissions and mechanical transmissions were unsuccessful and components of the source were not isolated. Various methods were used to determine the strain composition of the New Venture 41-2 source. The strain specific primers which amplify regions of the 5' end of the genome were able to detect VT, RB, T68 and HA16-5 strains as components of this population. The presence of VT, RB, T68 and HA16-5 were confirmed with Illumina sequencing, but the source may also contain other minor components based on this data.

3.3 Amongst candidate pre-immunizing source B390-3
Identify CTV strains in B390-3 CTV source using next generation sequencing.
Confirm homogeneity by inoculation of SAT sub-isolate on citrus indicator hosts and commercial varieties and monitor symptomology.

The B390-3 CTV source was isolated by single aphid transmission (SAT) from the South African Mouton source in Beltsville, USA and reimported back into South Africa. The source was biologically indexed and characterised by molecular genotyping using RT-PCR, Sanger and NGS sequencing. This work was done in parallel with current field trials which include B390-3 on Marsh and Star Ruby Grapefruit. CTV source, B390/3, did not produce severe symptoms on any of the indicators tested including, 'Mexican' lime, Sour orange, 'Duncan' grapefruit, and sweet orange grafted on a sour orange rootstock. No symptoms were observed on any of the commercial citrus varieties tested, including 'Palmer' Navel, 'Midknight' Valencia, 'Star Ruby' grapefruit, 'Esbal' clementine or 'Eureka' lemon under glasshouse conditions. Mild chlorosis, leaf cupping and mild stem pitting symptoms were only observed on 'Mexican' lime, the sensitive indicator

host. Sanger and Illumina sequence analysis of the p33 gene region, indicates a homogeneous viral population of a RB strain with most homology to isolate Taiwan-Pum/SP/T1 (JX266712.1).

3.4 Amongst new sources containing novel strains.

Establish a virus source from a Swaziland Rio Red orchard previously reported to contain the T36 strain (Smocilac, unpublished results) and confirm the finding using strain specific tests. Establish a single-strain source.

A survey was previously conducted to determine CTV strain populations in commercial grapefruit varieties. During this survey the T36 strain was detected in a Rio Red grapefruit in an orchard in Swaziland, a strain not previously detected in South African. The aim of this study was to confirm this finding. It was, however, determined that the initial identification was a misdiagnosis, and that T36 was not present in the original sample or in other grapefruit trees within that orchard. The RB strain, with close homology to T36 was, however, commonly found. Attempts were made to attain and establish a single-strain source of the RB strain. RB infected samples were screened for other known strains using strain-specific RT-PCR. A single graft-inoculated 'Mexican' lime plant, 12-7006, was assessed for its homogeneity by direct sequencing of the p33 gene. Sequencing of 20 clones of these amplicons and Illumina NGS sequencing of the p33 gene amplification products supported the finding that this source contains a homogenous RB strain.

3.5 Amongst naturally aphid infested, field grown trees (shotgun approach)

Due to the inefficient means of isolating pure sources of CTV strains using SAT, and the requirement downstream in further studies for single-strain sources, we employed a shotgun approach to isolate CTV strains, using naturally occurring aphid infestations. This was done by collecting aphids from CTV positive field trees in the Rustenburg area.

The aim of this study was to obtain homogenous CTV strain sources by single aphid transmissions (SAT), using natural aphid infestations (i.e. a shotgun approach to finding ANY single sources), bark-flap and stem slash inoculations to healthy 'Mexican' lime seedlings. All seedlings were screened post-inoculation for CTV presence using a CTV generic RT-PCR test. Only six CTV positive samples were obtained from the SAT trials. These seedlings were screened for strain composition using strain-specific RT-PCRs. All six positive sources appeared to be potentially homogenous for the RB strain. Amplification of the p33 gene region followed by Illumina next-generation sequencing (NGS) was performed on the samples. Reference mapping performed on each of the six filtered sample sequences confirmed that sample 14-7102 primarily contained Taiwan-Pum/SP/T1 (JX266712), an RB variant, while the other 5 samples (14-7103, 14-7104, 13-7105, 14-7107 and 14-7132) primarily contained the CTV isolate AT-1 (JQ061137). All six samples contained mixed CTV strain populations with low levels of other strains being present in addition to the dominant RB strain.

Conclusion

Comprehensive conclusions are included in the different sections (see addendum). During the course of this project techniques for the identification of *Citrus tristeza virus* strains and their composition in mixed populations were developed and evaluated. These were used to do a survey in Star Ruby orchards in the main grapefruit production areas of Southern Africa. The dominant strain was shown to be the resistance breaking (RB) strain. The techniques were further employed to evaluate the composition of CTV strains in candidate mild strain cross protecting sources and the homogeneity of these determined. Various virus isolation methods were evaluated and attempts made to isolate pure sources of CTV strains.

Future research

No research on CTV planned for the future.

Technology transfer

Read D. and Pietersen G. (2015). Genotypic diversity of *Citrus tristeza virus* within red grapefruit, in a field trial site in South Africa. European Journal of Plant Pathology DOI 10.1007/s10658-015-0631-x

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4.2.5 FINAL REPORT: Further studies on alternative hosts of "*Candidatus* Liberibacter africanus" and related Liberibacters on tree members of indigenous Rutaceae

Project 886B (April 2013 – March 2016) by G. Pietersen and R. Roberts (ARC-PPRI and UP)

Summary

Citrus greening is controlled at economically acceptable levels in South Africa through stringent vector control strategies and the removal of inoculum sources, but remains a problem in cooler citrus production areas of South Africa. The perpetuation of the disease may be due to the presence of alternate hosts for '*Candidatus* Liberibacter africanus' (Laf) other than citrus. During a previous project (886) samples of *Calodendrum, Vepris, Zanthoxylum* and *Clausena* were collected and analyzed for the presence of Liberibacters. Laf *sensu stricto* was not detected in any indigenous members of the Rutaceae; however, Liberibacters related to Laf were found in all genera of these *Rutaceae* tested, supporting the hypothesis that Laf may have originated from indigenous African Liberibacters of the *Rutaceae*. With the addition of another Liberibacter, Laf-T, found on *Teclea gerrardii*, five subspecies of Laf have now been identified from South Africa. Liberibacter subspecies specific tests were developed to LafC, LafCl, LafT, LafV and LafZ, and are useful for further diagnostics. It is still unknown whether any of the Liberibacter subspecies associated with indigenous Rutaceous hosts are capable of infecting commercial citrus species and it is therefore important to do controlled transmission tests to citrus. Simple Sequence Repeat (SSR) studies on South African Laf samples indicates that Laf populations from South Africa are conserved, but that the diversity observed might be influenced by citrus type, rather than geographical origin. With the introduction of "*Candidatus* Liberibacter asiaticus" (Las) to the USA, research efforts are targeted at fundamental understanding of the pathogen and development of control strategies for this disease. Novel control strategies are being evaluated, many based on molecular interventions. To this end it is important to characterize the local sources of Liberibacters by whole genome sequencing in order to exploit the sequence differences amongst the Liberibacters. The whole genome of Laf was compiled by a collaborative team, but the genome of LafC has not been completed. Additional sequence data has, however, been obtained and results indicate that LafC and Laf is less homologous than previously thought.

Opsomming

Die voorkoms van sitrus vergroening in Suid Afrika is verminder tot ekonomiese aanvaarbare vlakke deur die implementering van streng vektor beheer en die verwydering van inokulumbronne. Ten spyte hiervan, bly vergroening ŉ probleem in koeler produksie areas wat moontlik daarop dui dat '*Candidatus* Liberibacter africanus' (Laf) vanaf alternatiewe gashere versprei. Gedurende ŉ vorige projek (886) is verskeie *Calodendrum, Clausena, Vepris* en *Zanthoxylum* monsters vir Liberibacters getoets. Tipiese Laf is nie uit enige van die monsters geïdentifiseer nie, maar Laf verwante Liberibacters is in hierdie plant genera geïdentifiseer. Die bestaan van alternatiewe Liberibacters ondersteun die hipotese dat Laf se oorsprong vanaf ŉ inheemse Rutaceae bron is. Met die opsporing van nog 'n Liberibacter, Laf-T, op *Teclea gerrardii*, is vyf subspesies van Laf nou uit Suid-Afrika geïdentifiseer. Liberibacter subspesies spesifieke toetse is ontwikkel om LafC, LafCl, LafT, LafV en LafZ afsonderlik te kan opspoor en word gebruik vir verdere diagnostiese werk. Dit is nog onbekend of enige van die Liberibacter subspesies, gevind in die inheemse gashere, in staat is om kommersiële sitrus te besmet en daarom is dit belangrik om beheerde oordraging studies na sitrus te doen. 'Simple Sequence Repeat' (SSR) studies met Suid-Afrikaanse Laf monsters dui daarop dat Laf bevolkings van Suid-Afrika redelik gekonserveerd is, en dat die diversiteit wat wel waargeneem is, moontlik deur die sitrus tipe, eerder as geografiese voorkoms beïnvloed is. Met die voorkoms van '*Candidatus* Liberibacter asiaticus' (Las) in Amerika, word navorsing gefokus op beheer strategieë met die klem op molekulêre sisteme. Vir hierdie doel is dit belangrik om die inheemse Liberibacters te karakteriseer deur genoom basisvolgordes te bepaal, ten einde die verskille tussen die Liberibacters te bepaal. Sulke verskille kan lei tot die ontwikkeling van beter beheer strategieë. Die genoom van Laf is saamgestel deur samewerking van 'n span wetenskaplikes, maar die genoom van LafC is nog nie voltooi nie. Die bykomende data dui wel daarop dat LafC en Laf minder homoloog is as voorheen vermoed.

Introduction

Greening disease in South Africa is caused by a phloem-limited (Garnier and Bové, 1983) member of the Alphaproteobacteria (Jagoueix et al., 1994) namely, '*Candidatus* Liberibacter africanus' (Laf). Laf is primarily spread through the feeding and flight activities of the triozid, *Trioza erytreae* Del Guercio (McClean and Oberholzer, 1965b). This Liberibacter has thus far only been identified from citrus orchards in Africa and the Mascarene islands (Garnier and Bové, 1996; Garnier et al., 1996). Laf is considered to be heat sensitive (Garnier and Bové, 1983; Lopes et al., 2009), and is mainly a problem in cooler production areas. Infection of citrus trees with Laf is characterized by a mottled appearance of leaves from infected branches, the production of small, unripe fruit and tree stunting (McClean and Oberholzer, 1965b; Lopes et al., 2008). Greening disease is managed by a three-pronged approach, which includes the planting of disease free material, the removal of inoculum sources through elimination of infected trees and branches from an orchard, and chemical control of vectors (Buitendag and von Broembsen, 1993; Belasque et al., 2010; Hung et al., 2000; Shokrollah et al., 2011).

The perpetuation of Greening disease, despite the implementation of stringent control strategies, suggest that reservoir hosts may exist for this bacterium. However, to date, Laf has not unequivocally been identified from any other hosts other than commercial citrus species. Despite this, additional Liberibacter sub-species have been described. The first of these is a Liberibacter from an ornamental Rutaceae tree, *Calodendrum capense* (L.f.) Thunb (Cape Chestnut) indigenous to South Africa (Garnier et al., 2000). Sequence analyses, revealed that this Liberibacter, whilst having some homology with Laf, was unique from Laf and is known as '*Candidatus* Liberibacter africanus subsp. capensis' (LafC) (Garnier et al., 2000). LafC, despite being widely associated with *Ca. capense* (Phahladira et al., 2012), has not been identified from commercial citrus in South Africa (Pietersen et al., 2010), indicating that this bacterium does not play a direct role in the epidemiology of greening disease. In a recent CRI funded project (886), Liberibacters closely related to both Laf and LafC were identified from *Clausena anisata, Vepris lanceolata* and *Zanthoxylum spp,* all of which are known native hosts to *T. erytreae* (Moran, 1968a; Moran, 1968b). Despite the close sequence homology shared by the Liberibacters identified with both Laf and LafC, unique clusterings were observed based on phylogenetic analyses of Liberibacter *rplJ* and outer membrane protein (*omp*) genes. This distinct groupings correlated to the tree host species from which the Liberibacters were identified suggesting that each tree harbours a distict Liberibacter population. It was proposed that these Liberbacters be given subspecies status to reflect the great homology shared with Laf and additionally be named after the tree host from which each Liberibacter was identified to reflect the biological isolation of these Liberibacters. The names proposed were'*Candidatus* Liberibacter africanus subsp. clausenae' (LafCl), '*Candidatus* Liberibacter africanus subsp. vepridis' (LafV) and '*Candidatus* Liberibacter africanus subsp. zanthoxyli' (LafZ) (Roberts et al., 2015)

It has previously been hypothesized that Laf may have originated from an indigenous source as this Liberibacter has to date only been associated with citrus crops from Africa. The presence of mutliple Liberibacters on indigenous Rutaceous species further supports this claim. In the current study we aimed to further characterize the African Liberibacter species with regards to both biological and molecular properties. Firstly, the presence of Liberibacters from *Teclea spp.* and *Oricia bachmannii* was determined. By including *Teclea spp.* and *O. bachmannii* we are widening the scope of identifying possible reservoir hosts for Laf. Secondly, we attempted to determine whether sweet orange can act as a host to LafC, LafCl, LafV and LafZ by performing graft inoculation studies, but graft transmissions from the Rutaceous hosts have not succeeded to date. This study is ongoing in project 1157. Our third objective was to obtain sequence data of African Liberibacters. Whole genome sequencing of LafC was not completed, but valuable data was obtained.

Through characterizing both molecular and biological properties of the African Liberibacters we aim to identify various differences between Laf and the other African Liberibacters. Such differences can be utilized in creating novel control strategies for Laf.

Objectives

- **Finalize study on alternate tree host species of Laf and other Liberibacters amongst indigenous Rutaceae**
    - Determine alternate hosts of Laf amongst indigenous Rutaceous species *Teclea* and *Oricea*
    - Confirm identity of Liberibacters detected by multiple gene sequencing.
- **Conduct transmission studies to assess whether Liberibacters obtained are able to infect Citrus sp. and are transmissible by *T. erytreae*.**
    - Do graft transmission of LafC, Laf (ex Zanthoxylum), Laf (ex Vepris), Laf ex (Clausena) to sweet orange to determine if this citrus species can serve as host to these Liberibacters.
    - Once known that citrus can serve as a host, do *Trioza erytreae* vectored transmission of LafC, Laf (ex Zanthoxylum), Laf (ex Vepris), Laf ex (Clausena) in order to determine if *T. erytreae* can vector the Liberibacters.
- **Determine sequences of Liberibacters of South African Rutaceae**
    - Continue with a determination of the whole genome sequence of LafC
    - Determine variability of Laf sources (DNA extracts prepared during a survey of Liberibacters of citrus in 2006).
    - Develop Liberibacter variant specific detection technique.
- **Monitor promising international protocols for control of Huanglongbing and adapt for use in control of greening in South Africa.**

Materials and methods

1. Finalize study on alternate tree host species of Laf and other Liberibacters amongst indigenous Rutaceae

i) *Determine alternate hosts of Laf amongst indigenous Rutaceous species* Teclea *and* Oricia*:*

Leaf samples of *Teclea* spp and *Oricia bachmannii* were collected from natural forests in Southern KwaZulu-Natal where these genera are known to occur. The GPS coordinates of each sample was recorded and a unique accession number was allocated per sample. Total DNA was extracted from leaf petioles and midribs following the CTAB extraction method described by Doyle and Doyle (1990).

All samples were subjected to a generic Liberibacter TaqMan real-time PCR assay to identify Liberibacter-positive samples. For these reactions, 1µl of DNA template was added to a final reaction volume of 10µl containing 5.0µl 2X Taqman® universal Master Mix II (ABI, Foster City, CA, USA), 500nM forward primer LibUF (5'-GGC AGG CCT AAC ACA TGC-3'), 500nM reverse primer HLBr (5'-GCG TTA TCC CGT AGA AAA AGG TAG-3'), 150nM probe HLBp (5'-AGA CGG GTG AGT AAC GCG-3'), 2ng/ml BSA and 3.4µl dH_2O. Reactions were performed on a LightCycler 1.5 capillary-based thermocycler using the following conditions; initial denaturation of 10 min at 95˚C, 45 cycles 95˚C for 10s, 62˚C for 50s and 72˚C for 5s, followed by final cooling of 30s at 40˚C.. A crossing threshold (Ct) of Ct<35 was selected as a positive/negative threshold.

ii) Confirm identity of Liberibacters detected by multiple gene sequencing:

Liberibacters identified from *Teclea* and *Oricia* samples were characterized by amplifying portions of the 16S rRNA, outer-membrane protein (*omp*) and 50S ribosomal protein L10 (*rplJ*) genes. Purified amplicons per gene region were sequenced in both directions with corresponding primers. The obtained DNA sequences were compiled into different datasets along with relevant reference sequences obtained from Genbank. Reference sequences consisted of known sequences of other members within the *Liberibacter* genus, and in the case of 16S rRNA gene data, sequences from related genera for outgroup purposes. Each dataset was aligned using the online alignment tool Mafft. Following alignment, each dataset was trimmed in BioEdit to obtain equal length sequences. The best-fit substitution model for each dataset was determined by jModelTest and maximum-likelihood phylogenetic analyses was performed using MEGA software version 6.06.

To verify the nature of the tree species sampled, the extracted DNAs were subjected to DNA barcoding through amplification of two DNA barcodes for plants (*rbcL* gene, large subunit of ribulose-1,5-biphosphate carboxylase and *psbA-trnH* intergenic spacer).

2. Conduct transmission studies to assess whether Liberibacters obtained are able to infect citrus sp. and are transmissible by *T. erytreae*

i) Do graft transmission of LafC, LafCl, LafV and LafZ to sweet orange to determine if this citrus species can serve as host to these Liberibacters:

Thirty sweet orange seedlings were graft inoculated per Liberibacter sub-species. Each Liberibacter was also grafted to 5 original host replicates (LafV to *Vepris lanceolata* etc.) as controls. Additionally, for each Liberibacter subspecies, 10 sweet orange seedlings were mock-inoculated with healthy plant material to serve as negative controls.

All plants within this study were tested for the presence of a Liberibacter at three month intervals. This was achieved by extracting total DNA from each recipient seedling and performing a generic Liberibacter real-time PCR assay as previously described. These results were verified by amplification of the *rplJ* gene region using primer pair A2/J5.

ii) When it is determined that citrus can serve as a host for various Liberibacter sub-species, perform vector transmission studies to determine if Trioza erytreae can vector these Liberibacters:

Laf subspecies were not transmitted to citrus following graft inoculation studies and citrus has not yet been shown to be a host for these Liberibacters.

3. Determine sequences of Liberibacters of South African Rutaceae

i) Whole genome sequencing of LafC:

Total DNA extracted from LafC infected *Calodendrum capense* and *Moraniella calodendri* were sent to Dr. Hong Lin (USA) who performed deep sequencing using Illumina NGS technologies. The reads obtained were subject to *de novo* assembly using CLC genome software. The contigs obtained were aligned against all available Liberibacter genomes (Las, Lam and Laf). Total coverage of the LafC genome was then determined.

Additional Illumina sequencing was performed at the ARC-Biotechnology Platform on total DNA extracted from a LafC infected *Calodendrum capense* sample. The two datasets (USDA and ARC) were combined to fill additional gaps within the LafC genome.

ii) Determine variability of Laf sources (DNA extracts prepared during a survey of Liberibacters of citrus in 2006):

Microsatellite sequences within the genome of Laf were identified using MSat Commander software. 50 Primer sets were designed, flanking microsatellite sequences. To assess whether any of these sequences could differentiate between closely related Laf populations, PCR amplification was performed on Laf positive citrus sampled obtained from different cultivars as well as various geographical citrus producing areas (Pietersen *et al*., 2010).

The amplification products were run on either a polyacrylamide gel or 3% agarose gel to determine polymorphisms. Amplification products showing potential polymorphisms were sequenced to confirm that the targeted microsatellite was amplified.

iii) Develop Liberibacter variant specific detection technique

Primers specific to each African Liberibacter-subspecies were designed across the outer membrane protein (omp) sequences available for these Liberibacters using Primer blast (*ncbi.nlm.nih.gov/tools/primer-blast*). The primers were additionally designed to amplify varying amplification product sizes so a multiplex PCR can be performed to identify a number of Liberibacters within a single reaction. The primer sequences and respective amplicon sizes are presented in Table 4.2.5.1.

Each primer set was assessed for specificity by testing the primers against each known Liberibacter subspecies i.e. LafC, LafCl, LafT, LafV, LafZ, and Laf. These Liberibacters were tested in simplex and multiplexed. Amplification products were sequenced to confirm the specificity of the primer sets.

Table 4.2.5.1. Primer sequences and respective amplicon sizes developed for specific detection of the various sub-species of Laf.

Target	Forward primer	Reverse primer	Size (bp)
Laf	TCTCCGACGCGTATCAATCT	CGCGATGACACCTTAACTGC	250
LafC	TCACGGATCAAGTCCATCTG	TGCAAAAGAAGACTGCGAACG	310
LafCl	CGGTAGTCCTCACTCTTTCGTA	ATGAATCACCGAAACAGCGG	199
LafT	ACGCTATTGACGAGGGTGTT	ACGGACTCGTTCTCTACTGTAA	158
LafV	CCGCATTGAAATTCGCGGT	TAAGCATCGTCGGCGAAACA	401
LafZ	GCGCAGAAGTTGTTAGAGCG	AACACCCTCGTCAATCGCAT	543

Results and Discussion

1. Finalize study on alternate tree host species of Laf and other Liberibacters detected in indigenous Rutaceae

A total of 95 *Teclea* and 27 *O. bachmannii* specimens were sampled. A few samples displayed triozid feeding damage, similar to those made by *T. erytreae* on citrus; however, no triozid specimens were found. Sampling was conducted in the Oribi Gorge and Umtamvunu nature reserve. A positive reaction in the generic Liberibacter test was obtained for a single *Teclea* spp specimen (Accession number 13-2189) which was further analyzed.

PCR amplification of the 16S, *rplJ* and *omp* gene regions for the Liberibacter positive *Teclea* sample yielded amplification products corresponding in size to Liberibacter-positive controls. All healthy and 'no template' controls were negative. The 16S rRNA sequence obtained from the *Teclea* sample shared 99.4% nucleotide identity with Laf and its various subspecies for the 1060bp product. Compared to Las and Lam, the nucleotide homology was 98.3% and 94.7%, respectively. Phylogenetic analyses of the 16S rRNA gene confirmed that the sequence obtained from *Teclea* is more closely related to Laf and all its known subspecies than the other *Citrus* spp. infecting Liberibacter species (Fig. 4.2.5.1). While the 16S rRNA

gene of African Liberibacters are highly conserved, as previously demonstrated (Roberts *et al.,* 2015), the 16S rRNA sequence obtain from *Teclea* is found in a separate clade, albeit closely related to LafZ.

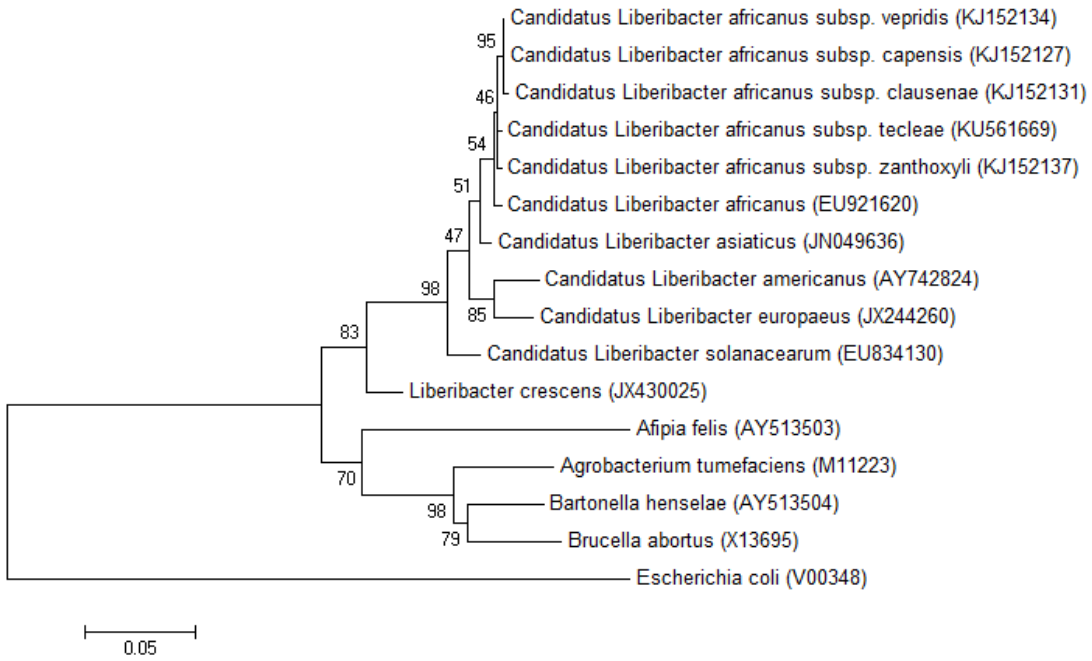

Fig. 4.2.5.1. Maximum-likelihood phylogeny based on 16S rRNA gene sequences of all members within the *Liberibacter* genus including the sequence obtained from the *Teclea gerrardii* sample examined in this study. Bootstrap support values based on 1000 replicates are indicated at branches. GenBank accession numbers are shown on the tree for sequences included in this analyses. *Escherichia coli* was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

For *omp* and *rplJ* sequences, the respective nucleotide similarities between the corresponding *Teclea* Liberibacter sequences and Laf (87.4% and 86.9%) and LafZ (89.2% and 84.8%) were greater than that for LafC (78.9% and 78.2%), LafCl (79.0% and 79.2%) and LafV (79.5% and 76.3%). Compared to Las *omp* and *rplJ* sequences, the *Teclea* Liberibacter sequences shared 73.3% and 72.0% overall sequence identity, respectively. Phylogenetic analysis of these two genes placed the Liberibacter sequence obtained from *Teclea* in a separate clade, closely related to Laf and LafZ (Fig. 4.2.5.2; Fig 4.2.5.3).

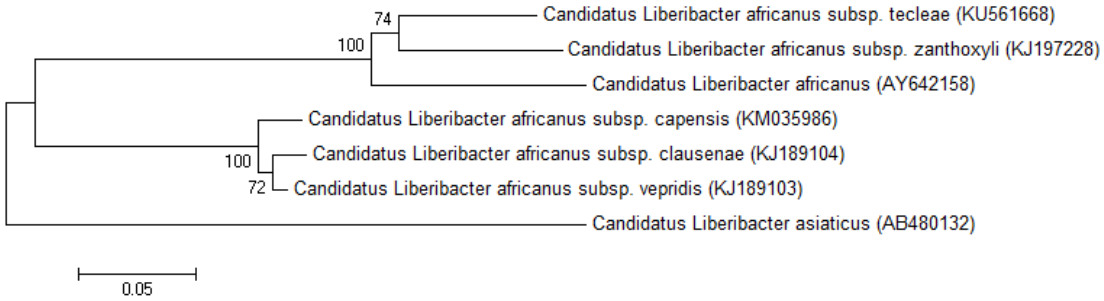

Fig. 4.2.5.2. Maximum-likelihood phylogeny based on available *omp* gene sequences of members within the *Liberibacter* genus which are associated with Rutaceae species. Bootstrap support values based on 1000 replicates are indicated at branches. GenBank accession numbers are shown on the tree for sequences included in this analyses. Bar, 0.05 substitutions per nucleotide position.

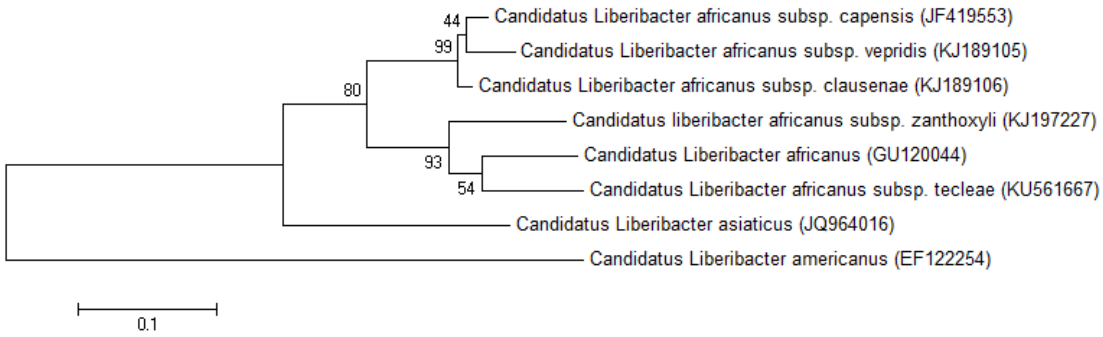

Fig. 4.2.5.3. Maximum-likelihood phylogeny based on *rplJ* gene sequences of members within the *Liberibacter* genus which are associated with Rutaceae species. Bootstrap support values based on 1000 replicates are indicated at branches. GenBank accession numbers are shown on the tree for sequences included in this analyses. Bar, 0.01 substitutions per nucleotide position.

To confirm the identity of the *Teclea* host from which the novel Liberibacter sequences were obtained, all samples collected were subjected to DNA barcoding by sequencing *rbcL* and *psb-trnH* gene region. The *rbcL* gene for all samples collected was successfully amplified, however this gene sequence could not resolve between *O. bachmannii* and the two *Teclea* species known to occur is South Africa (i.e. *T. natalensis* and *T. gerrardii* I. Verd) (Fig. 4.2.5.4). Of the 122 samples subjected to DNA barcoding of the *psb-trnH* gene, only 94 samples were successfully sequenced, with 14 putative *O. bachmannii* and 14 putative *Teclea* spp. failing to amplify. Phylogenetic analyses of this plastid gene region resolved various closely related Rutaceae species within the *Oricia, Teclea* and *Vepris* genera into separated clades (Fig. 4.2.5.5). Based on these results, it was shown that the tree host of the Liberibacter positive sample studied here is *T. gerrardii.*

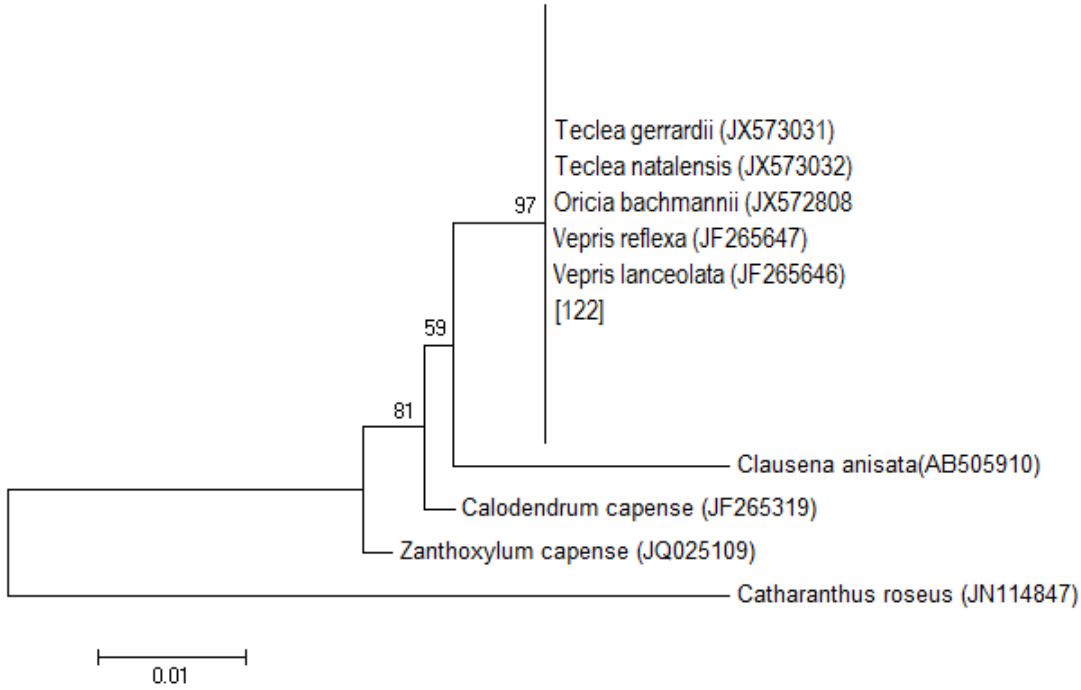

Fig. 4.2.5.4. Maximum-likelihood phylogeny of tree host species based on *rbcL* sequences obtained from all *Oricia* and *Teclea* samples collected for this study. Bootstrap values based on 1000 replicates are indicated at branch nodes. Branches with >70% bootstrap support for terminal taxa were collapsed. The 122 specimens which were successfully sequenced are indicated in brackets. Bar, 0.01 substitutions per nucleotide position.

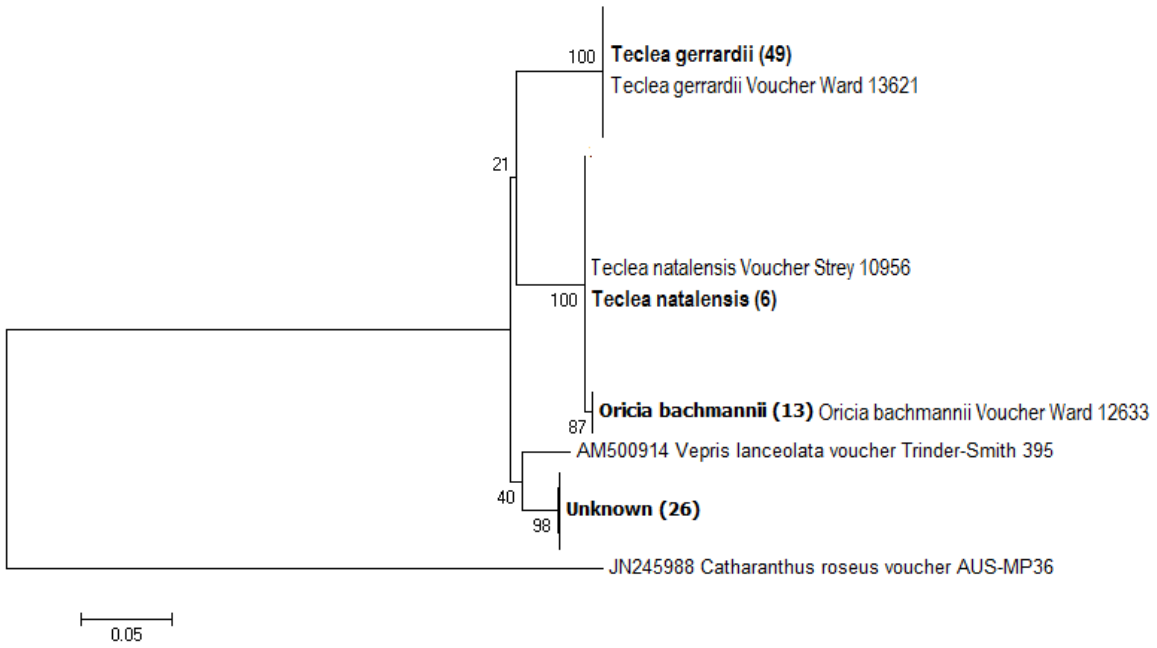

Fig. 4.2.5.5. Maximum-likelihood phylogeny of tree host species based on *psb-trnH* sequences obtained from *Oricia* and *Teclea* samples collected in this study (indicated in bold) as well as voucher specimens representing the tree species studied obtained from the South African National Biodiversity Institute (SANBI).). Bootstrap support values based on 1000 replicates are indicated at branch nodes. Branches with >70% bootstrap support for terminal taxa were collapsed. The number of specimens sequenced per tree species is indicated in brackets. Bar, 0.05 substitutions per nucleotide position.

The percentage nucleotide identity of the 16S rRNA sequence obtained for the single Liberibacter positive *T. gerrardii* sample, conforms to the >99% nucleotide identities found amongst previously characterized Laf subspecies (Garnier *et al.,* 2000; Roberts *et al.,* 2015). From the overall sequence similarity described here and the phylogenies for all gene regions studied, it is apparent that the sequences obtained from the single Liberibacter positive *T. gerrardii* represents a novel Liberibacter sequence closely related to Laf and its subspecies. In maintaining previous convention, we therefore propose that the Liberibacter obtained from *Teclea* also be assigned subspecies status under the proposed name of '*Candidatus* Liberibacter africanus subsp. tecleae' (LafT).

2. Conduct transmission studies to assess whether Liberibacters obtained are able to infect *Citrus* sp. and are transmissible by *T. erytreae.*

LafCl, LafV and LafZ positive material was collected from Knysna and LafC infected material was collected from Springbok park, Pretoria. Bark strips of the respective Liberibacter sources were graft inoculated onto 30 Madam Vinous sweet orange recipient trees per Liberibacter tested. Five original Rutaceae hosts was graft inoculated with each of its corresponding Liberibacter (i.e LafV to *Vepris lanceolata*) and 10 Madam Vinous seedlings per Liberibacter subspecies was mock inoculated with bark strips from healthy Rutaceae hosts. Samples were monitored for graft-take and symptom development.

One-year post inoculation (PI), 12 LafCl infected, 14 LafV infected and 5 LafZ infected recipient Madam Vinous seedlings died, whereas all LafC inoculated trees remained healthy. Total DNA was extracted from the living recipient trees and tested for the presence of Liberibacters as described above. None of the Liberibacter recipient Madam Vinous seedlings tested positive for Liberibacters. Similarly, all positive controls also remained negative for Liberibacters one-year PI.

In order to repeat graft transmission experiments using larger sample size, 1000 Madam Vinous sweet orange seeds were obtained. These seeds were planted in insect-free growth chambers at ARC-PPRI. After 3 months, only 28 seeds germinated. Additionally, seeds from *C. capense, V. lanceolata* and *Z. capense* were obtained. Only *V. lanceolata* and *C. capense* seeds germinated.

In an attempt to establish Liberibacter-subspecies positive material at ARC-PPRI, samples of *Clausena anisata, Vepris lanceolata* and *Teclea natalensis*, which previously tested positive for LafCl, LafV and LafT, respectively, were collected from Natal and an attempt was made to make root cuttings from these trees.

Additionally, bark strips from these trees were grafted onto periwinkles and well as 12, 5 and 7 citrus seedlings with LafCl, LafT and LafV, respectively. None of the cuttings rooted and the sources were therefore not established. None of the periwinkle plants tested positive for any Liberibacter sp. One-year PI. Of the citrus recipients, 7 LafCl inoculated-, 2 LafV inoculated- and 1 LafT inoculated citrus recipients died suddenly. In an attempt to determine whether Liberibacter sequences could be detected from these seedlings, DNA was extracted from the roots. A single LafCl inoculated citrus tree tested positive for a Liberibacter in real-time PCR analysis; however, these results could not be duplicated with a conventional A2/J5 PCR. None of the remaining citrus trees tested positive for Liberibacters.

3. Determine sequence of Liberibacters of South African Rutaceae

i) Whole genome sequencing of LafC

Previously identified LafC positive samples were subjected to total DNA extraction followed by real-time PCR analyses to determine the ratio of LafC DNA to host DNA within the samples. Based on these results, samples identified with high LafC concentration were submitted for Illumina sequencing at both the ARC-Biotech Platform and the USDA.

A total of five pairwise runs were performed on a single sample at ARC-PPRI. Adaptor sequences were trimmed from each dataset using CLC genomics. The amount of reads obtained from these runs after trimming were as follows: Run1: 32 875 674; Run2; 7 977 414; Run3: 7 977 962; Run4: 7 978 920; Run5: 961 684. Each dataset was mapped against the whole genome of Laf (GenBank accession: CP004021) under highly stringent conditions. The amount of reads mapped were extremely low i.e. Run1: 0.11%, Run2: 0.11%, Run3: 0.10%, Run4: 0.11% and Run5: 0.10%. All read mappings were merged and a consensus sequence was obtained from these merged mappings.

The Illumina data obtained from the USDA were processed similarly to the ARC-BTP data. Three runs were performed on three separate samples at the USDA; however, only two of the datasets received could be imported into CLC genome for processing. The amount of reads obtained from the two remaining datasets were as follows: USDA_Run1: 146 424 701 and USDA_Run2: 145 342 244. Of these two runs only 0.02% and 0.03% of reads mapped against Laf genome under highly stringent settings. These reads were merged and a consensus sequence was obtained.

Mapped reads from both ARC and USDA runs were merged and a single consensus sequence was obtained from these merged reads. All three consensus sequences were aligned with the whole genome of Laf, but this action could not be completed as the current computer used for CLC genomics does not possess the necessary computational capabilities required to align large genomes.

ii) Determine variability of Laf sources (DNA extracts prepared during a survey of Liberibacters of citrus in 2006):

Simple sequence repeat (SSR) markers were used to determine genetic variation within Liberibacter populations from South Africa. The study was modelled on the population genetic studies done with '*Candidatus* Liberibacter asiaticus'. Ronel Roberts spent 6 weeks (12 May-21 June 2014) at the USDA, San Joaquin Valley, CA, USA, where initial testing of a SSR system for the detection of Laf populations was developed. A total of 30 primer pairs targeting potential SSR markers within the Laf genome were designed. These primers were screened using polyacrylamide gel-electrophoresis to determine which of the primers were capable of differentiating between Laf sources from South Africa. Only 3 primer pairs were shown to effectively differentiate Laf populations.

An additional 20 primer sets were designed flanking microsatellite sequences within the Laf genome. Only two primer sets were shown to be polymorphic following gel electrophoresis and sequencing. The five polymorphic primer sets indicated in Table 4.2.5.2 were identified for samples obtained from different citrus types suggesting that geographical distribution may have a limited effect on the formation of distinct Laf populations, but that the citrus host may play a greater role in influencing pathogen diversity.

Table 4.2.5.2. Five SSR primers that detect polymorphisms in the Laf genome

Primer	Forward (5'-3')	Reverse (5'-3')	Repeat Motif
Laf-SSR1	CTTGGGATTTTGGAGCTTCAGG	GAGTGGTACGCACGTATACTATAC	AT
Laf-SSR8	AATCCATCTCCTATCTCCTTAACC	GTTGTACTTTGGCGATGAAGC	GAGTT
Laf-SSR9	AACCAGAACACAATGATATAATACC	CCACCCACAGTATCTACAGG	CAT
Saf_14	GCCTCCGTTTGGAGTATTGG	AGTCTGCCAGGTGATATTGAAG	GAATAA
Saf_6	GGGTGACTATAGCCCACAAG	CTGTTTGGTCTCCCGGTTTG	TATGAG

iii) Develop Liberibacter variant specific detection technique

Liberibacter subspecies specific primers were shown to specifically detect the correct target Liberibacter in both single infection samples and in sample mixtures. No cross-reactions were obtained with the primer sets apart from those for Laf-specific detection as seen in Fig 4.2.5.6. A faint amplification product was obtained with the LafCl sample. PCR amplicons were sequenced to confirm Liberibacter specificity. Primers LafC, LafCl, LafT, LafV and LafZ were shown to detect the intended targets, even in sample mixtures. This was also shown to be the case for primer Laf. The LafCl sample which was amplified with the Laf-specific primer set, was shown to be a Laf sequence. This sequence was then aligned with all know Liberibacter *omp* sequences and was shown to be more related to Laf *omp* sequences than LafCl (Fig. 4.2.5.7). This specific sample should be further investigated to determine whether it contains multiple Liberibacters.

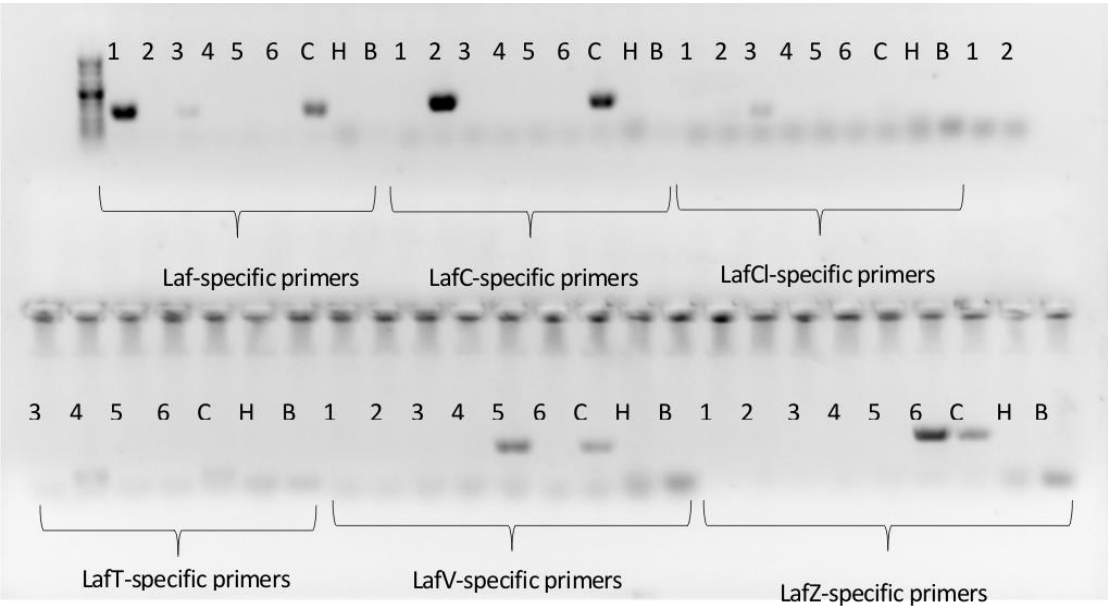

Fig. 4.2.5.6. Gel electrophoreses image indicating the specificity of Liberibacter sub-species specific primer sets. The samples are: 1: Laf positive DNA from citrus, 2: LafC positive DNA from *C. capense*, 3: LafCl positive DNA from *C. anisata*, 4: LafT positive DNA from *T. gerrardii*, 5: LafV positive DNA from *V. lanceolata*, 6: LafZ positive DNA from *Z. capense*, C: DNA sample containing DNA of all six Liberibacters identified in South Africa, H: Healthy citrus control, B: No template control. Specific PCR tests are indicated.

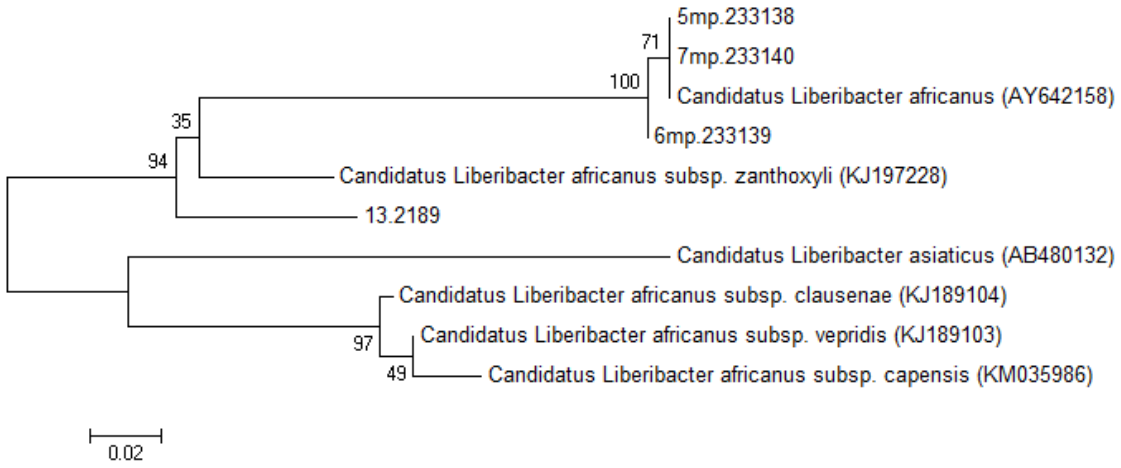

Fig. 4.2.5.7. Maximum likelihood phylogeny of Liberibacter *omp* sequences corresponding to Laf-specific primer amplification product. LafCl sample amplified is denoted as 6mp.233139 whereas Laf simplex and combined samples are denoted as 5mp.233138 and 7mp.233140, respectively.

Conclusions

With the addition of LafT, there are five subspecies of Laf that have been identified from South Africa. It is important to fully characterize various biological properties of these liberibacter subspecies i.e. vector and host range to fully understand the possible impact these Liberibacters may have on commercial citrus. Additional sequence data for LafC, LafCl, LafV, LafT and LafZ can also help clarify the exact taxonomic position of the various subspecies in relation to Laf and give further insight into the genetic divergence.

It is still unknown whether any of the Liberibacter subspecies associated with indigenous Rutaceous hosts are capable of infecting commercial citrus species. It is not certain whether LafCl and LafV were responsible for the sudden death of 'Madam Vinous' host plants in the glasshouse trial.

The whole genome sequence of LafC has not yet been determined, although additional sequence data has been obtained for LafC in areas where reads mapped against the Laf genome. Mapping results indicate that LafC and Laf is less homologous than previously thought (99.0% sequence identity for 16S gene). Completion of the whole genome of LafC will be informative as to whether LafC represents a haplotype of Laf or a different species. It is also important to note that DNA preparations for NGS should contain high concentration of the target DNA. Only a few reads (less than 0.1%) mapped to Laf in the current analysis using sample prepared from the host plant. DNA obtained from the vector of LafC should yield better NGS results.

SSR data indicate that Laf populations from South Africa are conserved. Fifty microsatellite markers were identified of which only 5 were polymorphic. In similar studies on '*Candidatus* Liberibacter solanacearum' populations in the USA, 8 polymorphic markers could be identified from 40 microsatellite markers (Lin et al., 2012). Despite the low diversity observed, it would appear that Laf diversity in South Africa is influenced by the citrus type as a host, rather than geographical origin. Different citrus types are ranked according to the severity of Laf infections with sweet orange mandarin and tangelo being severe, grapefruit, sour orange and lemon being ranked as moderate and lime, trifoliate orange and pomelo being tolerant. These differences in severity can possibly be due to the association of different genetic Laf populations with different citrus types over internal defense mechanisms.

The current PCR tests available for the detection of Liberibacters are flawed as the 16S, *rplJ* and *omp* tests commonly used amplifies all Liberibacters from South Africa resulting in a bias towards the Liberibacter present in the highest concentration. A Liberibacter-subspecies specific test was developed based on the outer membrane protein (*omp*) gene. It was demonstrated that the primers were specific and do not cross-react with the other sub-species. A single *Clausena anisata* used to optimize these test was found to be infected with Laf and LafCl. This is the first report of an indigenous Rutaceae species being infected with a combination of Liberibacters.

The Liberibacter studies discussed herein addressed various aspect pertaining to both biological and molecular properties of the Liberibacters identified from South Africa. It is apparent that the host range and

molecular diversity of Liberibacters in South Africa is greater than previously known. By studying the association of Liberibacters within indigenous Rutaceae hosts, we hope to understand how Laf came to be a pathogen of citrus. It will be important to ascertain whether Liberibacters from indigenous Rutaceae species can be transmitted to commercial citrus and whether they are pathogenic to citrus. Understanding the differences in host specificity between Laf and the other Liberibacter sub-species at the genomic level can lead to the development of novel control strategies. Different genetic populations of Laf were shown to exist and were associated with different citrus types. Should differences in virulence be shown, the different genetic Laf isolates can potentially be exploited in a cross-protection program; however, more research is needed to determine the validity of such an approach.

Technology transfer

Peer reviewed Scientific Articles

KOGENARU, S., YAN, Q., RIERA, N., ROPER, C., DENG, X., EBERT, T., ROGERS, M., IREY, M., PIETERSEN, G., RUSH C., AND WANG, N., 2014. Repertoire of novel sequence signatures for the detection of *Candidatus* Liberibacter asiaticus by quantitative real-time PCR. *BMC Microbiology* 14(1):39

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ROBERTS, R, STEENKAMP, E. T., AND PIETERSEN, G., 2015. Three novel lineages of '*Candidatus* Liberibacter africanus' associated with native rutaceous hosts of *Trioza erytreae* in South Africa. *International Journal of Systematic and Evolutionary Microbiology* 65:723-731

Oral Presentation

ROBERTS, R., AND PIETERSEN, G., 2014. Alternative hosts of '*Candidatus* Liberibacter africanus' in South Africa: Continuing the search. 8th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 17-20 August, 2014.

Further objectives and work plan

Transmission studies to citrus of the Liberibacter sub-species will continue in project 1157.

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4.2.6 PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations for the Orange River Valley

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

Summary

Disease expression of *Citrus tristeza virus* (CTV) is influenced by the CTV strains present, the specific citrus variety and climatic conditions. Although CTV disease symptoms such as stem pitting or decline may not always be visible in all citrus varieties, different CTV sources do influence tree growth and yield. CTV sources (SM46, SM47, SM48, SM49) derived from various good-performing, sweet orange trees, were used to inoculate virus-free 'Delta'-, 'Midknight'-, and 'Turkey' Valencia on C35 citrange rootstocks. These sources are compared to LMS6 (standard CTV pre-immunisation source for sweet oranges) and virus-free controls. The trees were planted in the Kakamas area in the Northern Cape province in 2007. Trees were evaluated annually and tree canopy volume and yield per tree determined. This season was the eighth year after planting and crop production of all the trees in the trial remain poor. Yields are too small to draw any conclusions regarding the effect of the various CTV sources on production. The C35 rootstock is not the recommended rootstock for the area and this is likely the reason for the low production, but the influence of the different CTV sources are nonetheless evident on tree growth. Trees of all three cultivars inoculated with SM48 were noticeably smaller than those of other treatments. The LMS6 CTV source had a slight impact on the growth of the 'Delta' trees compared to trees planted virus-free, but had no significant influence on 'Midknight' or 'Turkey' tree growth. The grower has requested removal of the trial as the site is now designated for production purposes. The next season will therefore be the last evaluation of these trees. Results thus far do not indicate any detrimental impact with the LMS6 source, the standard CTV pre-immunisation source for sweet oranges. None of the other CTV sources gave better results than LMS6.

Opsomming

Simptoom uitdrukking van *Citrus tristeza virus* (CTV) word beïnvloed deur verskillende CTV rasse en verskil ook tussen sitrus kultivars. Verskillende klimaatstoestande beïnvloed ook CTV simptoom uitdrukking en alhoewel simptome soos stamgleuf nie altyd sigbaar is op alle sitrus variëteite nie, word boom groei en opbrengs wel beïnvloed deur CTV. Dit is dus nodig om verskillende CTV bronne in verskillende sitrus produserende streke te evalueer. Potensiële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemoenbome versamel is (SM46, SM47, SM48, SM49), is gebruik om virusvrye

‘Delta’-, ‘Midknight’-, en ‘Turkey’ Valencia op C35 citrange onderstam te preïmmuniseer. Hierdie bronne word met LMS6 (die standaard preïmmuniseringsbron vir soetlemoene) vergelyk, asook met bome wat virusvry geplant is. Preïmmunisering is deur middel van ELISA bevestig, waarna die boompies gedurende September 2007 in die Kakamas omgewing in die Noord-Kaap geplant is. Die boomgroottes is vir 8 jaar na uitplant gemeet tesame met jaarlikse oesopbrengs. Opbrengste is te klein om enige gevolgtrekkings te maak. Die C35 onderstam word nie aanbeveel vir die area nie en kan die rede wees vir die lae produksie, maar die invloed van die CTV bronne is wel sigbaar op die boom grootte. Bome met SM48 was in al drie kultivars merkbaar die kleinste. Die LMS6 CTV bron het ŉ klein invloed op die ‘Delta’ boomgroei gehad in vergelyking met die wat virusvry geplant is, maar het geen betekenisvolle invloed op die ‘Midknight’ en ‘Turkey’ se groei gehad nie. Die produsent het versoek om die proefblok te verwyder omdat die boord geoormerk is vir kommersiële produksie. Die volgende seisoen sal dus die laaste jaar van evaluasie wees. Resultate dui aan dat die huidige kruisbeskermings bron, LMS6, geen skadelike invloed getoon het in hierdie proef nie. Nie een van die ander CTV bronne het beter resultate gelewer as LMS6 nie.

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

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

Summary

‘Turkey’ Valencia is an early Valencia type and an important component of the citrus export portfolio. There were indications that the variety might be more sensitive to CTV as bud-union crease was noted with certain rootstocks. It was therefore important to determine whether LMS6, the standard CTV cross-protection source for sweet oranges, is the most suitable CTV source for this variety. Virus-free scions were budded to ‘Troyer’ citrange rootstocks and inoculated with various CTV sources including LMS6 (standard), SM46, SM47, SM48 and SM49, previously obtained from good-performing sweet orange trees. Trees inoculated with GFMS12 and virus-free trees were included as controls. The trial was established in the Malelane area in Mpumalanga province in 2007. Trees are evaluated annually and tree canopy volume and yield per tree determined. This season was the eighth year after planting. Substantial fruit was lost due to baboon foraging and trees were not harvested during the 2015 season. Tree canopy volumes were determined. Significant stunting was induced by the SM48 CTV source. This was similarly found in a trial in the Orange River Valley where SM48 was associated with stunting of ‘Delta’- ‘Midknight’- and ‘Turkey’ Valencia trees (Project 739). Results do not indicate any negative impact with the LMS6 source, the standard CTV pre-immunisation source for sweet oranges. None of the new CTV sources performed better than the LMS6 source. The next season (2016) will be the last evaluation of these trees.

Opsomming

‘Turkey’ Valencia is ŉ vroeë kultivar en speel ŉ belangrike rol in die sitrus uitvoer mark. Daar was aanduidings dat ‘Turkey’ Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is. Dit is dus belangrik om te bepaal of LMS6, die standaard preïmmuniseringsbron vir soetlemoene, ŉ geskikte CTV bron is vir ‘Turkey’. Virusvrye ‘Turkey’ op ‘Troyer’ citrange onderstam is in ŉ glashuis voorberei en met die CTV bronne, LMS6 (standaard), SM46, SM47, SM48, SM49 (almal vanaf soetlemoene versamel), geïnokuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS12 bron geïnokuleer is en virusvrye bome is onderskeidelik as positiewe en negatiewe kontroles ingesluit. Preïmmunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 in die Malelane omgewing geplant. Die bome word jaarliks geëvalueer vir boomgrootte en oes-opbrengs. Hierdie was die agste jaar van evaluasie. As gevolg van verlies van vrugte veroorsaak deur bobbejane, is die bome nie geoes tydens die 2015 seisoen nie. Boomgroottes is bepaal en betekenisvolle verdwerging is deur die SM48 CTV bron veroorsaak; hierdie is ook in ‘Delta’- ‘Midknight’ en ‘Turkey’ Valencia bome gevind in ŉ proef wat in die Oranjerivier Vallei geplant is (projek 739). Resultate dui aan dat die huidige kruisbeskermingsbron, LMS6, geen negatiewe invloed op soetlemoene het nie. Nie een van die nuwe CTV bronne het beter as die LMS6 bron presteer nie. Die volgende seisoen sal die laaste jaar van evaluasie wees.

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

Project 1100 (2014/15 – 2016/2017) by G. Cook (CRI), T. Jooste (US), Marike Visser (US), S.P. van Vuuren (CRI), C. Steyn (CRI), J.H.J. Breytenbach (CRI), J.T. Burger (US) and H.J. Maree (US)

Summary

This project purposes to firstly identify the required components for cross-protection and also characterise CTV strains to enhance the understanding of the CTV complexity found within South Africa. Additionally, we aim to develop new diagnostic capabilities in terms of CTV strain identification and explore the application of Next Generation Sequencing (NGS) for diagnostics in citrus viral pathology. A glasshouse trial using two commercial grapefruit varieties, 'Star Ruby' and 'Marsh', is underway to evaluate the influence of specific CTV strains in single or mixed infections on stem-pitting and a further challenge trial is in progress to test the 'cross-protection' ability of various strains used in the first trial. The CTV sources applied in these studies were characterised and full genome sequences generated. Results obtained with two trial evaluations suggest possible interactions between the different strains as diminished symptom expression was seen in some treatments containing strain mixtures. An additional single-strain source of T3 was isolated and the full-genome characterised with NGS. This isolate will be used as the challenge strain in the second trial. The relative quantitative analysis of the various CTV strains in the 'Marsh' and 'Star Ruby' trial plants has not been completed, but the tests have been developed and the extractions prepared. A bio-informatic pipeline for virus diagnostics based on NGS data was optimized and packaged in a user-friendly interface named Truffle (http://truffle.sourceforge.net). The software can design e-probes based on user-defined virus targets, or be used with preloaded probes. This work will be of great benefit for the exchange of citrus material between citrus repositories once certain standards and methodologies are in place and has the potential to fast track introductions by replacing certain biological processes.

Opsomming

Hierdie projek is daarop gemik om eerstens die nodige komponente vir kruis-beskerming te identifiseer asook om CTV rasse te karakteriseer om die CTV ras populasies in Suid-Afrika beter te verstaan. Verder beoog ons om nuwe diagnostiese vermoëns te ontwikkel in terme van CTV ras identifikasie en ook die toepassing van Metagenomiese volgende-generasie volgordebepaling (NGS) vir diagnostiese doeleindes in sitrus virale patologie te ondersoek. 'n Glasshuis proef met twee kommersiële pomelo variëteite, 'Star Ruby' en 'Marsh', is onderweg om die invloed van sekere CTV rasse, beide enkele en gemengde infeksies, op stamgleuf te ondersoek. 'n Tweede proef evalueer die "kruis-beskermings" vermoë van verskillende rasse wat in die eerste proef gebruik word. Die CTV bronne gebruik in hierdie studie is gekarakteriseer en vol-genoom basisvolgordes is gegenereer vir elkeen. Voorlopige resultate van die pomelo-proef dui op moontlike interaksies tussen die verskillende rasse, vanweë verlaagde simptoom uitdrukking wat waargeneem is in behandelings van sekere ras-mengsels. 'n Bykomende enkel-ras bron van T3 is geïsoleer en die vol-genoom basisvolgorde bepaal met NGS. Die isolaat sal gebruik word in die tweede proef as die strawwe ras. Die relatiewe kwantifisering van die verskillende CTV rasse in die 'Marsh' en 'Star Ruby' proef plante is nog nie voltooi nie, maar die toetse is ontwikkel en die ekstraksies voorberei. 'n Bio-informatiese pyplyn vir virus diagnose, gebaseer op die NGS data is geoptimiseer en verpak in 'n gebruikersvriendelike program, genoem Truffle (http://truffle.sourceforge.net). Die sagteware kan 'e-probes' ontwerp wat gebaseer is op gebruiker-gedefinieerde virus teikens, of gebruik word met bestaande 'probes'. Hierdie werk sal tot groot voordeel van die uitruil van sitrus materiaal tussen sitrus genebronne wees sodra sekere standaarde in plek gestel is. Die tegnologie het die potensiaal om vinniger vrystelling van nuwe sitrus materiaal te bewerkstellig deur sekere biologiese prosesse te vervang.

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

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

Summary

Budwood was collected from 108 superior grapefruit trees from different grapefruit production areas of southern Africa to obtain mild CTV sources for cross-protection studies. These CTV sources were established in the glasshouse at CRI and inoculated to virus-free 'Mexican' lime indicator plants to evaluate the severity of the CTV sources. Nineteen sources were selected for further evaluation and were inoculated again to Mexican lime plants and compared to CTV sources; GFMS12, GFMS35, GFMS12-7, GFMS12-9,

and four Beltsville sub-isolates (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). The 'Mexican' lime plants were evaluated for growth and stem pitting and virus titre was determined by ELISA. The four most promising field sources, Tabankulu 1, New Venture 41-2, ORE 8 and Tshipise 19-5, indexed free for citrus viroids and are evaluated as pre-immunising agents for Marsh and Star Ruby trees in field trials. The sources are compared to GFMS12, previous standard CTV cross-protection source for white grapefruit at the time, GFMS35, current standard cross-protection source for all grapefruit, as well as the four best Beltsville sub-isolates; B389-1, B389-4, B390-3 and B390-5, the ITSC sub-isolates; GFMS 12-7 and GFMS 12-9 and virus-free controls. The Star Ruby trees were planted in the Letsitele area in Limpopo province and the Marsh trees in the Malelane area in Mpumalanga province in 2007. This was the fifth crop from the Marsh trees and the third crop for Star Ruby. Both the Marsh and Star Ruby trees inoculated with GFMS12 developed severe stem-pitting resulting in suppressed tree growth and smaller fruit. Star Ruby and Marsh trees containing the sub-isolated sources (single-strain sources) generally performed better than trees inoculated with the field sources, including the current grapefruit cross-protection source, GFMS35. CTV source B390-5 was associated with good tree size, higher yields and good fruit size in both Marsh and Star Ruby and shows the most potential for further assessment.

Opsomming

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

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

Project 968 (2004 - 2020) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

During re-indexing of the Citrus Foundation Block mother trees in 2003 it was found that many Clementine and mandarin trees did not contain CTV despite pre-immunisation with the LMS6 CTV source. This caused concern as the budwood that was multiplied from these mother trees and supplied to the commercial nurseries, were virus-free, rendering the trees unprotected against natural CTV infection with severe strains introduced by aphids. A change to another CTV source compatible with mandarin types was required. The GFMS12 CTV source was approved for pre-immunisation in the interim until a suitable CTV pre-immunising source for soft citrus is identified. A glasshouse trial was conducted in 2006 to evaluate additional CTV sources in four different soft citrus cultivars. The current field trials are extensions of the glasshouse trial. Trees of two Clementine selections ('Clemenluz', 'Esbal') and two mandarin selections ('Valley Gold', 'Morr 22') on Troyer citrange rootstock have been prepared and pre-immunized with different CTV sources; i.e. CTVSC, SM47, SM48 and SM49. Trees with these sources will be compared to trees that were pre-immunised with GFMS12 (standard) and trees planted virus-free. Pre-immunisation was confirmed by means of ELISA and the trees were planted during 2010/11 at two localities in different climatic regions suitable for the production of soft citrus, i.e. Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Trees at both trials sites were lost due to frost and poor drainage one year after planting.

New trees were prepared and the trials re-planted at two new sites. The first trial was planted in the Citrusdal area in December 2012 and the second was planted in spring 2014 at Burgersfort. Tree growth was recorded at the Citrusdal trial this season, but not at Burgersfort. Although tree size differences were observed in 'Clemenluz' and 'Esbal' with various CTV sources, it is only the third year after planting and too early to draw any definitive conclusions. No differences in growth was observed between the treatments in the 'Valley Gold' and 'Mor 22' varieties.

Opsomming

Tydens die her-indeksering van die Grondvesblok se moederbome gedurende 2003 is gevind dat 'n groot aantal Clementine en mandaryn bome geen CTV bevat het nie ten spyte van preïmmunisering met die LMS 6 bron. Dit het kommer gewek dat enthout, wat aan die kommersiële kwekerye verskaf word, virusvry is en nie beskerming bied teen natuurlike CTV rasse wat in die veld deur plantluise oorgedra word nie. Die GFMS 12 CTV bron is goedgekeur om tydelik gebruik te word tot 'n geskikte bron vir sagte sitrus gevind is. '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 seleksies ('Clemenluz', 'Esbal') en twee mandaryn hibried seleksies ('Valley Gold', 'Morr 22') is op 'Troyer' citrange onderstamme ge-okuleer en gepreïmmuniseer met verkillende CTV bronne, nl. CTVSC, SM47, SM48 en SM49. Bome met hierdie bronne word met GFMS12 (standaard) en bome wat virusvry geplant is, vergelyk. Nadat preïmmunisering deur middel van ELISA bevestig is, is die bome gedurende 2010/11 in twee verskillende klimaatstreke, wat vir sagte sitrus geskik is, geplant (Groblersdal in Mpumalanga en Citrusdal in die Wes Kaap). As gevolg van dreineringsprobleme en koue skade is proefbome in beide persele verloor. Nuwe bome is voorberei en gedurende Desember 2012 in die Citrusdal omgewing geplant en gedurende die lente van 2014 is die proef te Burgersfort geplant. Groei data is by die Citrusdal proefbome geneem maar nie by Burgersfort nie. Alhoewel daar betekenisvolle verskille was tussen die bronne in 'Clemenluz' en 'Esbal', is dit nog te vroeg om enige gevolgtrekking te maak. Geen verskille was waargeneem tussen behandelings in 'Valley Gold' en 'Mor 22' nie.

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

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

Summary

Some cultivar owners and agents claim that the use of old clone material of cultivars is more profitable than that of Citrus Improvement Scheme [Citrus Foundation Blok (CFB)] material. CFB material has been cleaned from all graft transmissible agents by shoot tip grafting and thereafter inoculated with an approved *Citrus tristeza virus* (CTV) source for cross-protection. The objective of this study is to compare tree and fruit characteristics of shoot tip grafted material with that of old clone material. Three cultivars are involved; *viz.* Benny Valencia, Cambria navel and Glen Ora Late navel. Budwood was collected from original sources of the cultivars and budded according to normal nursery practices to Swingle citrumelo, Carrizo citrange and C35 citrange rootstocks. The same was done with material of the three cultivars that was obtained from the CFB. Strict sterilisation measures of cutting tools were maintained during budding. The CTV and Citrus viroid (CVd) status of all the budwood sources were established by reverse transcription polymerase chain reaction (RT-PCR). All the sources originating from the CFB is supposed to have the same CTV strains than the LMS6 cross-protecting source; *viz.* T68, HA16-5 and two RB variants. It was found that Benny Valencia 2 only had the HA16-5 strain, which is just a part of the complex, while the Cambria 3 navel and the Glen Ora Late navel were virus-free. Treatments containing material from the CFB were re-inoculated with the LMS6 CTV source. The original Cambria material from Baviaanskloof, which was used to make trees of this treatment, was found to be free of CEVd. Trees of the treatment containing the original Cambria source were re-inoculated with the original material from Dunbrody Estates. CVd-IIa was found to be absent in the Baviaanskloof and Dunbrody Cambria sources used and could not be used evaluate the effect of CVd-IIa. Trees in the treatment containing CVd-IIa were re-inoculated with CVd-IIa from the OR 4 source. All the re-inoculations delayed the planting of the trees. Planting of the trees was further delayed by drought conditions. One trial was planted at Burgersfort during January 2016.

Opsomming

Sommige kultivar eienaars en agente maak aanspraak daarop dat ou kloon materiaal meer winsgewend is as Sitrus Verbeteringskema materiaal vanaf die Grondvesblok (GVB). GVB materiaal is skoongemaak van alle entoordraagbare siektes deur middel van groeipunt-enting en is daarna geïnokuleer met 'n goedgekeurde *Citrus tristeza virus* (CTV) bron vir kruisbeskerming. Die doel van die studie is om boom en vrug eienskappe van GVB materiaal met die van ou kloon materiaal te vergelyk. Drie kultivars is betrokke,

nl. Benny Valencia, Cambria nawel en Glen Ora Late nawel. Okuleerhout is van oorspronklike bronne van die kultivars versamel en volgens normale kwekery praktyke op Swingle citrumelo, Carrizo citrange en C35 citrange onderstamme ge-okuleer. Dieselfde is gedoen met materiaal wat vanaf die GVB ontvang is. Streng sterilisasie voorsorgmaatreëls van snygereedskap is gevolg tydens okulering. Die CTV en sitrus viroïed (CVd) status van al die enthout bronne is bepaal deur middel van polimerase kettingreaksie (PKR). Al die soetlemoen enthout bronne vanaf die GVB is veronderstel om dieselfde CTV rasse as die oorspronklike LMS6 kruisbeskermingsbron te hê, nl. T68, HA16-5 en twee RB variante. Dit is gevind dat Benny Valencia 2 slegs die HA16-5 ras bevat het, wat slegs ŉ gedeelte van die kompleks is, terwyl Cambria 3 nawel en die Glen Ora Late nawel albei virusvry was. Al drie die behandelings is weer geïnokuleer met die LMS6 CTV bron. Die ou kloon Cambria materiaal van Baviaanskloof, wat as bron vir die behandeling gebruik is, is vry van CEVd gevind. Bome wat van hierdie materiaal gemaak is, is weer geïnokuleer met ou kloon materiaal afkomstig van Dunbrody Estates. CVd-IIa was afwesig in die Baviaanskloof en Dunbrody bronne en die gebruik van die twee bronne was daarom nie geskik nie. Bome met die CVd-IIa behandeling is geïnokuleer met CVd-IIa vanaf die OR 4 bron. Al die her-inokulasies asook die droogte toestande het die projek vertraag. Een proef is gedurende Januarie 2016 geplant.

4.3 PROGRAMME: FRUIT AND FOLIAR DISEASES

Programme coordinator: G.C. Schutte (CRI)

4.3.1 Programme summary

No field trials could be conducted because the last mandarin orchard in the Lowveld has been removed. More can be done to alter spray programmes to cater for RB1 and RB2 in current strobilurin programmes as replacement for mancozeb (4.3.2).

Due to Citrus black spot growers tend to use high application fungicide volumes ranging from 6000 to 12000 L ha^{-1}. The potential of reduced volume applications has been shown in various previous studies but this potential must be proven through seasonal bio-efficacy trials. Various machines have been evaluated for this study. Reduced volume applications (1000 to 4000 L ha^{-1}) were performed with the Martignani and Cima sprayers. Results showed that conventional machines did realise better deposition uniformity throughout the canopy, indicating the importance of matching the sprayer profile to the canopy and also the role of proper canopy management in reduced volume application (4.3.3). Bio-efficacy trials were performed on Delta Valencia orchard near Marble Hall. A modified spray programme consisting of four fungicide and pesticide applications have been made from October to January. Sprays were applied with a Cima and ATASA sprayer at 2000 L ha^{-1} (at 3.5× concentration) and a Jacto at 7000 L ha^{-1} at 1×. Unfortunately, a mist blower was not used as a standard spray machine. A spray deposition study on leaves was also done following sprays with these sprayers in the same orchard. The Cima at 2000 L ha^{-1} generally performed the best; often significantly better than the other machines.

Replacing ground-area based sprayer calibration with tree volume based calibration for fungicide and pesticide spray application is a necessary step to improve spray application in terms of cost and efficiency. To move to tree volume based sprayer calibration, tree canopies need to be characterised in terms of dimensions (volume) and density. A light detection and ranging (LiDAR) system was procured for this specific task. LiDAR to PC communication has been established and ASCII and binary translation of scanning data has commenced. Various mounts for the LiDAR have been designed and one has been built. First scans were initiated and a cloud image constructed. Further in orchard scans have been done with the mounted system. GPS have been coupled with the system. Programming of software to convert scan-data to usable tree canopy parameters will be done in collaboration with Department of Viticulture at Stellenbosch University.

Programopsomming

Geen veldproewe kon uitgevoer word nie omrede die laaste mandarin boord in die Laeveld uitgestoot is. Meer kan gedoen word om spuitprogramme aan te pas om RB1 en RB2 in bestaande strobilurinprogramme as plaasvervanger vir mancozeb (4.3.2).

A.g.v. die zero toleransie status van sitrus swartvlek is produsente geneig om hoë swamdoder spuitvolumes te gebruik wat strek tussen 6000 tot 12000 L ha^{-1}. Die potensiaal van verlaagde volume toediening is in vorige studies bewys. ŉ Reeks masjinerie is ondersoek vir die proef doeleindes. Verlaagde volume toedienings (1000 tot 4000 L ha^{-1}) met die Martignani en Cima masjiene. Resultate toon dat konvensionele masjiene beter deposisie uniformiteit deur die boom lower (binne/buite blare;

bo/middel/onder blare) getoon het, wat die belang van gepaste spuit profiel met die boom lower uitwys, asook die noodsaaklikheid van lowerbestuur vir laer-volume toediening (4.3.3). Bio-effektiwiteit-spuitproewe is in ŉ Delta Valencia boord buite Marble Hall begin. ŉ Program van vier fungisied en insekdoder toedienings is vanaf Oktober tot Januarie gespuit. ŉ Cima en ATASA teen 2000 L ha^{-1} (3.5×) en ŉ Jacto teen 7000 L ha^{-1} (1×) is in die proef gebruik. Ongelukkig is ŉ standaard newelblaser nie in die proef gebruik nie. ŉ Spuit deposisie proef is ook op blare gedoen en die Cima teen 2000 L ha^{-1} het deurgaans die beste gevaar.

Die vervanging van grond-area gebaseerde spuit kalibrasie met boom-ry-volume gebaseerde kalibrasie vir fungisied en insekdoder spuit toediening is ŉ nodige stap vir die verbetering van spuit toediening in terme van koste en effektiwiteit. Om te beweeg na boom-ry-volume gebaseerde kalibrasie moet bome lowers gekarakteriseer word in terme van dimensies (volume) en lower-digtheid. ŉ "Light detection and ranging" (LiDAR) sisteem is aangeskaf vir die spesifieke taak. LiDAR na rekenaar kommunikasie is bewerkstellig en ASCII en binêre vertaling van data is begin. Verskeie monterings vir die LiDAR is ontwerp en een is gebou. Eerste skanderings is gedoen en ŉ 'cloud' figuur is geskep. Verdere boord skanderings is gedoen met die gemonteerde sisteem met ŉ GPS gekoppel aan die sisteem. Programmering van sagteware om die skandeer data om te skakel na bruikbare boomlower parameters sal in samewerking met die Departement Wingerdkunde by Stellenbosch Universiteit gedoen word.

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

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

Summary

No field trials could be conducted because the last 'Nova' mandarin orchard in the Lowveld has been removed. New trial sites in other provinces must be found for future trials. No new products were presented from companies for evaluation. The new BASF product may be registered but is very expensive. More can be done to alter spray programmes to cater for RB1 and RB2 in current strobilurin programmes as replacements for mancozeb.

Opsomming

Geen veldproewe kon uitgevoer word nie omrede die laaste 'Nova' mandarin boord in die Laeveld uitgestoot is. Nuwe proefpersele sal in ander provinsies gesoek moet word vir toekomstige proewe. Geen nuwe produkte is ook aan CRI gebring vir evaluasie nie. Die nuwe BASF produk mag geregistreer word, maar is duur. Meer kan gedoen word om spuitprogramme aan te pas om RB1 en RB2 in bestaande strobilurin programme as plaasvervanger vir mancozeb.

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

Project 1089 (2014/15 – 2015/16) by JG van Zyl and PH Fourie (CRI)

Summary

Replacing ground-area based sprayer calibration with tree volume based calibration for fungicide and pesticide spray application is a necessary step to improve spray application in terms of cost and efficiency. To move to tree volume based sprayer calibration, tree canopies need to be characterised in terms of dimensions (volume) and density. A light detection and ranging (LiDAR) system was procured for this specific task. LiDAR to PC communication has been established and ASCII and binary translation of scanning data has commenced. Various mounts for the LiDAR have been designed and one has been build. First scans were initiated and a cloud image constructed. Further in orchard scans have been done with the mounted system. GPS have been coupled with the system. Programming of software to convert scan-data to usable tree canopy parameters will be done in collaboration with Department of Viticulture at Stellenbosch University.

Opsomming

Die vervanging van grond-area gebaseerde spuit kalibrasie met boom-ry-volume gebaseerde kalibrasie vir fungisied en insekdoder spuit toediening is ŉ nodige stap vir die verbetering van spuit toediening in terme van koste en effektiwiteit. Om te beweeg na boom-ry-volume gebaseerde kalibrasie moet bome lowers gekarakteriseer word in terme van dimensies (volume) en lowerdigtheid. ŉ "Light detection and

ranging" (LiDAR) sisteem is aangeskaf vir die spesifieke taak. LiDAR na rekenaar kommunikasie is bewerkstellig en ASCII en binêre vertaling van data is begin. Verskeie monterings vir die LiDAR is ontwerp en een is gebou. Eerste skanderings is gedoen en 'n 'cloud' figuur is geskep. Verdere boord skanderings is gedoen met die gemonteerde sisteem met 'n GPS gekoppel aan die sisteem. Programmering van sagteware om die skandeer data om te skakel na bruikbare boomlower parameters sal in samewerking met die Departement Wingerdkunde by Stellenbosch Universiteit gedoen word.

4.3.4 PROGRESS REPORT: The use of adjuvants to improve fungicide foliar spray deposition and control of Alternaria brown spot on citrus

Project 1096 (2014/04 – 2014/10) by JG van Zyl and PH Fourie (CRI)

Summary

Previous work has shown that certain adjuvants together with copper oxychloride sprays improved Alternaria brown spot control. However, anomalous results were found and the spray deposition benchmark model over- and under-predicting observed control levels. As a hypothesis, these anomalous results were ascribed to the effect of deposition quality on disease control, as well as possible physical and/or chemical effects of adjuvant use together with copper oxychloride sprays on the pathogen and leaf surfaces. These factors were investigated through improvement of the deposition quality parameter and a histopathology study and microtiter plate assays to assess direct effects on the pathogen. These aspects formed part of a PhD study. The student is presently concluding the dissertation, which will be submitted as a final report in 2017.

Opsomming

Vorige navorsing wat gedoen is op benatters saam met koperoksichloried, het gewys op verbeterde Alternaria bruinvlekbeheer. Onreëlmatige resultate was gevind met die verbeterde deposisie asseserings-protokol met deposisie maatstawwe wat onder- en oor siektebeheer voorspel. Hierdie onreëlmatige resultate is hipoteties aan die effek wat deposisie kwaliteit op siektebestuur, en/of moontlike fisiese of chemiese effekte van die benatter met koperoksichloriedspuite op Alternaria bruinvlek en blaaroppervlaktes toegeskryf. Hierdie faktore is deur die verbetering van die deposisie kwaliteitparameter en 'n histopatologie studie en mikroplaat toetse ondersoek om die direkte effekte op die patogeen te bepaal. Voorgenoemde vorm deel van 'n PhD studie. Die student is besig om sy proefskrif af te handel, wat dan ook as finale verslag in 2017 sal dien.

4.4 PROGRAMME: SOILBORNE DISEASES

Programme coordinator: J.M. van Niekerk (CRI)

4.4.1 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 is also being investigated, specifically looking at factors that could be used as early indicators of tree decline. Unknown diseases with unknown causal organisms and epidemiology is furthermore studied to determine the causes of the observed disease and which management practices have potential to lessen the impact of the disease.

Projects 762 and 1030 are specifically aimed at finding alternative means of control for *Phytophthora* and citrus nematode. Data have been recorded in project 762 since 2011 and the juvenile and female nematode counts still do not clearly show up differences between treatments. However, from the *Phytophthora* incidence data and physical tree measurement data a picture is emerging that indicates the preplant fumigation treatments are slightly better than the other treatments. In October 2014 a new field trial was started in project 1030. This included a new product OLLYS trailed at three different concentrations along with Cropguard. A fungal nematicide, PL Gold Plus was also included and applied according to seven different application regimes and in combination with Rugby 10 ME. To date some of the regimes of PL Gold Plus is showing promise. The regime where Rugby 10 ME is applied in September followed by PL Gold Plus applied in October and November are emerging as the best treatment. This regime consistently reduced juvenile and female counts over the five sampling periods between 2014 and 2016. Further evaluation will be done to confirm the observed results.

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 (project 1068). Extensive sampling and isolations from diseased material have led to the conclusion that a complex of pathogens are involved in the disease in the Eastern Cape while in the Swaziland/Hoedspruit areas only one pathogen is at work. Pathogenicity trials are currently underway to determine the pathogen status of the isolated pathogens on citrus. A field trial, testing various soil drench treatments, was started in Kirkwood in 2015. The aim is to find a treatment that could halt or slow down the disease development. After one season of applications and monitoring no clear results are yet available.

Following on project 910, project 1092 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. Soil, root and leaf samples were collected from these trees. Chemical leaf and soil analyses were done along with physical soil analysis, nematode counts in the soil and roots, *Phytophthora* incidence in the soil and starch content of the roots. Due to outstanding results, in depth data analyses using multivariate and principle component analyses were not possible. These will be completed once all data is available and reported on in 2017.

The last project in the portfolio, 1101, focusses on the soilborne pathogens in citrus nurseries. A large number of *Phytophthora* and *Pythium* isolates were collected during the project. These were characterized using molecular techniques. This indicated that a variety of *Pythium* species are present in citrus nurseries. These need to be studied further to determine their pathogenicity along with mefenoxam and chlorine sensitivity. *Phytophthora* isolates were found to have varying degrees of sensitivity to mefenoxam and this needs further investigation to determine the level of resistance. Standard potting medium treatments with mefenoxam and captan showed variable effectiveness in different potting media and this trial is being repeated to confirm results. Phosphonates were furthermore applied to citrus seedlings according to three regimes, using two application methods. The results from this trial showed that foliar applications lead to bigger, healthier trees as *Phytophthora* infections in treated trees were also less than in untreated trees.

Program opsomming

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

Projek 762 en 1030 se doel is spesifiek om alternatiewe chemiese middels vir die beheer van *Phytophthora* en sitrus nematode te vind. Binne projek 762 word data al sedert 2011 versamel en die nematode tellings toon steeds geen verskille tussen behandelings nie. Die *Phytophthora* data tesame met fisiese boommetings begin egter toon dat die voorplant berokingsbehandelings matig beter is as die ander behandelings. In Oktober 2014 is ʼn nuwe veldproef binne projek 1030 begin. Produkte wat ingesluit is, was ʼn nuwe produk OLLYS teen drie verskillende konsentrasies asook Cropguard. ʼn Swambevattende nematisied, PL Gold Plus is ook ingesluit en word getoets volgens sewe verskillende programme in kombinasie met Rugby 10 ME. Tot op datum toon van die PL Gold Plus programme belofte. Die program waar Rugby 10 ME in September toegedien word opgevolg met PL Gold Plus in Oktober en November toon sedert 2014 konstante resultate ten op sigte van nematode tellings wat dit betekenisvol verminder. Verdere evaluasie van die program sal gedoen word ten einde die resultate te bevestig.

Twee duidelik verskillende siektes is onlangs in boorde in Kirkwood en Patensie in die Oos-Kaap en boorde in Swaziland en Hoedspruit waargeneem. Intensiewe monsterneming van simptomatiese materiaal tesame met isolasies uit hierdie materiaal het tot die gevolgtrekking gelei dat in die Oos-Kaap ʼn kompleks van patogene teenwoordig is. In die Swaziland/Hoedspruit areas is slegs een patogeen geassosieer met die waargenome simptome. Patogenisiteitsproewe is tans aan die gang om te bepaal wat die patogeenstatus van die swamme is. Verder is daar ook ʼn veldproef in Kirkwood begin waar verskillende grondbehandelings getoets word om te bepaal of enige van die behandelings die siekte kan stop of die ontwikkeling daarvan vertraag. Na een seisoen se toedienings en monitering is duidelike resultate nog nie beskikbaar nie.

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

Die laaste projek in die portefeulje, 1101, fokus op grondgedraagte patogene in sitruskwekerye. ŉ Groot getal *Phytophthora* en *Pythium* isolate is versamel en d.m.v. molekulêre metodes gekarakteriseer. Dit het getoon dat verskeie *Pythium* spp. in kwekerye voorkom. Hierdie sal verder ondersoek word t.o.v. hul mefenoxam en chloor sensitiwiteit. *Phytophthora* isolate is bevind om wisselende vlakke van mefenoxam sensitiwiteit te toon en verdere ondersoek is nodig om te bepaal wat die vlak van weerstand is. Standaard behandelings van plantmedium met mefenoxam en kaptan het getoon dat dit wisselend effektief is in verskillende plantmedia. Hierdie proef word herhaal om resultate te bevestig. Fosfonate is verder ook toegedien op sitrussaailinge in drie verskillende programme deur gebruik te maak van twee verskillende aanwendingstegnieke. Die proefresultate het getoon dat blaarbespuitings gelei het tot groter en gesonder bome omdat die fosfonaat aanwendings ook die *Phytophthora* infestasie in behandelde saailinge verminder het.

4.4.2 PROGRESS REPORT: The evaluation of different pre-plant products for the control of the citrus nematode, as part of an integrated nematode control approach in citrus replant situations

Project 762 (2007/8 – 2017/8) by JM van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

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

Opsomming

Die doel van hierdie projek is om voor-plant behandelings te vind wat grond in boorde vir so lank as moontlik na plant vry sal hou van sitrusaalwurm en *Phytophthora*. Die proef is al sedert 2010 aan die gang. Verskeie grondbehandelings is gedoen voor plant in Januarie 2010 terwyl sommige behandelings jaarliks in Januarie en November toegedien word. Parameters wat jaarliks sedert die begin van die proef gemonitor is, sluit in stam deursnee, boom hoogte, nematode tellings in die grond en wortels, *Phytophthora* status in die grond en ŉ visuele boom gradering. Op grond van nematode tellings in die grond en boomwortels staan geen behandeling duidelik uit nie. Die gemiddelde persentasie *Phytophthora* besmette blaarskyfies tesame met die fisiese boommetings begin aantoon dat die beter behandelings die voor-plant berokingsbehandelings is.

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

Project 1030 (2008/9 – 2017/18) by JM van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

Tylenchulus semipenetrans, the citrus nematode infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus orchards. The use of toxic compounds as nematicides is becoming more and more under pressure internationally and locally. Developing alternatives to chemical nematicides is therefore essential. The following products were included in a new trial that commenced in September 2014. A new product OLLYS was trialed at three different concentrations along with Cropguard and Mosblend. A fungal nematicide, PL Gold Plus was also included and applied according to seven

different application regimes and in combination with Rugby 10ME. After two seasons of testing results are still inconclusive. However, at this early stage the regime where Rugby 10ME is applied in September followed by PL Gold Plus applied in October and November are emerging as the best treatment. This regime consistently reduced juvenile and female counts over the five sampling periods of between 2014 and 2016. However, in order to obtain conclusive results evaluation for another season is needed.

Opsomming

Die sitrus nematode, *Tylenchulus semipenetrans*, infekteer sitrus wêreldwyd en is die volopste plantparasitiese nematode in sitrus boorde. Die gebruik van toksiese nematisiedes kom toenemend plaaslik en internasionaal onder druk. Ontwikkeling van alternatiewe tot chemiese nematisiedes is dus noodsaaklik. Die volgende produkte is ingesluit in ŉ nuwe proef wat gedurende September 2014 begin is. ŉ Nuwe produk, OLLYS is getoets teen drie verskillende konsentrasies tesame met Cropguard en Mosblend. ŉ Swambevattende nematisied, PL Gold Plus, is ook ingesluit en toegedien in sewe verskillende programme in kombinasie met Rugby 10ME of alleen. Na twee seisoene se evaluasie is duidelike gevolgtrekkings aangaande die beste behandeling nog nie moontlik nie. Op hierdie vroeë stadium wil dit voorkom asof die program waar Rugby 10ME toegedien word in September gevolg deur PL Gold Plus aanwendings in Oktober en November die meeste potensiaal het. Hierdie program het konstant in al die monster periodes tussen 2014 en 2015 die nematode getalle in die wortels en grond verminder. Ten einde duidelike resultate te verkry sal die evaluasie egter vir nog ŉ seisoen voorgesit moet word.

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

Project 1068 (2012/3 – 2016/7) 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. Initial thoughts were that the symptoms are caused by *Armillaria* spp. Despite several attempts to isolate this pathogen, no isolates could be obtained from symptomatic tissue collected from declining trees. However, several other fungal genera were isolated, which led to the conclusion that *Armillaria* spp. might not be involved. Molecular identification of the isolated fungi identified *Kretzschmaria deusta* as being the only pathogen associated with the symptoms observed in Swaziland and Hoedspruit. This pathogen, previously known as *Ustulina deusta,* is known to cause Ustulina root and collar rot of citrus. In the two Eastern Cape areas a complex of pathogens was found to be associated with the observed symptoms. In this case the dominant ones were *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* and *Eutypella* sp. *Phaeoacremonium parasiticum* and *Schizophyllum commune* were also isolated but at levels much lower than the abovementioned group. *F. solani* has been associated with tree collapse and dry root rot or sudden death of citrus trees. *C. micaceus* are a coprinoid fungus that has the ability to degrade wood. *D. neotheicola* is known as a weak pathogen on several woody hosts but has been shown to cause a severe dieback of persimmons. Three different *Eutypella* spp. have been reported from California where they were associated with branch cankers and dieback of citrus trees. *Ph. parasiticum* is a known pathogen of many woody hosts such as grapevine, apple and forest trees. The last pathogen in the group is *S. commune* that is known to be associated with wood rot of citrus. A field trial with different soil treatments at this stage does not show any differences between the treatments based on improvement or decline in visual tree health. The trial will be repeated in 2015/2016.

Opsomming

Agteruitgang en afsterwe van sitrus bome is vir ŉ aantal jare al in Swaziland, Hoedspruit en die Gamtoos en Sondagsriver valleie gerapporteer. *Armillaria* spp. is aanvanklik gereken as die veroorsakende organisme. Ten spyte van verskeie pogings om hierdie patogeen te isoleer kon geen isolate uit simptomatiese materiaal verkry word nie. Verskeie ander swam genera is wel geïsoleer wat gelei het tot die gevolgtrekking dat *Armillaria* spp. dalk nie betrokke is nie. Molekulêre identifikasie van die geïsoleerde swamme het *Kretzschmaria deusta* as die enigste patogeen geassosieer met die simptome in Swaziland en Hoedspruit geïdentifiseer. Hierdie patogeen, vroeër bekend as *Ustulina deusta*, is bekend as die veroorsakende patogeen van Ustulina wortel en kraagvrot van sitrus. In die twee Oos-Kaap areas is bevind dat ŉ kompleks van patogene met die waargenome simptome verbind word. Die dominante patogene was *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* en *Eutypella* sp.. *Phaeoacremonium parasiticum* en *Schizophyllum commune* is ook geïsoleer, maar teen baie laer vlakke as bogenoemde

groep. *F. solani* is bekend as die veroorsakende organisme van “dry root rot” en “sudden death” van sitrus. *C. micaceus* is welbekend as ŉ houtverrotter. *D. neotheicola* is bekend as ŉ swak patogeen, hoewel dit ernstige terugsterwing van persimmons veroorsaak. Drie verskillende *Eutypella* spp. is uit Kalifonië aangeteken in assosiasie met tak kankers en terugsterwing op sitrus. *Ph. parasiticum* is ŉ bekende patogeen van houtagtige gashere soos wingerd, appels en bosbou bome. Die laaste patogeen, *S. commune*, veroorsaak weer houtverrotting van sitrus. ŉ Veldproef met verskillende grondbehandelings toon nie op die oomblik enige verskille tussen die behandelings en onbehandelde kontrole nie. Hierdie proef sal in 2015/2016 herhaal word.

4.4.5 PROGRESS REPORT: Diachronic study of abiotic and biotic factors associated with citrus decline

Project 1092 (2014/5 – 2017/8) by MC Pretorius, Adele McLoed (SU) and C Kotze(CRI)

Summary

Trees from four different citrus orchards in the Nelspruit area of Mpumalanga were selected and characterised in three categories (1-3) according to their level of decline. Chemical soil and leaf analysis were done of each of the categories for each of the orchards. Further analysis included the determining of leaf size, nematode and *Phytophthora* incidence as well as the starch content of sampled roots. To date only certain results have been received, therefore a full analysis of all the factors and their associations could not be completed. However, preliminary observations indicated certain promising factors such as the sodium content of soil and leaf samples. Further, more in-depth analysis of the data will be conducted once all of the results have been received.

Opsomming

Bome is binne vier verskillende sitrus boorde geleë in die Nelspruit omgewing van Mpumalanga geselekteer. Hierdie bome is volgens hul graad van agteruitgang in drie verskillende kategorieë (1-3) geklassifiseer. Verskeie analises is op hierdie bome voltooi insluitend die chemiese ontleding van blaar en grondmonsters, die aalwurm en *Phytophthora* voorkoms, blaargroottes en die stysel inhoud van die wortels. Ongelukkig is nog net sekere van die resultate ontvang en kon ‘n in-diepte analise van al die faktore en hul assosiasies nie voltooi word nie. Daar kon egter sekere afleidings gemaak word vanuit die data wat wel ontvang is, soos bv. die assosiasie tussen die verskillende agteruitgang kategorieë en die natrium inhoud van die blaar- en grondmonsters. Verdere, meer intensiewe analises sal voltooi word sodra al die uitstaande data ontvang is.

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

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

Summary

A large number of *Pythium, Phytophthora nicotianae* and *P. citrophthora* isolates have been collected. PCR-RFLP and ITS sequence analyses identified a number of *Pythium* spp. amongst the collected isolates. Mefenoxam resistance testing for the collected isolates showed varying degrees of resistance at different fungicide concentrations. Standard potting medium treatments with mefenoxam and captan showed varied effectiveness in eradicating *Phytophthora* infestations in the different potting media used and the trial is being repeated to confirm results. The application of different phosphonate and phosphite chemicals to nursery trees showed that foliar applications did lead to larger trees when applications were done from July to October or from October to February. These applications, regardless of application method or regime, also significantly reduced root infections by *Phytophthora* in comparison with the untreated control. The phosphite/phosphonate trial is being repeated to confirm results. Mefenoxam and chlorine sensitivity testing of *Phytophthora* isolates will be concluded in the current year together with the alternative treatments of potting media.

Opsomming

ŉ Groot getal *Pythium, Phytophthora nicotianae* en *P. citrophthora* isolate is versamel. “PCR-RFLP” en ITS DNA volgorde analise het verskeie *Pythium* spp. uit die versamelde isolate geïdentifiseer. Mefenoxam weerstandstoetsing vir die versamelde isolate het aangedui dat daar verskillende vlakke van weerstand by die verskillende swamdoder konsentrasies is. Die standaard behandelings van plant medium met kaptan en mefenoxam het wisselende resultate getoon in die verskillende mediums en die proef word

herhaal ten einde resultate te bevestig. Die aanwending van verskillende fosfonaat en fosfiet middels op kwekeryboompies het getoon dat blaarbespuitings gelei het tot groter bome as die aanwendings gedoen is vanaf Julie tot Oktober en vanaf Oktober tot Februarie. Hierdie aanwendings, ongeag die metode of program van aanwending, het ook wortelinfeksies deur *Phytophthora* betekenisvol verminder in vergelyking met die onbehandelde kontrole. Hierdie fosfiet/fosfonaat proef word ook herhaal vir die bevestiging van resultate. Die toetsing van chloor en mefenoxam sensitiwiteit in *Phytophthora* isolate sal voltooi word in die komende jaar, tesame met die alternatiewe behandelings van plant mediums.

4.5 PROGRAMME: POSTHARVEST PATHOLOGY

Programme coordinator: Wilma du Plooy (CRI)

4.5.1 **Programme summary** (Arno Erasmus / Wilma du Plooy)

The departure of Dr Arno Erasmus at the end of 2015 was a major event for the post-harvest team of the CRI Nelspruit. During the 2015/6 period Charmaine Christie completed her MSc on aspects of precision application in the packhouse (Optimisation of Postharvest Drench Application of Fungicides on Citrus Fruit) (4.5.6), under supervision of Dr Arno Erasmus, Dr Cheryl Lennox and Prof Paul Fourie. The second student on this project (Catherine Savage) will complete the practical part of her study in 2016. The field work for a master's project by Wouter Schreuder on Citrus Black Spot will also be completed in 2016. His work is focused on the possibility of post-harvest control of CBS lesion expression (4.5.5). The TUT team has had a serious setback when a critical component to build the encapsulating particles became unavailable, but has since recovered and is making good progress, with a master's degree (Sinclair Bopaima) and a doctorate (Katlego Phala) emanating from the work (4.5.7). At USPP Dr Cheryl Lennox has a master's student investigating several aspects of propiconazole. This study is conducted with the support of the manufacturer of the fungicide in South Africa, ICA (Stellenbosch).

The investigation of commercial products as alternative or augmentative postharvest remedies is continuing, with the peracetic acid (PAA) and ozone products now at the forefront of the sanitation strategies being developed (4.5.4). Current studies include the break-down of applied fungicides, and compatibility with preharvest chemicals. While PAA gives good results in the practical application, registration of the products is still being awaited and is a serious concern, particularly in light of the loss of guazatine. Apart from propiconazole, which cannot be used to all markets, no other alternative has yet been developed, making sanitation management the most important aspect in combatting sour rot. Although chlorine dioxide has some acceptance in other food-related industries, it was found to be inferior in performance in a citrus packhouse environment. Fludioxonil was not included in the 2016 packhouse recommendations, but will be investigated for possible synergism with imazalil in a resistance strategy. In another resistance strategy investigation, a product based on secondary plant metabolites (Pimi Agro) is being investigated in collaboration with the Israeli developer. A project on pallet mould has recently been initiated at the CRI. Initial investigations point to a more complex problem than just storage facilities provided by the packhouses. A conclusion on the extent of the problem will be reached the end of phase one, by March 2017.

Programopsomming

Dr Arno Erasmus se vertrek na die VSA aan die einde van 2015 was ŉ groot ingreep in die na-oes patologie span by die CRI Nelspruit. Tydens die 2015/6 periode het Charmaine Christie haar MSc oor aspekte rondom presisie aanwending in die pakhuis voltooi ("Optimisation of Postharvest Drench Application of Fungicides on Citrus Fruit") (4.5.6), onder leiding van Dr Arno Erasmus, Dr Cheryl Lennox en Prof Paul Fourie. Die tweede student op hierdie projek (Catherine Savage) sal haar praktiese werk in die loop van 2016 voltooi. Die veldwerk vir die MSc deur Wouter Schreuder op Sitrus Swartvlek sal ook tydens 2016 voltooi word. Sy werk fokus op die moontlike na-oes beheer van letseluitdrukking van SSV (4.5.5). Die TUT span het ŉ ernstige terugslag gehad met die produksie staking van ŉ kritiese komponent in die projek. Intussen het hulle herstel, met goeie vordering en word ŉ meestersgraad (Sinclair Bopaima) en doktoraal (Katlego Phala) word beplan uit die werk (4.5.7). By die USPP doen ŉ meestersgraadstudent onder Dr Cheryl Lennox ŉ studie op verskeie aspekte rondom propikonasool. Die studie word ondersteun deur die Suid-Afrikaanse vervaardiger van die aktief, ICA (Stellenbosch).

Die ondersoek van kommersieële produkte as alternatiewe of aanvullende na-oesbeheermiddels word voortgesit, met PAA en O_3 aan die voorfront van sanitasie strategieë wat ontwikkel word (4.5.4). Ondanks die goeie resultate wat PAA gee in praktiese aanwending, word daar steeds gewag op registrasie van die produkte. Dit is ŉ groot bekommernis, veral met die verlies van guasatien, aangesien daar geen

alternatiewe is nie en sanitasiebestuur dus die mees belangrike aspek in die bekamping van suurvrot is. Alhoewel chloordioksied aanvaarding geniet in sommige ander voedselmarkte, het die prestasie daarvan nie beïndruk in sitruspakhuise nie. Fludioksonil was nie in die 2016 pakhuisaanbevelings ingesluit nie, maar word ondersoek vir moontlike sinergisme met imazalil in weerstandsbeheerstrategieë. In ŉ ander weerstandsbeheerstrategie studie word ŉ produk van Pimi Agro wat op sekondêre plantmetaboliete gebaseer is, saam met die Israeli ontwikkelaar ondersoek. Die projek op palletswamme is onlangs van stapel gestuur by die CRI. Aanvanklike ondersoeke dui daarop dat die probleem meer ingewikkeld is as blote swak opbergingspraktyke deur die pakhuise. ŉ Gevolgtrekking aangaande die omvang van die probleem sal teen die einde van fase een in Maart 2017 gegee word.

4.5.2 **FINAL REPORT: Optimisation of postharvest drench application of fungicides on citrus fruit**
Project 1103 (2014/4 – 2015/3) by Arno Erasmus (CRI), Paul Fourie (CRI), Mareli Kellerman (CRI at USPP), Cheryl Lennox (USPP), Catherine Savage (CRI/USPP) and Charmaine Christie (USPP)

This project was terminated with Arno Erasmus' resignation but the work is continuing under project 1126.

4.5.3 **FINAL REPORT: Further optimisation of in-line aqueous fungicide application in citrus packhouses**
Project 1104 (2014/5 – 2014/15) by Arno Erasmus (CRI), Paul Fourie (CRI), Mareli Kellerman (CRI at USPP), Cheryl Lennox (USPP) and Catherine Savage (CRI/USPP)

This project was terminated with Arno Erasmus' resignation but the work is continuing under project 1126.

4.5.4 **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, Arno Erasmus, Catherine Savage, Charmaine Christie and Paul H. Fourie (CRI)

Summary

Several prospective alternative postharvest products were offered to the CRI for testing during 2015. The most important investigations were on guazatine replacements, specifically propiconazole, a propiconazole/pyrimethanil blend, and fludioxonil. These synthetic chemicals presented the best results of all fungicides evaluated in this project. In addition, HPPA has impressed as a sanitiser, leading to more rigorous product development and a new powder formulation to address some of the physical issues experienced with liquid formulations. Another sanitiser alternative drawing interest is ozone and an applicator was donated for research purposes. A programme utilising GRAS chemicals was evaluated in a single trial. The variability of results from a ring test between 7 analytical laboratories highlighted the need for more stringent control on protocols and execution.

Opsomming

Verskeie produkte wat as na-oes beheermiddels aangebied word, is aan CRI aangebied om in 2015 getoets te word. Die mees belangrike ondersoeke was geloods om vervanging te kry vir guazatine. In besonder is propikonasool, ŉ mengsel van propikonasool / pirimethaniel en fludioksonil getoets. Hierdie sintetiese produkte het steeds die beste resultate in hierdie projek gelewer. Verder het die sogenaamde "HPPA" (ŉ gestabiliseerde waterstof peroksied / per-asynsuur mengsel) as ŉ saniteermiddel beïndruk. Dit het gelei tot die verdere onwikkeling van die poeier formulasie, wat meer hanteerbaar en minder korrosief as die vloeistof weergawes is. ŉ Ander oksidatiewe saniteermiddel wat potensiaal inhou is osoon en ŉ toediener is vir CRI gebou om die aktief te toets. ŉ Program met GRAS chemie is in ŉ pakhuis getoets. Die ringtoets tussen 7 analitiese laboratoria is afgehandel en die wisselvallige resultate dui op ŉ behoefte aan beter beheer oor protokolle en uitvoering.

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 2015/16 report year.

Objectives

Objective	Achievement
1. Selected new products with potential will be tested as sanitation agents and/or fungicides by CRI postharvest plant pathology. CRI will be involved in setting up protocols for such testing and interpreting the data and findings.	1. CRI selectively conducted pilot trials in the interest of the industry.
2. Test HPPA formulations for efficacy and compatibility: Products containing hydrogen peroxide and peracetic acid applied as sanitisers in packhouse drench systems	1. A powder form of HPPA is being developed with CRI's involvement
3. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	1. A test was done, in collaboration with QMS, to evaluate a chemistry-free packline at Letaba.
4. Test new technology on offer for packhouse sanitation	1. An ozone generator was installed on the high pressure spray unit on the pilot line by ArqAqua and tested.
5. Analytical laboratory ring test with the aim to reduce variability	1. Nine amended ("spiked") citrus samples were sent to seven different analytical laboratories; six were in South Africa and one in Europe.

Materials and methods

Standard protocols according to which products are tested have been developed. Pilot trials are done with cultivars available at the time of doing the trial. It consists of 4 repeat treatments with 12 fruit each. In the case of fungicidal claims, the fruit are inoculated for both protective and curative evaluations. Company claims towards products being fungicidal are evaluated against standard norms for both protective and curative action of the products involved. In the case of sanitizers, fruit are dipped in 5 L of water to which 20 ml of a 10^6 spore suspension (*Penicillium digitatum*) was added, left to dry and then treated.

Results and discussion

Objective 1
Selected potential products will be tested as sanitation agents and/or fungicides by CRI postharvest plant pathology.

CITRASHINE
The Citrashine 2,4-D product, Deccomone, was evaluated against the Calyfix formulation (Erintrade) at 250 ppm to determine if there was any difference in the button retention efficacy of the formulation. None was found and no report was submitted. Citrashine provided an OPP (Ortho-phenyl phenate) by Decco and it was evaluated at 0.2 % as a fungicide, but the results were poor. No report is available.

DUTRION
A product based on a chlorine dioxide formulation in tablet form has been tested against navel orange fruit as a sanitiser. The report is available in Addendum 1.

ICA
In expectation of the loss of guazatine early in 2016, propiconazole has been investigated as replacement chemistry. These tests were run in conjunction with ICA. The final report was submitted to ICA. Propirly and fludioxonil were similarly evaluated and the results reported. These results were reported in the 2014/15 progress report.

JANSSEN
A postharvest fungicide with the active ingredient pyrimethanil, and produced by Janssen PMP, has been tested against postharvest pathogens. The result was satisfactory, pointing towards pyrimethanil as a potential replacement for guazatine. However, there are management issues with the chemical on the

packline, requiring dedicated attention to line protocols. Contractual work has been concluded and the final report submitted (Addendum 2).

RBT
Chlorine dioxide-based products from RBT Chemicals were tested. The results below indicate the failure of the product to control inoculated green mould infections. Two formulations were evaluated, RBT A ("Optimal Concentrate") and RBT B ("APL Concentrate"), at concentrations of 0.01 and 0.001 ppm respectively. Compared with 87.5% disease in the untreated control fruit and 0% disease in the 500 ppm imazalil dip treatment, the RBT formulations proved ineffective at 59% to 78% disease. No report was submitted.

Objective 2
Test products containing hydrogen peroxide and peracetic acid applied as sanitisers in packhouse drench systems

Due to the withdrawal of quaternary ammonium compounds (BAC and DDAC) from food surface and contact environments, the need arose for alternative sanitation agents. The specific focus was on the drench solution in an attempt to reduce the build-up of spores in the solution. Products containing hydrogen peroxide combined with peracetic acid (HPPA) are proclaimed as such alternatives by a number of researchers and chemical companies. A new formulation of HPPA in powder form is being developed under contract, and CRI has become instrumental in this development.

Objective 3
Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
A packhouse (Letaba) went into an agreement with the CRI to test GRAS chemicals on the line. These tests were run in conjunction with QMS. A report on the results is included in Addendum 3.

Objective 4
Test new technology on offer for packhouse sanitation
OZONE
An ozone generator has been installed on the pilot line at the CRI in Nelspruit. This unit delivers the gas immediately into the pressurised water flow, resulting in minimal loss of the reactive species. Initial trials were done on Clementines and Valencias, using the standard sanitisation protocol. Though the results from these trials were promising, the unit (specifically built for the CRI) required some rework. These trials will be repeated in 2016.

Objective 5
Analytical laboratory ring test with the aim to reduce variability
A ring test between seven laboratories (six local and one abroad) was initiated. Spiked samples of fruit with various postharvest chemicals at different residue levels were sent to each. The results clearly indicated the need for improved methodology and execution by three laboratories, with only four (three local and one abroad) returning satisfactory results (Addendum 4).

Conclusion to date

Whilst the propiconazole-containing fungicides proved to be effective, no single product tested to date could match guazatine's superior efficacy against sour rot. The sanitisers offer a flexible augmentation to postharvest management.

Technology Transfer

Selected results from the various 123-trials were reported by Arno Erasmus at the 2016 workshops.

Further objectives 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 Phytophthora brown rot and sour rot.

2. Investigate GRAS options, and where feasible, implement the use of GRAS chemicals in the citrus postharvest industry.

3. Investigate alternative disease control strategies and technologies in citrus postharvest.

4. Analytical laboratory ring test with the aim to reduce variability.
5. Assist CRI DC with packhouse resistance testing.

ADDENDUM 1

The evaluation of Dutrion as a potential sanitation agent for the control of citrus green mould

Arno Erasmus and Catherine Savage

Product information

Product name:	Dutrion 0.5 g Tablet
Company:	Greenland Technologies SA
Active ingredient:	Chlorine dioxide
Trial date:	August 2015
Fruit:	Navel orange fruit

Evaluation of sanitation action

Materials and methods

- A 5 L *Penicillium digitatum* (green mould) spore suspension of 1×10^4 spores. mL^{-1} was prepared
- Each treatment replicate contained 10 fruit wounded four times beyond the flavedo (orange part) by means of a wounding tool. The wounds were inflicted around the calyx end of the fruit and each were ≈ 1 mm^2 in diameter and 10 mm deep. Two replicates were used for each treatment.
- Untreated control: Wounded citrus fruit dipped for 1 min in the spore suspension
- Product treatment: In each treatment the product was added to a specific untreated control treatment spore suspension and after 4 - 8 min wounded citrus fruit was dip treated for 1 min in the specific solution.
- After treatment fruit was incubated for 4 days at ambient temperature (≈ 22°C)
- Infected wound rating data were converted to percentage infection

Results

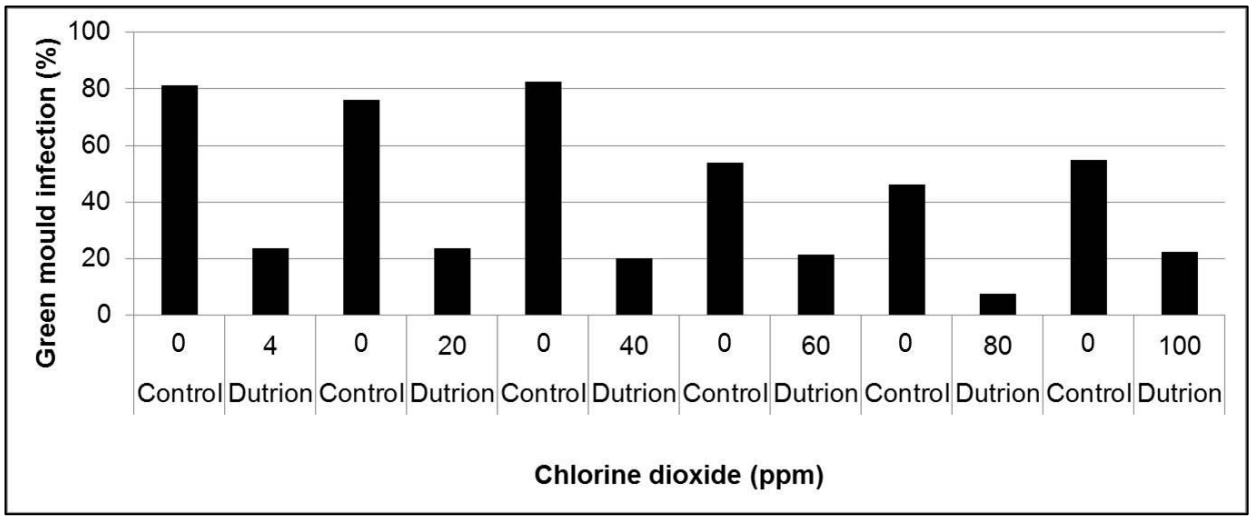

Figure 1: Mean percentage green mould infection on wounded navel oranges fruit dipped for 60 s in either an untreated or Dutrion treated (≈6 min product exposure) spore suspension of *P. digitatum*.

Observations and comments

- In all Dutrion concentration treatments, except the 80 ppm treatment, the level of infection could not be reduced to lower than 20%. Concentration seemed to have no effect on the efficacy of Dutrion.
- In this pilot trial Dutrion showed potential as a sanitation agent for the control of green mould, but there are other sanitation products available to the industry that give 100% control in similar treatment protocols.
- No more work will be done in the foreseeable future by CRI on Dutrion

Important comments

These were pilot trials. None of these results can be used in marketing or as a CRI recommendation in any citrus chemical program.

Non-GEP study for the determination of the minimum effective dose of Penbotec 400 SC used in post-harvest treatments on citrus

Arno Erasmus, Wilma du Plooy and Catherine Savage
Citrus Research International, Nelspruit, South Africa

Introduction

Penbotec 400 SC is a postharvest fungicide with the active ingredient pyrimethanil, and is produced by Janssen PMP. Pyrimethanil was classified as a reduced-risk fungicide by the U.S. Environmental Protection Agency on 8/26/2004 for use on almond, grape, onion (dry bulb, green), pome fruit, stone fruit, strawberry, tomato, tuberous & corm vegetables (EPA, 2016). As an anilinopyrimidine, pyrimethanil is a FRAC 9 fungicide, and has no risk of building cross-resistance with other existing citrus postharvest fungicides. The aim of the contract work entered into between CRI and Janssen PMP was towards testing Penbotec 400 SC at reduced concentrations to control green mould (*Penicillium digitatum*). This was done by means of various application methods, using thiabendazole as comparative fungicide and untreated fruit as control treatments. The application methods and fungicide concentrations are summarised in Table 1.

Table 1. Application methods and concentrations of pyrimethanil and thiabendazole used in an assessment on green mould control on Valencia orange fruit

APPLICATION	PYRIMETHANIL ($\mu G.G^{-1}$)	THIABENDAZOLE ($\mu G.G^{-1}$)
DIP A	500	900
	325	
DRENCH A	500	900
	325	
DIP B	1000	1800
	650	
DRENCH B	1000	1800
	650	
WAX	2000	4200
	1300	
SPRAY	2000	3000
	1300	

Materials and methods

Untreated, export quality Valencia oranges were collected from packinghouses in the Mpumalanga province of South Africa. Fruit were transported to the CRI laboratories in Nelspruit and washed using a total loss ozone spray (ArcAqua, Miltons Way, 11, Bell Crescent Westlake Business Park,7945). Fruit were stored (at 4°C) for no longer than four days before trials commenced. Fruit were removed from cold storage two days before trials to allow fruit temperature to return to ambient and for any condensation to evaporate.

The trials (Table 1) were conducted once each. For each treatment, five replicates of 50 fruit each was used. An additional six fruit per replicate were treated and sent to Hearshaw and Kinnes Analytical Laboratory (Cape Town) for residue analyses.

Each fruit was inoculated once on the side of the fruit six hours before treatment. The pathogen used was an imazalil and thiabendazole sensitive culture of *Penicillium digitatum* (STEU 6560) (Erasmus *et al*., 2011) and the inoculum was prepared as a spore suspension (Erasmus *et al*., 2013). Fruit were inoculated by dipping a metal rod into the suspension, whereafter four equidistant wounds were made in the shoulder area of the fruit, thereby creating a 1mm diameter wound, 2 mm deep. Fruit were incubated at ≈23°C for 6 hours prior to treatment, whereafter fruit was left to dry before being repacked in open display, five ply corrugated fruit boxes, 500 × 300 mm in size. Treated fruit were incubated at ≈23°C for 10 days, after which evaluation for infection and sporulation was conducted.

Dip and drench trials

Concentrations of the two actives, throughout all the applications, were adjusted as per Table 1. The water temperature was at ambient (≈22°C throughout), with the drench water flow at 26.5 L/min with a fruit exposure time of 60 seconds for both the drench and the dip applications. The dip and drench trials were done together on the same day, using the same solution, same batch of fruit, same control and same inoculum. The trial used a custom-built, specific function appliance that was able to switch between dip and drench applicator functions. This enabled the use of one tank of solution, with the concentration of the active increased as necessary throughout. After all the pyrimethanil trials were complete, the tank was rinsed thoroughly three times and then refilled with the thiabendazole solution, which was similarly increased in concentration as needed.

Wax trial

Wax trials were conducted on a wax applicator similar to those installed on pack lines, using a commercial spray nozzle and 8 brushes on a line two feet wide. Wax was applied at a rate of 1.2 L per ton of fruit, similar to industry recommendations. This trial was conducted on its own, and on a different day to that of the dip and drench trials.

Spray trial

The spray trial was conducted on the same day as the wax trial, using the same batch of fruit and the same inoculum. Sprays were applied high pressure (3 bar) through 16 nozzles over 6 rotating brushes. Total fruit exposure time was 5 seconds.

Results and discussion

The results of the four application methods are reported separately (Tables 2, 3, 4 and 5). Reported within each table are both the mean percentage of infection and the corresponding average residue gained from the particular fungicide and concentration combination.

Table 2. Dip applicator treatments showing the green mould (*Penicillium digitatum*) infection and correlating residue loaded from treatments

Active	Concentration (µg.mL^{-1})	Infection (%)		Residue (mg.kg^{-1})
Control	0	100	a	0.5
Pyrimethanil	325	1.2	b	4.7
Pyrimethanil	500	0.4	bc	6.4
Pyrimethanil	650	0.0	c	7.5
Pyrimethanil	1000	0.0	c	9.3
Thiabendazole	900	0.4	bc	2.0
Thiabendazole	1800	0.0	c	2.7

Table 3. Drench applicator treatments showing the green mould (*Penicillium digitatum*) infection and correlating residue loaded from treatments

Active	Concentration (µg.mL^{-1})	Infection (%)		Residue (mg.kg^{-1})
Control	0	100	a	0.5
Pyrimethanil	325	6.4	bc	1.2
Pyrimethanil	500	6.8	b	1.6
Pyrimethanil	650	5.2	bc	1.3
Pyrimethanil	1000	3.2	c	1.6
Thiabendazole	900	4.8	bc	0.7
Thiabendazole	1800	3.2	c	1.0

Table 4. Spray applicator treatments showing the green mould (*Penicillium digitatum*) infection and correlating residue loaded from treatments

Active	Concentration (µg.mL^{-1})	Infection (%)		Residue (mg.kg^{-1})
Control	0	100.0	a	0.1
Pyrimethanil	1300	8.8	b	2.1
Pyrimethanil	2000	8.4	b	2.1
Thiabendazole	3000	3.6	c	2.0

Table 5. Wax applicator treatments showing the green mould (*Penicillium digitatum*) infection and correlating residue loaded from treatments

Active	Concentration (µg.ml^{-1})	Infection (%)		Residue (mg.kg^{-1})
Control	0	100.0	a	0.0
Pyrimethanil	1300	90.0	b	1.2
Pyrimethanil	2000	83.6	c	1.5
Thiabendazole	4200	33.2	d	4.3

Wax amended with Penbotec 400 SC resulted in low control of *P. digitatum*. It should be noted that in South Africa the registered dose for the application of PYR in wax is 4000 µg.ml^{-1}, double that of the highest concentration treated here, which may explain the poor control achieved. Further studies will have to be done to explore the reasons behind this result.

Some slight anomalies (Table 4) are seen in the residue results, but since this is only a single data set it may be of no significance. Residue loading generally correlated with the concentration of fungicide used.

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Citrus Post harvest - Evaluation of Letaba No-Chem line for treatment of *Penicillium* (Blue mould) and *Geotrichum* (Sour Rot)

QMS Laboratories™, P.O. Box 416, Letsitele, 0885

Aim: To determine the efficacy of the "no chemical" pack line at Letaba Estates Packhouse against *Penicillium* (Blue mould) and *Geotrichum* (Sour Rot)

Materials and methods

The postharvest trial was conducted at Letaba Estates Packhouse, to test the efficacy of their "no chem" pack line. The efficacy of their organic products combination, which included Fortisol Ca Plus (1.5 L/100 L), Fungicid Gras (3.5 L/100 L) and Citrocide Plus (300 ml/100 L), was tested against *Penicillium* (Blue mould) and *Geotrichum* (Sour Rot). The trial also tested preventative and curative action of the organic products combination. The trial was conducted as follows. The trial consisted of four treatments (Table 1), with three replicates, each replicate containing twelve fruit. The freshly picked oranges were surface disinfected with a 200 ppm chlorine solution and left to dry before the treatments were applied.

Table 1. Treatments applied during the trial

Treatment Name	Pathogen	Treatment
Geo Punch (Curative)	*Geotrichum*	Treated fruit were wounded on two sides (one punch forms five wounds) and inoculated by dipping wounds in a 106 spores ml^{-1} freshly prepared *Geotrichum* spore suspension. Fruit were incubated in plastic bags at 25 °C for four hours after which the fruit were dipped for one minute in the 'no chem' fungicide bath at Letaba Estate Packhouse. Fruit were incubated once again and evaluated after five days.
Geo Dip (Preventative)	*Geotrichum*	Treated fruit were put through the 'no chem' pack line at Letaba Estate Packhouse. Fruit were dipped for one minute in the 'no chem' pack line after which the fruit were wounded on four sides (punch form five wounds) and inoculated by dipping wounds in a 106 spores ml^{-1} freshly prepared *Geotrichum* spore suspension. Fruits were incubated at 25 °C in plastic bags and evaluated after five days.
Pen Punch (Curative)	*Penicillium*	Treated fruit were wounded on two sides (one punch forms five wounds) and inoculated by dipping wounds in a 104 spores ml^{-1} freshly prepared *Penicillium* spore suspension. Fruit were incubated in plastic bags at 25 °C for four hours after which the fruit were dipped for one minute in the 'no chem' fungicide bath at Letaba Estate Packhouse. Fruit were incubated once again and evaluated after five days.
Pen Dip (Preventative)	*Penicillium*	Treated fruit were put through the 'no chem' pack line at Letaba Estate Packhouse. Fruit were dipped for one minute in the 'no chem' pack line after which the fruit were wounded on four sides (punch form five wounds) and inoculated by dipping wounds in a 106 spores ml-1 freshly prepared *Geotrichum* spore suspension. Fruits were incubated at 25 °C in plastic bags and evaluated after five days.
Control	*Geotrichum/ Penicillium*	Fruit was wounded on two sides (punch form five wounds) and inoculated with the respective pathogen and incubated similarly to the treatments.

Spore suspensions were prepared from 7-10 day old pure cultures. The cultures were flooded with sterilized deionized water. Spore concentrations were determined using a haemocytometer and adjusted accordingly.

Results and discussion

Please note that statistical analyses were not done for this trial. Table 1 indicates the average percentage infection obtained with each treatment. Raw data is attached.

Table 2. Average incidence (%) of infection observed with different treatments

	Treatment	Avg % Infection
Penicillium		
	Control	97.50
	Punch	27.78
	Dip	11.81
Geotrichum		
	Control	90.00
	Punch	62.50
	Dip	4.86

From the data obtained, it appears that the organic products combination was not equally effective in controlling *Penicillium* (Blue mould) and *Geotrichum* (Sour Rot). The product mixture prevented infections very well. As a curative action, the product mixture is more effective against *Penicillium* (Blue mould) than *Geotrichum* (Sour Rot). From the control batches, near 100% infection and rot were observed (Table 2 and Table 3). Except for the control fruit, the treatment Pen Punch was the only other treatment that had two fruit (out of the 12 fruit of one replication) that turned soft. All other symptoms appeared dry.

Symptoms observed on the fruit, indicated that infection took place. However, the infection was limited and fruit did not become soft (Figure 1). Other symptoms observed are shown in Figure 2.

Figure 1. Signs of non-spreading infections on fruit

Figure 2. Symptoms of infection observed on Pen Dip fruit.

Table 3. Symptoms observed on fruit on which various treatments were applied during the trial.

Treatment Name	**Pathogen**	**Symptom**
Geo Punch (Curative)	*Geotrichum*	
Geo Dip (Preventative)	*Geotrichum*	
Pen Punch (Curative)	*Penicillium*	
Pen Dip (Preventative)	*Penicillium*	
Geo Control	*Geotrichum*	
Pen Control	*Penicillium*	

ADDENDUM 4

2015 CRI ring test for analytical laboratories

Arno Erasmus (CRI), Paul Fourie (CRI) Keith Lesar (CRI), Paul Hardman (CGA) and Catherine Savage (CRI)

The Exporters Technical Forum (ETF) requested that CRI conduct a ring test between the different analytical laboratories servicing the fresh citrus fruit export industry. During the 2015 packing season, nine amended ("spiked") citrus samples were sent to seven different analytical laboratories; six were in South Africa and one in Europe. The samples were amended with specific concentrations of imazalil (IMZ), thiabendazole (TBZ) and pyrimethanil (PYR). Each laboratory received a portion of exactly the same sample batch. Results were compared to the amended concentrations as well as all the participating laboratories.

Materials and methods

Sample preparation

A 1 L stock solution of 1000 µg.mL^{-1} of IMZ, TBZ and PYR was prepared separately for each active. Untreated Navel oranges (cv. Cara Cara) were macerated by means of an electrical blender. Three mixtures were made, each containing a different concentration of the active ingredients (Table 1). The total pulp volume for each mixture was increased systematically in 1 kg increments. For each kilogram the initial volume of pulp was added to a blender jug (≈ 800 g) then the specific amount of active was added to the blender where after more pulp was added to make the final weight up to 1000 g. The specific ratios of each stock solution added can be seen in Table 1. The pulp was then blended for 3 min before it was transferred to stainless steel mixing bowl. This was done 6 times to gain a pulp volume of approximately 6 kg. The pulp in the mixing bowl was mixed thoroughly by means of a hand-held electrical mixer for 5 minutes. The protocol was repeated for each of the three mixtures. The amended pulp was divided into approximately 200 g portions to create 3 repetitions of each mix for each laboratory. One tub with un-spiked pulp was also filled to act as a control and blank sample. The samples were stored in clean unused containers and deep frozen before sent via courier to the different laboratories.

Analyses of results

For each lab the average (AVE) of the three replicate samples per active per concentration was determined. Similarly, the standard deviance (STDEV) was also determined for the three mentioned samples. The coefficient of variance (CV) was determined by dividing AVE by CV for the mentioned samples. The CV, presented as percentage, gives an indication on how consistent each lab was when analysing the same sample. The discrepancy in terms of the amended concentration for each specific set of samples was determined for each lab by calculating the percentage by which the lab's AVE was more or less than the amended concentration.

Rating

The CV and discrepancy value for each lab within each amended concentration category was used to determine a rating for consistency and accuracy, respectively (Table 2 and 3, respectively). The specific rating was converted to a figure where Excellent = 1, Good = 0.75, Fair = 0.5, Acceptable = 0.25 and Poor = 0. The highest mark a lab can get is 6. The mark out of 6 was transformed to be a mark out of five and this mark out of five was related to a star rating.

Table 1. The volume of stock solution for each active added to orange pulp to make a specific concentration as part of a specific mixture

	Volume per kg (mL)								
Mix no.	1			2			3		
Sample no.	1	3	7	4	8	9	2	5	6
IMZ Concentration (µg.g^{-1})	0.40			2.60			8.90		
PYR Concentration (µg.g^{-1})	2.60			8.90			0.40		
TBZ Concentration (µg.g^{-1})	8.90			0.40			2.60		

Table 2. Consistency rating as related to coefficient of variance (CV)

CV (%)	Consistency rating
0-5	Excellent
>5-10	Good
>10	Poor

Table 3. Accuracy rating as related to discrepancy

Discrepancy (%)	Accuracy rating
-5 to +5	Excellent
-10 to -5 and +5 to + 10	Good
-15 to -10 and +10 to +15	Fair
-20 to -15 and +15 to +20	Acceptable
< -20 and > +20	Poor

Results and ratings

The results of all laboratories are summarised in Tables 4, 5 and 6 for IMZ, TBZ and PYR, respectively and include the consistency and accuracy ratings. The star rating for each lab in terms of IMZ, TBZ and PYR analyses are shown in Table 7.

Table 4. Analytical results for imazalil (IMZ) amended fresh citrus samples from various national, and one international, laboratories.

Imazalil (µg.g^{-1})								
Amended concentration	**Sample no**			**Ave**				
0.40 µg.g-1	**1**	**3**	**7**		**Consistency (CV %)**		**Accuracy (Discrepancy %)**	
Lab 1	0.34	0.35	0.34	0.34	1.7	Excellent	-14.2	Fair
Lab 2	0.38	0.41	0.41	0.40	3.7	Excellent	-0.5	Excellent
Lab 3 – 2[a]	0.28	0.33	0.34	0.32	10.2	Poor	-20.8	Poor
Lab 4	0.43	0.44	0.44	0.44	1.3	Excellent	9.2	Good
Lab 5	0.33	0.32	0.35	0.33	4.6	Excellent	-16.7	Acceptable
Lab 6 – 2[a]	0.24	0.29	0.25	0.26	10.2	Poor	-35.0	Poor
Lab 7	0.34	0.36	0.41	0.37	9.1	Good	-7.8	Good
All samples all labs				0.35				
2.60 µg.g-1	**4**	**8**	**9**					
Lab 1	2.40	2.40	2.40	2.40	0.0	Excellent	-7.7	Good
Lab 2	2.48	3.17	2.60	2.75	13.5	Poor	5.8	Good
Lab 3 – 2[a]	3.52	1.32	2.85	2.56	44.0	Poor	-1.4	Excellent
Lab 4	2.84	2.89	2.77	2.83	2.1	Excellent	9.0	Good
Lab 5	2.10		2.10	2.10	0.0	Excellent	-19.2	Acceptable
Lab 6 – 2[a]	1.21	1.20	1.18	1.20	1.3	Excellent	-54.0	Poor
Lab 7	2.33	2.48	2.46	2.42	3.3	Excellent	-6.8	Good
All samples all labs				2.34				
8.90 µg.g-1	**2**	**5**	**6**					
Lab 1	8.40	9.20	8.10	8.57	6.6	Good	-3.7	Excellent
Lab 2	7.86	8.40	8.09	8.12	3.4	Excellent	-8.8	Good
Lab 3 – 2[a]	8.15	6.02	9.97	8.05	24.6	Poor	-9.6	Good
Lab 4	10.43	10.51	10.66	10.53	1.1	Excellent	18.4	Acceptable
Lab 5	7.50	7.40	7.40	7.43	0.8	Excellent	-16.5	Acceptable
Lab 6 – 2[a]	4.64	6.36	5.25	5.42	16.1	Poor	-39.1	Poor
Lab 7	7.87	7.62	7.55	7.68	2.2	Excellent	-13.7	Fair
All samples all labs				7.97				

[a] Labs 3 and 6 were given a second opportunity to improve their results and their first opportunity results were ignored when calculating the all samples all labs average.

Table 5. Analytical results for thiabendazole (TBZ) amended fresh citrus samples from various national, and one international, laboratories

Thaibendazole (µg.g^{-1})								
Amended concentration	**Sample no**			**Ave**				
0.40 µg.g-1	**4**	**8**	**9**		**Consistency (CV %)**		**Accuracy (Discrepancy %)**	
Lab 1	0.25	0.30	0.29	0.28	9.5	Good	-30.0	Poor
Lab 2	0.40	0.45	0.42	0.42	6.7	Good	5.5	Good
Lab 4	0.38	0.38	0.37	0.38	1.5	Excellent	-5.8	Good
Lab 5	0.33		0.30	0.32	6.7	Good	-21.3	Poor
Lab 7	0.33	0.34	0.35	0.34	2.8	Excellent	-15.4	Acceptable
All samples all labs				0.35				
2.60 µg.g-1	2	5	6					
Lab 1	2.50	2.40	2.40	2.43	2.4	Excellent	-6.4	Good
Lab 2	3.34	3.05	2.97	3.12	6.2	Good	19.9	Acceptable
Lab 4	2.68	2.65	2.70	2.68	0.9	Excellent	2.9	Excellent
Lab 5	1.90	1.90	2.00	1.93	3.0	Excellent	-25.6	Poor
Lab 7	2.22	2.16	2.09	2.16	3.0	Excellent	-17.0	Acceptable
All samples all labs				2.46				
8.90 µg.g-1	1	3	7					
Lab 1	9.10	8.50	8.60	8.73	3.7	Excellent	-1.9	Excellent
Lab 2	8.17	7.86	8.50	8.18	3.9	Excellent	-8.1	Good
Lab 4	8.00	7.90	7.95	7.95	0.6	Excellent	-10.7	Fair
Lab 5	7.30	7.00	6.60	6.97	5.0	Excellent	-21.7	Poor
Lab 7	7.56	7.50	7.36	7.47	1.4	Excellent	-16.1	Acceptable
All samples all labs				7.86				

Table 6. Analytical results for pyrimethanil (PYR) amended fresh citrus samples from various national, and one international, laboratories

Pyrimethanil (µg.g^{-1})								
Amended concentration	**Sample no**			**Ave**				
0.40 µg.g-1	**2**	**5**	**6**		**Consistency (CV %)**		**Accuracy (Discrepancy %)**	
Lab 1	0.28	0.30	0.30	0.29	4.0	Excellent	-26.7	Poor
Lab 2	0.28	0.31	0.30	0.30	4.6	Excellent	-26.0	Poor
Lab 4	0.56	0.53	0.52	0.54	3.9	Excellent	34.2	Poor
Lab 5	0.37	0.37	0.35	0.36	3.2	Excellent	-9.2	Good
Lab 6 – 2[a]	0.12	0.16	0.14	0.14	14.29	Poor	-65.0	Poor
Lab 7	0.47	0.45	0.44	0.45	3.0	Excellent	13.7	Fair
All samples all labs				0.35				
2.60 µg.g-1	1	3	7					
Lab 1	2.20	2.10	2.10	2.13	2.7	Excellent	-17.9	Acceptable
Lab 2	2.09	2.26	2.29	2.21	4.8	Excellent	-15.0	Fair
Lab 4	3.29	3.30	3.22	3.27	1.3	Excellent	25.8	Poor
Lab 5	2.50	2.20	2.50	2.40	7.2	Good	-7.7	Good
Lab 6 – 2[a]	0.84	1.05	0.94	0.94	11.1	Poor	-63.7	Poor
Lab 7	2.83	2.93	2.95	2.90	2.1	Excellent	11.6	Fair
All samples all labs				2.31				
8.90 µg.g-1	4	8	9					
Lab 1	6.20	6.00	5.50	5.90	6.1	Good	-33.7	Poor
Lab 2	7.03	9.93	8.10	8.35	17.5	Poor	-6.1	Good
Lab 4	9.94	10.38	9.81	10.04	3.0	Excellent	12.8	Fair
Lab 5	6.90		7.60	7.25	6.8	Good	-18.5	Acceptable
Lab 6 – 2[a]	3.59	4.05	3.45	3.70	8.49	Good	-58.5	Poor
Lab 7	9.61	9.12	9.21	9.31	2.78	Excellent	4.6	Excellent
All samples all labs				7.44				

[a] Lab 6 was given a second opportunity to improve their results and their first opportunity results were ignored when calculating the all samples all labs average.

Table 7. Star rating given to seven laboratories for imazalil, thiabendazole and pyrimethanil analyses

Lab	Imazalil	Thiabendazole	Pyrimethanil
1	****	****	***
2	****	****	***
3	**		
4	****	****	***
5	***	**	****
6	*		*
7	****	***	****

Conclusion

Laboratories 1, 2, 4 and 7 gave the best results in terms of consistency and accuracy and can be trusted for analyses of the three actives. Laboratory 5 needs to improve on its accuracy, especially with IMZ and TBZ analyses. Laboratory 3 was limited to only being able to do IMZ analyses and here it failed in terms of consistency. Laboratory 6 was limited to IMZ and PYR analyses and failed in terms of these analyses.

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

Project 1127 (2016/17 – 2017/18) by Arno Erasmus (CRI), Wouter Schreuder Jnr (USPP), Catherine Savage (CRI), Cheryl Lennox (USPP), Tian Schutte (CRI) and Paul Fourie (CRI)

Summary

Although citrus black spot is mostly regarded as a disease that should be dealt with pre-harvest, postharvest treatments and storage have a large effect and implications on symptom expression. This project is co-funded by the Postharvest Innovation Fund (PHI) to investigate the effect of postharvest treatments on symptom expression and lesion viability. The first year's trials focussed on currently implemented applications. So far 15 trials have been conducted: seven on lemon and eight on Valencia orange fruit. In addition, trial one was repeated 3X on lemons to evaluate the effect of ethephon. Further data collection, analyses of results and trials are in progress.

Opsomming

Hoewel sitrus swartvlek meestal beskou word as 'n siekte wat voor-oes behandel moet word, het na-oes behandelings ŉ groot effek op simptoom uitdrukking. Hierdie projek word mede-befonds deur die Postharvest Innovation Fund (PHI) om die effek van na-oes behandelings op simptoom uitdrukking en letsel lewensvatbaarheid te ondersoek. Die eerste jaar se werk het gefokus op die aanwendingsmetodes wat huidiglik geïmplementeer is. Tot dusver is 15 proewe uitgevoer: sewe op suurlemoene en agt op Valencia vrugte. Verder was die eerste proef 3X herhaal op suurlemoene uit die Brits area, ten einde die effek van ethephon te evalueer. Verdere data insameling, analiese van resultate en proewe is tans in proses.

4.5.6 PROGRESS REPORT: Precision fungicide application for the control of postharvest diseases on citrus

Project 1126 (2015/16-2016/17) by Charmaine Christie (USPP), Catherine Savage (CRI), Arno Erasmus (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 are the most widely used remedies in citrus postharvest decay, providing effective curative control and sporulation inhibition of *Penicillium*, but provides practically no control against sour rot. As part of a resistance management strategy, it is not recommended to include IMZ during drenching. Pyrimethanil (PYR) and thiabendazole is therefore regarded as the main components of this application. Part one of this study was conducted on drench application in terms of the influence of infection age, fruit orientation (pole), treatment exposure time and the addition of adjuvants and sanitisers on disease control. The ability of two different sanitisers (Chlorine and HPPA) to reduce sour rot inoculum in solution while maintaining fungicide concentration and residue levels for effective green mould control were compared. Incompatibility between sanitisers and fungicides was not observed in this study, with addition of both sanitisers providing effective sour rot control. HPPA is effective at a short exposure time (1 – 3 min) at the high pH used in this study (> 10), which indicates it can be incorporated with commercial drenching when pH is not regulated. Differential PYR residue loading was seen between the top and bottom bin levels during commercial packhouse trials with the upper level loading higher residue levels compared to the bottom level. This could be due to poor solution coverage associated with drenching stacked fruit bins. Part two of this study focusses on optimising the physical parameters of pH, temperature, exposure time, and their effect on chemicals used in the dip tank, and will only be completed in 2017.

Opsomming

Naoes swamdoders kan met behulp van verskeie metodes aangewend word, naamlik voor-pakhuis stortbehandeling, dip, in-lyn spuit en stortbehandelings en in die waks. Die mees gebruikte chemikalieë vir na-oes siektebeheer is tans imazalil en thiabendasool, met goeie kuratiewe werking teen en inhibisie van *Penicillium* sporulasie, maar dit bied geen beskerming teen suurvrot nie. As deel van weerstandsbestuur word imazalil egter nie in die voorpakhuisstort aanbeveel nie. Pirimetaniel en thiabendasool word dus as die hoofkomponente van hierdie behandeling beskou. In deel een van hierdie studie is gefokus op die voorpakhuis stort behandeling in terme van infeksie ouderdom, vrug oriëntasie, blootstellingstyd en die byvoeging van benatters en saniteermiddels ten opsigte van siektebeheer. Die vermoë van twee saniteerders (Chloor en HPPA) om suurvrot inokulum in oplossing te verminder, tesame met die fungisiedkonsentrasie en residuvlakke

wat nodig is vir effektiewe groenskimmel beheer, is vergelyk. Onverenigbaarheid tussen saniteermiddels en swamdoders is nie waargeneem nie, terwyl byvoeging van beide saniteermiddels suurvrot effektief beheer het. HPPA was effektief binne ŉ kort blootstellingstyd (1 – 3 min) teen hoë pH in hierdie studie (> 10), wat aandui dat dit ingesluit kan word in kommersiële stortbaddens waar die pH nie gereguleer word nie. Die PYR residue lading in die plukkratte het gewissel van bo na onder, met die boonste kratte wat beter laai in vergelyking met die onderstes. Hierdie is waarskynlik as gevolg van swak bedekking met die oplossing in die gestapelde kratte. Deel twee van die studie fokus op optimisering van die fisiese parameters (pH, temperatuur en blootstellingstyd) en hul effek op die chemikalieë vir die dompeltenk, en sal teen 2017 voltooi wees.

4.5.7 PROGRESS REPORT: Application of nanotechnology to decrease the volatility of effective essential oils in different applications against citrus postharvest fungi

Project 66/2014 – PHI 66 (1 January 2015 to 30 December 2015) by Sandra Combrinck (TUT), Thierry Regnier (TUT), Wilma Augustyn (TUT), Wilma Du Plooy (CRI)

Summary

This project is aimed at the development of slow-release essential oil (EO) encapsulated in nanoparticles. Essential oils are known to protect against various postharvest fungal decay pathogens. The EO materials will be tested in various forms including in coatings, aqueous environments and in headspace. For headspace analysis, an accurate and reliable method must be developed to measure the extent of volatilisation and therefore the degree of protection offered by active metabolites in the EO under storage conditions for fruit. A gas chromatograph was designed for the sampling of headspace and has since been constructed. Two particle types have been synthesized and partially characterized and *in vitro* trials confirming the efficacy of some oils, have been completed. Although an *in vivo* trial at CRI to confirm the inhibitory effects of two commercial essential oils was unsuccessful due to the inoculation technique selected, a reduction in the size of lesions was observed compared to the controls. Good progress was made with a study aimed at identifying other essential oils with good activity against green and blue moulds, as well as against sour rot. Chemometric analysis of an extensive dataset generated by gas chromatography-mass spectrometry is currently underway.

Opsomming

Hierdie studie is op die ontwikkeling van nanopartikels wat essensiële olies enkapsuleer en stadig vrystel gemik. Dit is reeds bekend dat sommige van hierdie olies beskerming teen nadelige na-oes swampatogene bied. Die EOs sal getoets word in verskeie vorms wat insluit bedekkings, waterige matrikse en in bodamp vorm. ŉ Akkurate en betroubare bodamp metode moet ontwikkel word om die mate van vervlugtiging en dus die graad van beskerming te bepaal wat deur aktiewe metaboliete in die essensiële olie gebied word. Hierdie metode sal toegepas word onder toestande waaronder vrugte geberg word. ŉ Gas chromatograaf is ontwerp vir bodamp analise en is reeds gebou. Twee tipes partikels is sinteties berei en gedeeltelik gekarakteriseer. *In vitro* studies wat die effektiwiteit van die olies bevestig is reeds afgehandel. Hoewel ŉ *in vivo* studie by CRI, wat gedoen is om die swaminhibisie aktiwiteit van twee kommersiele EOs te bepaal, groetendeels onsuksesvol was as gevolg van die inokulasietegniek wat gebruik is, was die grootte van die letsels tog kleiner teenoor die van die negatiewe kontroles. Goeie vordering is gemaak met ŉ studie wat daarop gemik is om ander essensiele olies met goeie aktiwiteit teen groen en blou skimmel en suurvrot te identifiseer. Chemometriese analise van die uitgebreide datastel wat met behulp van gas chromatografie-massa spectrometrie bekom is, word tans uitgevoer.

4.6 PROGRAMME: CITRUS BLACK SPOT

Programme coordinator: G.C. Schutte (CRI)

4.6.1 Programme summary

Genetic markers such as microsatellite markers enable the quantification of gene and genotypic diversities within and amongst pathogen populations of USA, China, Brazil and Australia. *P. citricarpa* populations in China and Australia have a greater genetic diversity than those in South Africa, Brazil and in the USA where more recent introductions have occurred (4.6.4). Mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution. The USA population harboured only a single mating type.

Various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on ‘Valencia’ oranges for the control of citrus black spot. AC1286 performed well as two applications sprayed in mid-November and mid-January (4.6.2). RB1 and RB2 also performed well in

strobilurin spray programmes where they replaced mancozeb and where mancozeb was replaced in tank mixtures with strobilurins and can be recommended for registration. NuFilm-17 and Entrée as replacements for mineral spray oil in spray programmes with mancozeb and strobilurins also performed well.

Improper calibration, machinery and equipment use and wrongful and neglected application techniques, leads to reduced spray efficiency and poor disease control. Various spray trials using various machines at spray volumes from 1000 to 24000 L ha^{-1} showed that excessively high spray volumes (>6-8 000 L/ha) did not result in better spray deposition on leaves (4.6.7). Similar and even improved spray deposition quantity and uniformity at better spray efficiency could be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers on leaves. Low-energy/low cost/reduced volume sprayers were evaluated on leaves. Little differences could be observed in deposition quality as influenced by spray volumes, with lower volumes realising better deposition quality, but higher volumes being more consistent throughout the tree canopy (washing effect).

Phosphonates do not need adjuvants to improve their deposition on leaves. It was attempted to determine the amount of spray volume that gets lost after sprays with 0.5 and 1 ml with pigments on different backgrounds without success (4.6.5). Low volume (½x) and normal (1x) spray applications of Fighter react like contact fungicides for the first two weeks after application after which the systemic action kicks in three weeks after application. Medium and high volume phosphonate applications gave better control than the low volume applications from the day of application for two weeks. Good coverage in this case insured good control of Phytophthora brown rot as the phosphonate acts as a contact fungicide. However, between week two and three, the low volume application also gave good control of Phytophthora brown rot and also performed well. This shows that the systemic action started to kick in and prevented further development of the disease under harsh conditions.

C. gloeosporioides and *C. boninense* were isolated from grapefruit with symptoms similar to that of citrus black spot (4.6.6). These cultures were subjected to molecular identification by means of polymerase chain reaction (PCR). Fruit were inoculated on the trees with a spore suspension (1×10^7 spore/ml) of either species; one set of fruit wounded and the other set left unwounded. After 4 weeks the fruit were evaluated in regards to lesion formed and lesion size, where after isolations were made to fulfil Koch's postulates. The results were however inconclusive as the inoculated pathogens were not re-isolated. The trial was duplicated on mature and immature fruit, as with the previous season lesions were only formed on wounded fruit. *C. gloeosporioides* was re-isolated from 15 and 11% of wounds inoculated with both isolates respectively during the immature phase of the trial. The mature fruit yielded none of the inoculated isolates, suggesting a changing in susceptibility of fruit with maturity. The cause of these type of lesions remains unknown.

Parameters influencing germination and infection of pycnidiospores and ascospores have not been sufficiently studied for *Phyllosticta citricarpa* (CBS). The influence of spore age, spore generation, germination media and incubation time on pycnidiospore germination was microscopically studied (4.6.8). Spores older than 1 day and from the second and subsequent generations of spores oozing from pycnidia had better germination. Water was a sufficient germination medium for pycnidiospores on leaves. The 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. Ascospores of *P. citricarpa* cannot be morphologically distinguished from ascospores of *P. capitalensis*, and therefore a method is being developed to quantify and distinguish between them. A qPCR protocol has been developed to quantify *P. citricarpa* pycnidiospores. This method will eventually be used to quantify spores on spore traps from orchards, as well as to distinguish between *P. citricarpa* and *P. capitalensis*.

Volumetric ascospore trapping was performed for two seasons in some of the new areas in South Africa during which ascospores were released at all sites (4.6.10). In areas with few releases, trends were picked up between KIM data and months with high rainfall with many hours of high relative humidity. Using 1 July as Biofix with the published DDTemp model, accurate (within 1-12 days in range) prediction of the date where mature ascospores should readily available was verified in the new monitoring areas, following the same trend observed with data from Letsitele and Hoedspruit last season. Initial screening of ascospores using monoclonal antibodies obtained against *Phyllosticta citricarpa* revealed potential of the technique to positively bind with CBS ascospores. Packhouse data on CBS counts for four years of harvest has been obtained and will be examined against weather data from this area.

The genus *Phyllosticta*, the causal agent of Citrus Black Spot (CBS), occurs worldwide, and contains numerous plant pathogenic, endophytic and saprobic species. This disease is widespread in citrus-growing regions, but is absent within countries of the European Union (EU). *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. To investigate the putative incursion and persistence of *Phyllosticta* species on infected plant material, we explored the occurrence and the diversity of *Phyllosticta* spp. associated with *Citrus* spp. in European orchards, nurseries and gardens (4.6.11). We used a multi-locus DNA dataset consisting of the ITS, *actA, tef1, gapdh*, LSU and *rpb2* gene regions to investigate 99 isolates of *Phyllosticta*, of which 52 isolates were collected during extensive surveys performed in 2015 in Europe. Two isolates were collected in Florida, USA, and three isolates in China. Based on the data generated here, we recovered several species associated with citrus plants in EU countries. In the EU, they were not found to be widespread, and were never associated with infections.

The Centre for Geographical Analysis has been contracted for development of the web-based prediction system. Shapefiles containing all citrus orchards in South Africa using SPOT6 satellite images were created (4.6.9). A web-based weather service has been identified with weather data recording, including sub-models for CBS ascospore and pycnidiospore development, dispersal and infection, was formulated.

Programopsomming

Genetiese merkers soos mikrosateliete maak die kwantifisering van geen en genotipiese diversiteit binne en tussen patogeen populasies moontlik te maak afkomstig van die VSA, China, Brasilië en Australië. Die studie het bevestig dat *P. citricarpa* populasies in China en Australië ŉ groter genetiese diversiteit het as die populasies in Suid-Afrika, Brasilië en die VSA waar meer onlangse introduksies voorgekom het (4.6.4). “Mating-type” analises het getoon dat beide “mating-types” in populasies van Suid-Afrika, China, Australië en Brasilië in 'n 1:1 verhouding teenwoordig is. Slegs een van die “mating-types” is in die VSA populasie gevind.

Verskeie nuwe sistemiese- en kontakswamdoders asook benatters in kombinasies met geregistreerde swamdoders is op ‘Valencia’ lemoene beproef vir die beheer van swartvlek. ŉ Nuwe swamdoder, AC1286, het goeie beheer van swartvlek gegee as twee bespuitings in middel November en middel Januarie (4.6.2). RB1 en RB2 het ook goed gevaar in strobilurin spuitprogramme waar alle mancozeb bespuitings met die produkte vervang is. NuFilm-17 en Entrée wat minerale spuitolie vervang het in tankmengesels van strobilurine en mancozeb, het ook goed gevaar.

ŉ Groot gedeeltes van die bespuiting gaan verlore weens afloop en drif, omgewingsbesoedeling, die hoeveelheid water en koste daarvan, brandstof en plant beskermingsproduk wat gebruik word, asook die slytasie op masjinerie. Verskeie spuitproewe is uitgevoer waar spuitvolues van 1000 tot 24000 L ha^{-1} getoets is dat hoe spuitvolumes (>6-8 000 L/ha) nie beter spuitdeposisie op blare gegee het nie (4.6.7). Soortgelyke en en verbeterde spuitdeposisie is met laer spuitvolumes oip blare verkry. Lae-energie/lae koste/verlaagde volume spuite is op blare getoets. Min verskille is waargeneem in deposisie soos beinvloed deur spuitvolumes, met hoer spuitvolumes wat beter deposisie regdeur die bome gegee het.

Fosfonate benodig nie bymiddels om beter op blare te kleef nie. Lae volume (½x) en normale (1x) bespuitings van Fighter reageer soos kontakdoders vir die eerste twee weke na toediening, waarna die sistemiese werking na drie weke eers intree (4.6.5). Medium en hoë volume fosfonaatbespuitings het sedert die dag van toediening vir ŉ verdere periode van twee weke beter beheer as lae volume bespuitings gegee. Goeie bedecking het goeie beheer van Phytophthora bruinvrot tot gevolg gehad en het die fosfonate soos kontakswamdoders gewerk. Nogtans het die lae volume tussen week twee en drie ook goeie beheer begin gee en kan aan sistemiese aksie van die produk toegeskryf word.

Colletotrichum gloeosporioides en *C. boninense* wat soortgelyk is aan sitrus swartvlek op pomelos, is ge-isoleer (4.6.6). Hierdie isolate is toe onderwerp aan molekulêre identifikasie deur middel van ŉ polimerase kettingreaksie (PKR). Vrugte is na 4 weke geoes en ondersoek vir letselvorming. Waarna isolasies uit gevoer is om Koch se postulate te voltooi. Die proef op onvolwasse en volwasse vrugte herhaal. Tydens hierdie seisoen is *C. gloeosporioides* vanuit letsels op onvolwasse vrugte geïsoleer. Dit was egter uit letsels wat deur elkeen van die isolate inokuleer is (11-15%). Isolasies vanuit letels op onvolwasse vrugte het geen van die inokuleerde isolate opgelewer nie wat aandui dat die vrugte se vatbaarhied dalk met ouderdom verander. In gevoltrekking, kon sitrus swartvlek tipe letsels nie nageboots word deur die uitvoering van hierdie projek nie en bly die oorsaak van hierdie tipe letsels onbekend te wees.

Parameters wat die ontkieming en infeksie van piknidiospore en askospore van *Phyllosticta citricarpa* (sitrusswartvlek (SSV)) beinvloed, is nog nie breedvoerig bestudeer nie. Die invloed van spoor ouderdom, spoor generasie, ontkiemingsmedia en inkubasie tydperk op ontkieming van piknidiospore was mikroskopies ondersoek (4.6.8). Spore ouer as 1 dag en van 2de of later generasies het die beste ontkieming getoon. Spore het suksesvol in water op blare ontkiem. Die konfokale laser mikroskopie beelde het soortgelyke

ontkiemingspatrone van spore op suurlemoen en lemmetjie blare gewys, en dit wys dat lemmetjiebome se toleransie tot SSV nie met die ontkiemingsproses van spore te doen het nie. Askospore van *P. citricarpa* kan nie morfologies onderskei word van dié van *P. capitalensis* nie, en daarom moes ŉ metode ontwikkel word om dit te kan doen en terselfdertyd spore te kwantifiseer. ŉ qPCR protocol is ontwikkel om *P. citricarpa* piknidiospore te kwantifiseer. Hierdie metode sal mettertyd gebruik word om spore van spoorlokvalle in boorde te kwantifiseer, en om tussen spore van *P. citricarpa* and *P. capitalensis* te onderskei.

Volumetriese spoorlokvalle is vir twee seisoene in nuwe areas in Suid Afrika gebruik waartydens askospore in alle areas vrygestel is (4.6.10). In areas waar spoorvrystelling laag was, is daar ŉ korrelasie opgespoor tussen KIM data en maande met hoë reënval en periodes met hoë relatiewe humuditiet. Deur 1 Julie as 'Biofix' tyd te gebruik in die gepubliseerde DDTemp model, was die datum wanneer askospore geredelik beskikbaar sou wees, redelik akkuraat (tussen 1-12 dae) bepaal in die nuwe moniterings areas wat saamstem met data van verlede seisoen gegenereer is vir Letsitele en Hoedspruit. Inisieële eksperimente met monoklonale teenliggaampies, spesifiek teen *Phyllosticta citricarpa*, lyk hoopvol om CBS askospore onder die mikroskoop te kan eien. Vier jaar se pakhuisdata vir swartvlek is vanaf die Oos Kaap verkry wat vergelyk sal word met weerdata vir die area.

Die genus *Phyllosticta* wat sitrusswartvlek veroorsaak, kom wereldwyd voor en bestaan uit ŉ verskeidenheid plantpatogeniese, endofitiese en saprofitiese spesies. Die siekte kom wydverspreid in sitrusverbouingsareas voor, maar is afwesig in lande van die EU. *Phyllosticta citricarpa* word verwar met *P. capitalensis*, wat ŉ nie-patogeniese endofiet is wat algemeen van sitrusblare en vrugte geisoleer word en ŉ verskeidenheid van ander gashere. Om die vermoedelike teenwoordigheid van *Phyllosticta* spesies op geinfekteerde plantmateriaal te ondersoek, is die voorkoms en diversiteit van *Phyllosticta* spp. wat geassosieer word met *Citrus* spp. in Europese boorde, kwekerye en tuine ondersoek (4.6.11). ŉ Multi-locus DNA datastel bestaande uit ITS, *actA, tef1, gapdh*, LSU en *rpb2* geen-areas is ondersoek in 99 isolate van *Phyllosticta*, waarvan 52 isolate versamel is in 2015 in Europa. Twee isolate van Florida, VSA, en drie isolate van China is ook gebruik. Gebaseer op die data wat gegenereer is, is verskeie spesies geassosieer met sitrusplante in EU lande. Simptome van SSV was nie waargeneem tydens die opnames nie wat daarop dui dat die swamme daar voor kom, maar nie siekte kan veroorsaak nie.

"Centre for Geographical Analysis" is gekontrakteur vir die ontwikkeling van die web-gebaseerde siekte voorspellingsisteem. "Shapefiles" wat sitrus produksie-areas sowel as weerpunte bevat is geskep deur die gebruik van SPOT6 satelliet fotos (4.6.9). ŉ Web-gebaseerde weerdiens is geïdentifiseer om weerdata te bekom en ŉ CBS voorspellingsmodel, met sub-modelle vir askospoor en pyknidiospoor ontwikkeling, vrystelling en infeksie, is geformuleer.

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

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

Summary

Various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of citrus black spot according to predetermined protocols from the various companies. Of the fungicides tested, AC1286 performed well as two applications sprayed in mid-November and mid-January. RB1 and RB2 also performed well in strobilurin spray programmes where they replaced mancozeb and where mancozeb was replaced in tank mixtures with strobilurins and can be recommended for registration. Good CBS control was also achieved where mineral spray oil was replaced with NuFilm-17 and Entrée in spray programmes with mancozeb and strobilurins.

Opsomming

Verskeie nuwe sistemiese en kontak swamdoders asook benatters in kombinasies met geregistreerde swamdoders is op 'Valencia' lemoene beproef vir die beheer van swartvlek volgens vooropgestelde protokolle van die onderskeie maatskappye. Van die swamdoders wat getoets is, het AC1286 goeie beheer van swartvlek as twee bespuitings in middel November en middel Januarie tot gevolg gehad. RB1 en RB2 het ook goed gevaar in strobilurien spuitprogramme waar alle mancozeb bespuitings met die produkte vervang is. Goeie beheer van swartvlek is ook verkry met NuFilm-17 en Entrée wat minerale spuitolie in tenkmengsels van strobiluriene en mancozeb vervang het.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa), affects all commercial citrus cultivars only in the summer rainfall regions of the world. Control of the disease is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in the southern hemisphere. The most important inoculum source of CBS is airborne ascospores. Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40-180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during rain spells. Ascospores are dependent on converging currents and 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 24.7 m. When protective fungicides such as copper and dithiocarbamates are used to control CBS, spray applications have to be carefully timed to coincide with the critical infection period. Spore trapping with an Interlock volumetric spore trap and sampler is used to determine the first onset of ascospore release in South Africa.

A four-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes. However, alternating copper fungicides with mancozeb in a four-spray programme, solved this problem. Protective fungicides became less popular after the release of post-infection benzimidazole fungicides such as benomyl. In 1971, the introduction of a single benomyl application in a tank mixture with mancozeb and mineral spray oil came as a breakthrough as it replaced copper and dithiocarbamates that must be applied in a four-spray protective schedule (9). Since the detection of *G. citricarpa* resistance to benomyl in South Africa in 1981, emphasis has shifted back to the use of contact fungicides for disease control. Field evaluations using strobilurins for the control of CBS in 1993 also came as a breakthrough. Two applications of kresoxim-methyl and azoxystrobin at respective rates of 0.10 and 0.075 g a.i./liter in tank mixtures with mancozeb (1.2 g a.i./liter) and mineral oil (0.5% [vol/vol]/liter of water) were initially recommended. The possibility that CBS may develop resistance to the strobilurins, justifies the incorporation of two additional mancozeb applications before and after the strobilurin applications in October and January. Since the registration of strobilurins in South Africa in 1999, no new fungicides have been registered for use against CBS. Testing of novel control measures against CBS is therefore regarded as a priority even if it includes tank mixtures with current registered fungicides.

Objectives

The aim is to evaluate any new potential fungicides for the control of CBS.

Materials and methods

Valencia orange orchards with a history of CBS were selected at Croc Valley Citrus Co. Rates and dates of applications are listed in Tables 1 to 4. A randomised design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500 - 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Certain treatments commenced in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. At fruit maturity in July or August, CBS severity will be rated on 100 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 will be analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Results and discussion

<u>AC1286</u>

Results from the trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P > 0.05$) between the standard registered mancozeb treatment and the strobilurin, Cabrio (pyraclostrobin) plus mancozeb and oil and the new experimental fungicide AC1286 (Table 4.6.2.1). AC1286 sprayed in November and January at a rate of 175 ml (2x) per 100 L water with and without mineral spray oil resulted in more than 99.8% clean exportable fruit, while the untreated control had only 73.6% clean exportable fruit. In comparison, the benomyl tank mixture with azoxystrobin and mineral oil that was also sprayed in November and January, also resulted in 97.6% clean exportable fruit which was not significant different from the new experimental

fungicide AC1286. All fungicide spray programmes were significant different from the untreated control, which had only 73.6% clean exportable fruit in a year that was very dry (Table 4.6.2.1).

Table 4.6.2.1. Evaluation of AC1286 applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co. during 2014 and 2015.

Treatment	Concentration (g/L per 100L of water)	Percentage fruit in each class[w]		
		Lesions/fruit		
		0	0-3	≥4
Untreated control		73,6b	8,8a	17,6a
Mancozeb[x]	200g	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+ Cabrio+Oil/ Mancozeb+Cabrio+Oil/ Mancozeb[y]	200g/150g+ 10ml+250ml/ 150g+10ml+250ml/ 200g	100,0a	0,0b	0,0b
Benomyl+azoxystrobin+Oil[z]	50g+20ml+250ml	97,6a	1,6b	0,8b
AC1286[z]	175ml	99,8a	0,0b	0,2b
AC1286[z]	175ml + Oil	100,0a	0,0b	0,0b

[w] Means in a column, based on 5 replicates, followed by the same letter are not significantly different (*P* > 0.05) according to Fisher's least significant difference test.
[x] Spray dates were 14 October 2014, 12 November 2014, 10 December 2014, 7 January 2015.
[y] Spray dates were 14 October 2014, 12 November 2014, 22 December 2014, 28 January 2015.
[z] Spray dates were 19 November 2014 and 14 January 2015.

Adjuvants

Table 4.6.2.2. Evaluation of different adjuvants sprayed in tank mixtures with registered spray programmes applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co. during 2014 and 2015.

Treatment	Concentration (g/L per 100L of water)	Percentage fruit in each class[x]		
		Lesions/fruit		
		0	0-3	≥4
Untreated control		73,6b	8,8a	17,6a
Mancozeb[y]	200g	100,0a	0,0b	0,0b
Mancozeb + oil[y]	150g +600ml	100,0a	0,0b	0,0b
Mancozeb + Entrée[y]	200g + 60ml	100,0a	0,0b	0,0b
Mancozeb + Nu Film 17[y]	200g + 50ml	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+Cabrio+Oil/ Mancozeb+Cabrio+Oil/Mancozeb[z]	200g/150g+10ml+250ml/ 150g+10ml+250ml/200g	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+Cabrio+ Entrée/ Mancozeb+Cabrio+Entrée/Mancozeb[z]	200g/150g+10ml+60ml/ 150g+10ml+60ml/200g	99,2a	0,8b	0,0b
Mancozeb/Mancozeb+Cabrio+NuFilm 17/ Mancozeb+Cabrio+NuFilm17/Mancozeb[z]	200g/150g+10ml+50ml/ 150g+10m250ml/200g	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+Ortiva+Oil/ Mancozeb+Ortiva+Oil/Mancozeb[z]	200g/150g+20ml+250ml/ 150g+20ml+250ml/200g	99,8a	0,2b	0,0b
Mancozeb/Mancozeb+Ortiva+ Entrée/ Mancozeb+Ortiva+Entrée/Mancozeb[z]	200g/150g+20ml+60ml/ 150g+20ml+60ml/200g	99,4a	0,2b	0,4b
Mancozeb/Mancozeb+Ortiva+NuFilm 17/ Mancozeb+Ortiva+NuFilm17/Mancozeb[z]	200g/150g+20ml+50ml/ 150g+20m250ml/200g	100,0a	0,0b	0,0b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different (*P* > 0.05) according to Fisher's least significant difference test.
[y] Spray dates were 14 October 2014, 12 November 2014, 10 December 2014, 7 January 2015.
[z] Spray dates were 14 October 2014, 12 November 2014, 22 December 2014, 28 January 2015.

Both adjuvants (Nu-Film 17 and Entrée) that replaced mineral spray oil in spray programmes with the strobilurins, pyraclostrobin (Cabrio) and azoxystrobin (Ortiva) plus mancozeb sprayed in November and December, resulted in more than 99.2% clean exportable fruit (Table 4.6.2.2). The addition of both Nu-Film 17 and Entrée to four mancozeb treatments sprayed monthly from October to January, also resulted in 100% clean exportable fruit. These treatments were, however, not significantly different from the registered mancozeb treatment alone and the mancozeb spray mixture with mineral oil (Table 4.6.2.2).

RB1 and RB2

According to Table 4.6.2.3, the trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P > 0.05$) between the standard registered mancozeb treatment and strobilurin spray programmes and the new spray programmes where RB1 and RB2 replaced mancozeb, where they were used in tank mixtures with strobilurins, as well as combined with strobilurins where mancozeb was totally replaced with RB1 and RB2. All spray programmes resulted in more than 99.2% clean exportable fruit and all the treatments were significant different from the control. No phytotoxicity was observed on any of the treatments.

Table 4.6.2.3. Evaluation of RB1 and RB2 sprayed in tank mixtures with registered spray programmes applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co. during 2014 and 2015.

Treatment	**Concentration (g/L per 100L of water)**	**Percentage of fruit in each class[x]**		
		Lesions/fruit		
		0	**0-3**	**≥4**
Untreated control	–	73,6b	8,8a	17,6a
Mancozeb[y]	200g	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+Cabrio+Oil/ Mancozeb+Cabrio+Oil/Mancozeb[z]	200g/150g+10ml+250ml/ 150g+10ml+250ml/200g	100,0a	0,0b	0,0b
Mancozeb/Mancozeb+Ortiva+Oil/ Mancozeb+Ortiva+Oil/Mancozeb[z]	200g/150g+20ml+250ml/ 150g+20ml+250ml/200g	99,8a	0,2b	0,0b
Mancozeb/RB1+Cabrio+Oil/ RB1+Cabrio+Oil/Mancozeb[z]	200g/100ml+10ml+250ml/ 100ml+10ml+250ml/200g	99,6a	0,2b	0,2b
Mancozeb/RB2+Cabrio+Oil/ RB2+Cabrio+Oil/Mancozeb[z]	200g/1000ml+10ml+250ml/ 1000ml+10ml+250ml/200g	99,8a	0,2b	0,0b
Mancozeb/RB1+Cabrio+Oil/ RB1+Cabrio+Oil/Mancozeb[z]	200g/200ml+10ml+250ml/ 200ml+10ml+250ml/200g	98,8a	0,6b	0,6b
Mancozeb/RB2+Cabrio+Oil/ RB2+Cabrio+Oil/Mancozeb[z]	200g/2000ml+10ml+250ml/ 2000ml+10ml+250ml/200g	100,0a	0,0b	0,0b
RB1/RB1+Cabrio+Oil/ RB1+Cabrio+Oil/RB1[z]	200ml/100ml+10ml+250ml/ 100ml+10ml+250ml/100ml	99,2a	0,6b	0,2b
RB1/RB1+Cabrio+Oil/ RB1+Cabrio+Oil/RB1[z]	200ml/200ml+10ml+250ml/ 200ml+10ml+250ml/200ml	100,0a	0,0b	0,0b
RB2/RB2+Cabrio+Oil/ RB2+Cabrio+Oil/RB2[z]	1000ml/1000ml+10ml+250ml/ 1000ml+10ml+250ml1000ml	100,0a	0,0b	0,0b
RB2/RB2+Cabrio+Oil/ RB2+Cabrio+Oil/RB2[z]	2000ml/2000ml+10ml+250ml/ 2000ml+10ml+250ml/2000ml	100,0a	0,0b	0,0b

[x] Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.
[y] Spray dates were 14 October 2014, 12 November 2014, 10 December 2014, 7 January 2015.
[z] Spray dates were 14 October 2014, 12 November 2014, 22 December 2014, 28 January 2015.

Conclusion

Although it was an extremely dry season, some fruit did develop CBS in the untreated control. A new fungicide AC1286 as two applications gave excellent control of CBS. No phytotoxicity was observed. The trial must be repeated. The adjuvants, Nu-Film 17 and Entrée, which replaced mineral spray oil in strobilurin spray programmes, also performed well. On the other hand, they did not enhance the efficacy of mancozeb even when four applications were applied. RB1 and RB2 performed well when mancozeb was partially and totally replaced with both fungicides in strobilurin spray programmes.

Future research

There is a constant need to evaluate new and old fungicide formulations as well as fungicides that may possess activity against citrus black spot (CBS). Chemical companies frequently modify and upgrade their old products to possess new characteristics such as rain fastness and particle size and they need to be re-evaluated for efficacy. Searching for new fungicides or fungicides with new characteristics as well as some new ideas on how we can alter aspects of old fungicide spray programmes to be included in effective spray programmes and to cope with fungal resistance strategies at the same time. Searching for and experimenting with cheaper and more effective fungicides sprayed alone or in tank mixtures with new or existing registered fungicides, will contribute a lot to reducing production costs and be more environmentally friendly and sustainable with regard to resistance development.

Technology transfer

Talks at study groups. Results will be presented on the bi-annual CRI Symposium in August 2016.

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4.6.3 **PROGRESS REPORT: Epidemiology and pest risk assessment of *Phyllosticta citricarpa***
Project 1026 (April 2011 - March 2015) by Paul Fourie, Vaughan Hattingh and Tian Schutte (CRI)

Opsomming

Sitrus swartvlek is die belangrikste sitrus-siekte in Suid-Afrika, veral gegewe sy impak op marktoegang. Baie aandag en *ad hoc* navorsing is onlangs hieraan gespandeer. Hierdie projek formaliseer die *ad hoc* navorsing en het gefokus op die ontwikkeling en verbetering van ʼn model vir *Phyllosticta* pseudotesium rypwording en spoorvrystelling gebasseer op meso- en mikroklimaat data. Op hierdie onderwerp is aanvanklike modelering afgehandel en ʼn artikel in ʼn toonaangewende wetenskaplike joernaal gepubliseer. ʼn Kwantitatiewe PKR metode om tussen die swartvlek patogeen en ʼn endofitiese *Phyllosticta* sp. te onderskei, is ge-optimiseer, maar was nie voldoende akkuraat sodat voortgegaan kan word om die versamelde filtreerpapier spoorvangers te analiseer nie. Verdere verbeterings word aan die PKR tegniek gedoen en vorm deel van ʼn nuwe RCE befondsde projek. Verder is sekere CRI navorsers betrokke in ʼn samewerkingsprojek wat deur die Florida sitrusbedryf in VSA befonds word. Hierdie doelwit beoog om ʼn kwantitatiewe pes risiko analiese vir *Phyllosticta citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Die projek is amper afgehandel en word in 2016 gefinaliseer, en ʼn wetenskaplike publikasie word tans geskryf. ʼn Finale verslag wat bogenoemde publikasies insluit sal in 2017 ingedien word.

Summary

Citrus Black Spot is the most important citrus disease in South Africa, specifically given its impact on market access. A considerable amount of effort and *ad hoc* research is conducted on an ongoing basis to service market access to these markets. This project formalises the *ad hoc* research and focussed on developing and improving a model for *Phyllosticta* pseudothecium maturation and ascospore dispersal based on meso-climatic weather data. On this topic, initial modelling research was completed and an article published in a leading scientific journal. Quantitative PCR methods to distinguish between the CBS pathogen and endophytic *Phyllosticta* sp. have been optimised, but further improvements are required before collected filter paper spore trap discs can be analysed. This aspect of the study will continue as part of a new RCE funded project. Additionally, three CRI-researchers are collaborating on a project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *Phyllosticta citricarpa*, with special emphasis on the fresh fruit pathway. The project is almost finalised and a scientific paper is being prepared. A final report will be submitted in 2017 and will include the aforementioned articles.

4.6.4 **PROGRESS REPORT: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely**
Project 977 (2010/11 – 2017) by E. Carstens (CRI)

Summary

Genetic markers such as microsatellite markers are very useful and widely used in population genetic studies. These markers enable the quantification of gene and genotypic diversities within and amongst pathogen populations. Fifteen markers were used to genotype 383 *P. citricarpa* isolates from five SA populations and seven international populations from four countries including USA, China, Brazil and Australia. The study confirms that *P. citricarpa* populations in China and Australia have a greater genetic diversity than those in South Africa, Brazil and in the USA where more recent introductions have occurred. Mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution. The USA population harboured only a single mating type. Funding has terminated, but research continues as part of a PhD study. A scientific article is being concluded as well as research to conclude the PhD dissertation, which will be submitted as final report for this project.

Opsomming

Genetiese merkers soos mikrosateliete is geskik en word baie in genetiese studies gebruik. Hierdie merkers maak die kwantifisering van geen en genotipiese diversiteit binne en tussen patogeen populasies moontlik. Vyftien merkers is gebruik om 383 *P. citricarpa* isolate van vyf SA populasies en sewe internasionale populasies afkomstig van vier lande, insluitend die VSA, China, Brasilië en Australië te genotipeer. Die studie het bevestig dat *P. citricarpa* populasies in China en Australië ʼn groter genetiese diversiteit het as die populasies in Suid-Afrika, Brasilië en die VSA waar meer onlangse introduksies voorgekom het. Paringstipe analise het getoon dat beide paringstipes in populasies van Suid-Afrika, China, Australië en Brasilië in 'n 1:1 verhouding teenwoordig is. Slegs een van die paringstipes is in die VSA populasie gevind. Howel befondsing vir die projek gestaak het, gaan die navorsing steeds voort as deel van ʼn PhD studie. ʼn Wetenskaplike artikel

word tans voltooi, asook navorsing vir die PhD proefskrif. Laasgenoemde sal as finale verslag van hierdie projek dien.

4.6.5 FINAL REPORT: Improving the retention of suspension liquid phosphonate fungicides on citrus fruit and leaves

Project 1012 (April 2011- March 2014) by G.C. Schutte, C. Kotze, and M.C. Pretorius (CRI)

Opsomming

Twee fosfonate is op hulle eie en in kombinasies met twee nuwe eksperimentele bymiddels, naamlik AquaStick en Balista, met behulp van fluorometrie getoets om die retensie van die fosfonate in laboratorium- en veldproewe te bepaal. In laboratoriumproewe waar beide fosfonate teen geregistreerde dosisse op voor- en agterkante van statiese blare gespuit is, toon die resultate dat hulle op hulle eie goeie retensie en kwantitatiewe verspreiding van neerslag tot gevolg gehad het. Meer as 0.5 ml spuitstof per kant per blaar of 1 ml per hele blaar word benodig om enigsins bedekking van Valencia blare te kry met fosfonate. Bymiddel B teen geregistreerde dosisse het die beste kwantitatiewe verspreiding van Fighter aan die onderkante van blare tot gevolg gehad. Dit lyk asof die aflooppunt vir beide bymiddels tussen 4-6 ml/blaar is indien beide kante bespuit word. Geeneen van die bymiddels kon egter die kwantitatiewe neerslag van Rootmaster verhoog nie en van die twee het bymiddel A die swakste gevaar. In ŉ veldproef waar dieselfde behandelings toegedien is en waar blaar- en vrugmonsters kort na die bespuitings getrek is, is getoon dat die waterbehandeling meer neerslag tot gevolg gehad het as die fosfonate op hulle eie. Waar bymiddel A gemeng is met fosfonaat Y, is betekenisvol minder verspreiding van spuitneerslag op blare waargeneem. Op hulle eie asook in kombinasies met eksperimentele bymiddels A en B het Fighter en Rootmaster in al die gevalle meer spuitneerslag op die boonste helfte van vrugte as die onderste helfte tot gevolg gehad. Die uitsondering was bymiddel B met Rootmaster, wat toon dat hierdie produk goeie bedekking oor die hele vrug gegee het in hierdie uitsonderlike geval. Tot dusver blyk dit dat bymiddels nie nodig is om fosfonate beter op blare te laat kleef nie. Daar is gepoog om die volume water wat verlore gaan na 0.5 en 1 ml bespuitings met pigmente op verskillende soorte agtergronde te meet, maar sonder sukses. Lae volume (½x) en normale (1x) bespuitings van Fighter reageer soos kontakdoders vir die eerste twee weke na toediening waarna die sistemiese werking na drie weke eers intree. Medium en hoë volume fosfonaatbespuitings het sedert die dag van toediening vir ŉ verdere periode van twee weke beter beheer as lae volume bespuitings gegee. Goeie bedekking het goeie beheer van Phytophthora bruinvrot tot gevolg gehad en het die fosfonate soos kontakswamdoders gewerk. Nogtans het die lae volume tussen week twee en drie ook goeie beheer begin gee en kan dit aan sistemiese aksie van die produk toegeskryf word.

Summary

In order to determine the retention of the phosphonates in laboratory and field trials, two phosphonates were tested on their own and in combinations with two experimental adjuvants, AquaStick and Balista, using fluorometry. Results from laboratory trials, where both phosphonates were sprayed at registered rates on upper and lower sides of static orange leaves, showed that the retention and quantitative deposition of these phosphonates were good if they were sprayed on their own. More than 0.5 ml spray application per side per leaf or 1 ml per whole leaf is required for effective coverage of Valencia leaves with phosphonates. On the other hand, registered rates of experimental adjuvant B resulted in the best quantitative deposition of Fighter on the lower side of orange leaves. It seems that the run-off point for both adjuvants A and B seems to be between 4-6 ml/leaf if both sides are sprayed. None of the adjuvants could result in an increase in quantitative deposition of Rootmaster and experimental adjuvant A performed the worst of the two adjuvants tested. Leaf and fruit samples from a field trial showed that the water-only treatment resulted in higher deposition quantities on upper and lower leaf surfaces than phosphonates sprayed on their own. Where experimental adjuvant A was added in the tank mixture with Rootmaster, it resulted in significantly less spray deposition on both leaves and fruit. Fighter and Rootmaster resulted in significantly more spray deposition on the upper side of fruit than the lower side of the same fruit. The exception was experimental adjuvant B with Rootmaster, which showed that this product gave good coverage of the whole fruit. Thus far it seems that phosphonates do not need adjuvants to improve their deposition on leaves. It was attempted to determine the amount of spray volume that gets lost after sprays with 0.5 and 1 ml with pigments on different backgrounds without success. Low volume (½x) and normal (1x) spray applications of Fighter react like contact fungicides for the first two weeks after application after which the systemic action kicks in three weeks after application. Medium and high volume phosphonate applications gave better control than the low volume applications from the day of application for two weeks. Good coverage in this case ensured good control of Phytophthora brown rot as the phosphonate acts as a contact fungicide. However, between week two and three, the low volume application also gave good control of Phytophthora brown rot and also performed well. This shows that the systemic action started to kick in and prevented further development of the disease under harsh conditions.

Introduction

Suspension liquid (SL) formulations like phosphonates are registered as foliar applications on citrus for the control of Phytophthora induced diseases but they do have a variety of phytotoxicity problems. Apart from field trials using different rates with different spray intervals to establish effective spray programmes, no one has ever looked scientifically at the target areas (leaves and fruit) and how SL fungicides adhere to the surfaces of citrus leaves and fruit and if adjuvants can increase/decrease their retention. Experiment 918 showed that fluorometry analyses can serve as an indicator of the deposition quantity and quality of residues on citrus fruit and leaves when SC formulations of copper and dithiocarbamates are compared with WP formulations. Residue analysis was done simultaneously with good effect and the variation of residues on citrus fruit and leaves was less if compared with fluorometry.

From associated studies (Fourie *et al.* 2008), we know that run-off curves on leaves and fruit of different ages and cultivars differ significantly. Knowledge of this aspect is vital in customized development of adjuvants, as over- or under-dosing for a given spray volume might respectively lead to excessive run-off or no additional benefit. Likewise, for a given adjuvant concentration, a pronounced difference in the adjuvant effect is experienced with varying spray volumes, especially on hydrophobic surfaces. Through the use of the deposition assessment protocol using fluorometry, digital photomacrography and image analyses the effect of adjuvants in tank mixtures with phosphonates on run-off and spray deposition can be studied. By using both fluorescent pigment deposition analyses and residue analyses of the same samples, one can determine the deposition quantity and quality as well as degradation of phosphonates over time under natural conditions.

Turrell (1961) studied the growth of the photosynthetic area of Valencia orange trees over a 29-year period. The number, size and distribution of leaves on Valencia orange (*Citrus sinensis*) trees of various ages were determined (Fig. 4.x.x.1). The trees used were 3, 6, 12 and 29 years old and 3 to 5 meter in height and 7 to 15 meter in circumference. He determined that the increase in the total leaf number with tree age is given by an equation where N is the estimated number of leaves, $c1$and $n1$ are constants, and α is the age of the tree in years:

$$\text{Log } N = 3.613 + 1.249 \log \alpha$$

Therefore, if this equation is proved to be useful to determine the amount of leaves of Valencia orange trees of a certain age and the amount of spray mix that each leaf should receive and correlates with the actual %Fluo that it did receive, then one can see if adjuvants can contribute in the adhesiveness of phosphonates (and other fungicides) onto citrus leaves and fruit.

Fig. 4.6.5.1. Relationships of leaf numbers of 3-, 6-, 12-, and 29-year-old Valencia orange trees with increase in tree age (Turrell, 1961).

Objectives

The objective of this study was to determine if deposition quantity and quality and retention of phosphonates can be increased/improved using different phosphonate plus adjuvant combinations. Additionally, the effects

of these combinations on potential phytotoxicity were evaluated. The efficacy of low, medium and high phosphonate applications was determined for the control of Phytophthora brown rot.

Materials and methods

Laboratory trial 1

a) Quantitative spray deposition

Fresh Valencia orange leaves were picked from trees at Crocodile Valley Co. and brought to CRI. Here the leaves (three replicates; n =5) were mounted onto a steel frame with a steel grid (4m x 40 cm) using stainless steel clamps spaced at equal distances from each other with leaves slanted at a 60° angle. Phosphonates Fighter and Rootmaster were mixed in water taken from taps at Crocodile Valley Citrus Co. and made up to registered rates in 1 L glass bottles. A gravity feed mist spray gun was mounted onto a compressor and a spray gun (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) with a fluid nozzle tip of 0.8 mm in diameter in a laboratory with post-run-off volumes (*circa* 3 ml per leaf/fruit) with selected phosphonate formulations and varying concentrations of selected adjuvants (2× and 1×) were used. Yellow Fluorescent pigment (400 g/L, EC, South Australian Research and Development Institute, Loxton SA 5333 Australia) at 1 ml/L was added. The spray gun was mounted onto a metal frame at a distance of 60 cm from the target with a spray angle of 90° relative to the target. The phosphonates were sprayed at 1, 2, 3 and 5 ml per leaf, first upper and then lower side and left to dry vertically after each application before they were removed for deposition assessment.

The leaves were then illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken of upper and lower surfaces of leaves and fruit using a Canon EOS 40 D camera equipped with a 50 mm macro lens. Spray deposition assessment involved digital image analyses with Image-Pro Plus version 6.2 software to determine deposition quantity of the fluorescent pigment particles. Deposition quantity analysis involved the measurement of the area covered by pigment particles, expressed as a percentage of total fruit and leaf area. Data were subjected to analysis of variance and Student's T-test for least significant difference (P = 0.05).

b) Residue analyses

After the deposition assessment, the same leaves were put into plastic bags and frozen and submitted for PO_3 analysis (Absolute Science, Silverton, Pretoria, South Africa).

Laboratory trial 2

Methods to determine loss of spray drift

Methods were investigated as to how one can measure excess spray drift sprayed with 0.5 and 1 ml per leaf. It was taken into account that the available surface of light box is 24 x 24 cm square and the surfaces and objects that we have to spray must not exceed that. Black paper without and white A3 paper with 24 x 24 cm squares were mounted and sprayed with Yellow Fluorescent (400 g/L, EC, South Australian Research and Development Institute, Loxton SA 5333 Australia) at 1 ml/L with a gravity feed mist spray gun that was mounted onto a compressor and a spray gun as described above. The fluorescent pigment was difficult to see under UV light. It was also observed that a lot of pigment of 0.5 and 1 ml was observed over the 24 x 24 cm square boundaries needed to determine the quantitative spray deposition. Fuschin acid was then used as replacement for the yellow pigment which was also sprayed on the 24 x 24 squares. Leaves were also mounted in front of the squares and then sprayed with fuschin acid using 0.5 and 1 ml. The leaves were removed and sprayed again to see how the spray deposition varied between the sprays.

Field trial

2011-2012 season

A uniform Glen Ora orchard on 'Cleopatra' mandarin rootstock (*C. reshni*) at Joubert en Seuns was selected. The trees were 8 years old and *ca.* 4 m high. The rows ran directly south-east to north-west. A randomized design with 3 single-tree plots per treatment was used with guard trees between the plots. Trees were uniform in canopy density and tree size. Fungicides were applied on 18 January 2012 to the point of run-off with a trailer-mounted, high-volume, high-pressure (2 500 to 3 000 kPa) sprayer with two hand-held spray guns and received about 25 litres spray mix per tree of treatments listed in Table 4.6.5.1. According to Turrell's (1961) equation (also see Fig. 4.6.5.1) if each tree had about 60 000 leaves per tree, each leaf should receive about

0.42 ml spray mixture as sprayed for a medium cover application (15 L per tree or 0.25 ml per leaf for light cover spray). Fifteen leaves and fruit were sampled from each treatment and the three replicates respectively to determine deposition quantity.

2013 and 2015 field trials for brown rot control

A Navel orange (*Citrus sinensis* (L.) Osbeck) orchard on 'Cleopatra' mandarin rootstock (*C. reshni*) at Crocodile Valley Citrus Co., Nelspruit, was selected. The trees were 17 years old in 2013 and *ca.* 4 m high. The rows ran directly north to south. Trees were selected for uniformity in canopy density and tree size. Fungicides were applied on 2 July 2013 with a Jacto Arbus 2000 airblast sprayer. One treatment consisted of a standard rate as generally used by the Estate (±8000 L/ha) was applied for high volume application. For low volume application the same spray machine was used and calibrated to deliver 2 500 L/ha operated at 10 bar at 3 km/h using one set of Teejet D3 with dc25 hollow cone whirlers alternated with another set of Teejet D2 with dc35 hollow cone whirlers (Fig. 4.6.5.2). For full cover sprays, a trailer-mounted, high-volume, high-pressure (2 500 to 3 000 kPa) sprayer with two hand-held spray guns was used to spray trees to the point of run-off (±25 L/tree). At 540 trees per ha, that accumulates to approximately 13 500 L/ha. The fungicide tested was phosphorous acid (Fighter, 200 SL 555 g/L Ag-Chem Africa, Silverton, South Africa). One hundred milliliters SARDI Yellow Fluorescent Pigment (40% EC; South Australian Research and Development Institute, Loxton SA 5333 Australia) per 100 L water was added to the spray mixture of each fungicide formulation.

Fig. 4.6.5.2. Jacto Arbus 2000 airblast sprayer applying Fighter at low volume applications

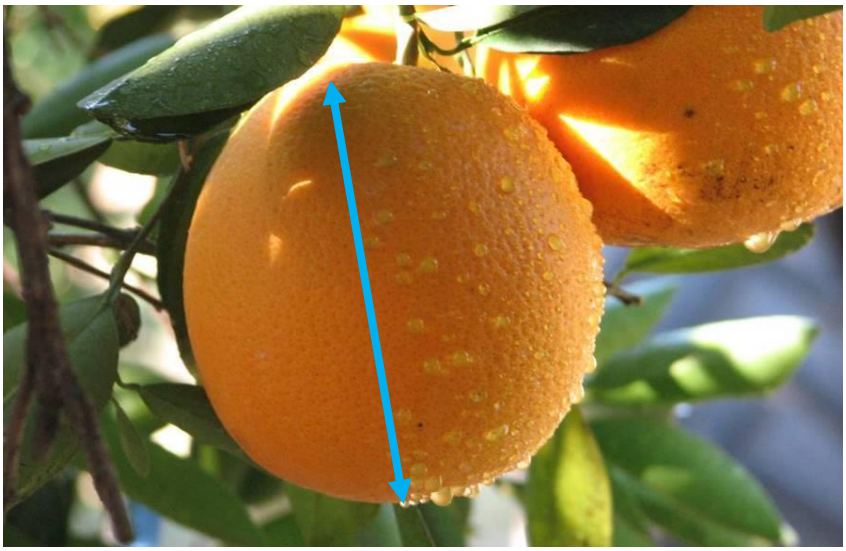

Fig. 4.6.5.3. Low volume application of Fighter with SARDI fluorescent pigment showing coverage of one half of Navel fruit only.

Fruit for bioassay

Twenty mature Navel oranges were randomly harvested from all 4 quadrants, 1 and 2 m above ground level from the outside circumference of each tree on a weekly basis from the day of application for six weeks. They were taken to CRI where they were subjected to a rigid test where fruit were marked to distinguish between the protected and unprotected areas (Fig. 4.6.5.3) using a UV light to illuminate the SARDI yellow fluorescent pigment using a water resistant permanent marking pen (Fig. 4.6.5.4). The fruit were subjected to an in-house method to determine the protection capacity of algaecides against *Phytophthora* as previously described (Fig. 4.6.5.4). The fruit was placed in water in such a manner that at least one half of the fruit with and without protection was exposed to the water containing *Phytophthora nicotianae* var. *parasitica* (Pnp). The soil was tested beforehand to contain Pnp using a citrus leaf disc baiting technique and PARPH selective media. The fruit were inspected on a daily basis for a period of 9 days to determine their Pnp infection status and were also sub-divided into two criteria viz. infection on and outside the protected zones (Fig. 4.6.5.5).

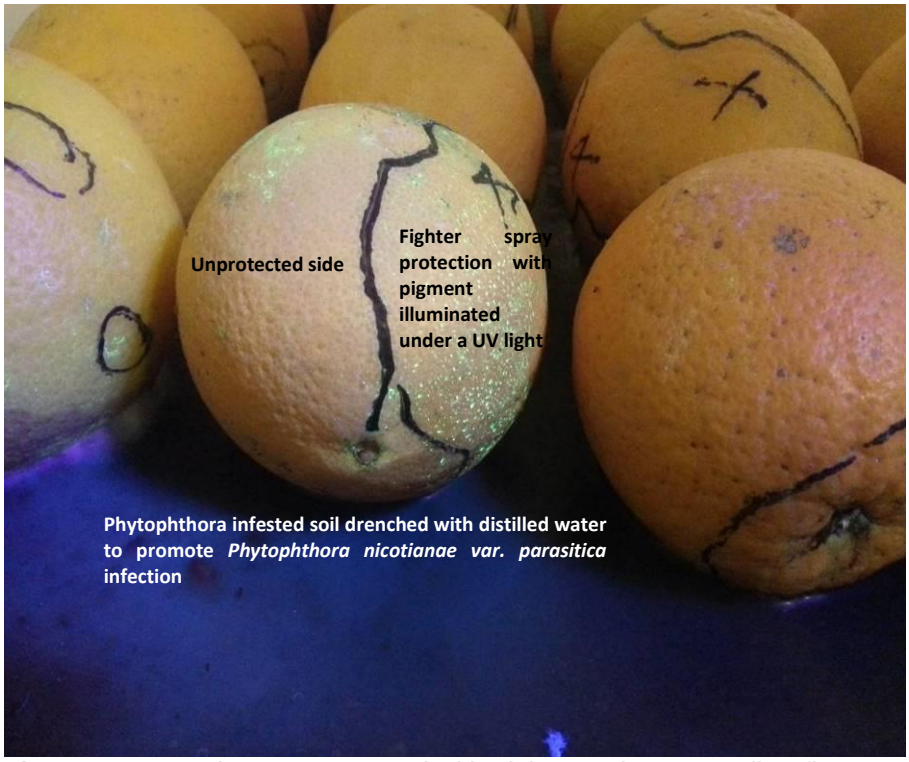

Fig. 4.6.5.4. Navel oranges sprayed with Fighter and SARDI yellow fluorescent pigment to distinguish between protected and unprotected sides of the fruit.

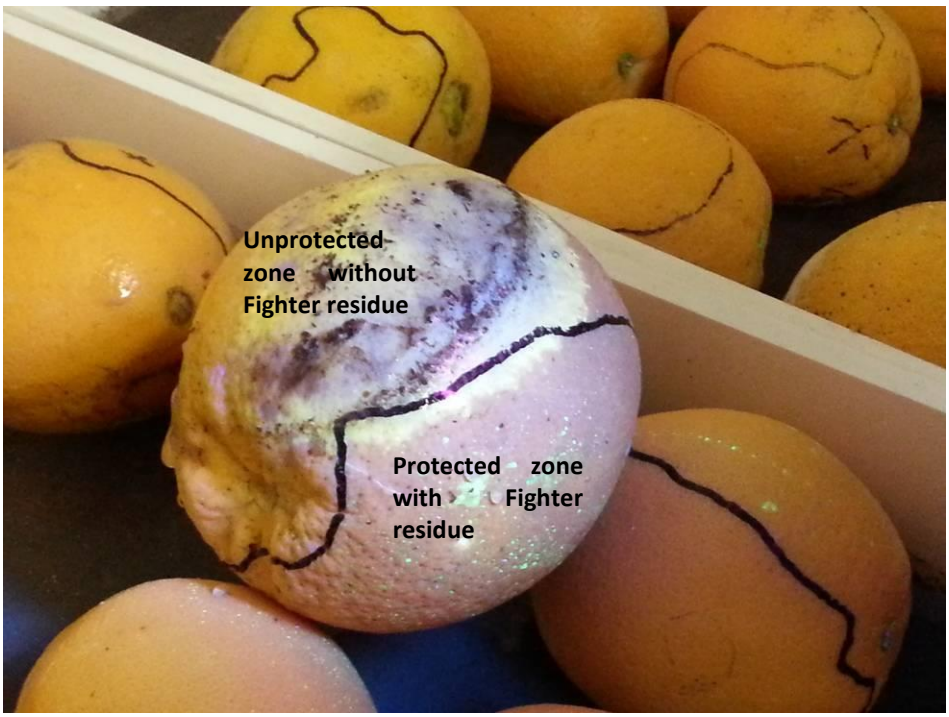

Fig. 4.6.5.5. Evaluation of Navel fruit after 7 days of exposure to infested soil with Pnp showing protective activity after spray application with algaecides.

Results and discussion

Objective / Milestone	Achievement
a) Determining the quantitative spray deposition of several different phosphonates. b) Residue analysis of treated leaves.	a) Individual leaves were sprayed with two different phosphonates in combination with/ without two experimental adjuvants at 4 different volumes. b) Leaves were photographed and subjected to deposition quantity analysis. a) Frozen leaves were sent for analysis, the process was however unsuccessful.
a) Evaluation of methods to determine spray loss due to drift.	a) Black and white paper sheets were respectively sprayed with yellow fluorescent pigment and fuschin, with/without a leaf hanging in front of the sheets. b) Volume of loss could however not be determined, only a visual estimate.
a) Determining the quantitative spray deposition of two different phosphonates under field conditions.	a) Phosphonates mixed with a fluorescent pigment were applied with hand guns to Glen ora navels. b) 15 leaves were removed when dry and photographed. c) Photographs were subjected to quantitative deposition analysis.
a) Evaluation of phosphonates at different volumes to control phytophthora brown rot in orchards.	a) Trees were sprayed with two different phosphonates mixed with a fluorescent pigment at different volumes and rates. b) Fruit were collected from the orchard once a week for 6 weeks after application. c) Collected fruit were inspected under an UV light and rated according to their coverage. d) Fruit were then placed in a soil and water mix and inspected daily for 9 days.

Laboratory trial 1

a) Quantitative spray deposition

Results showed that the total spray deposition of Fighter increased as the spray volume increased (Fig. 4.6.5.6 (A)). On the other hand, the total spray deposition of Rootmaster also doubled from 2 ml to 6 ml spray mix, but decreased at 10 ml/leaf (Fig. 4.6.5.7 (A)). The total spray deposition of Fighter with experimental adjuvant A (AquaStick) at a rate of 50 ml/100 L water showed a slight increase as the rate per leaf was increased but also showed a decrease in spray deposition at the higher rate of 10 ml/leaf. When the rate of experimental adjuvant A was doubled, all the rates showed a decrease in spray deposition showing that this rate will result in excessive run-off of spray material. On the other hand, experimental adjuvant B (Balista) at the registered rate of 3.5 ml/100 L water also doubled in deposition from 2 ml to 6 ml /leaf, but decreased at 10 ml /leaf, similar to 6 ml/leaf. The double rate of experimental adjuvant B (7 ml/100 L water) also showed an increase in deposition from 2 to 4 ml/leaf, but decreased if sprayed at 6 ml /leaf and stayed constant even at a rate of 10 ml/ leaf. This shows that the saturation point for experimental adjuvant B will be at 4 ml/leaf (upper and lower sides) (Fig. 4.6.5.6 (A)).

Fighter

When the spray deposition quantity analysis was divided into separate analyses of upper and lower leaf surfaces, results showed that both these adjuvants resulted in better spray deposition on the lower leaf surfaces than the upper leaf surfaces. The double rate of experimental adjuvant A (100 ml/ 100 L water) with Fighter, performed poorly when compared with the adjuvant's proposed registered rate of 50 ml /100 L water. Fighter without experimental adjuvant A performed even better, showing that the deposition was better on the lower leaf surface than the upper leaf surfaces and that this adjuvant does not contribute to the retention of this fungicide. Experimental adjuvant B evaluated at the 2x rate (7 ml/ 100 L water) with Fighter performed well when 1 and 2 ml spray mix were sprayed onto the leaves and that the lower leaf surfaces had more spray deposition than the upper leaf surfaces. Where the 2x rate declined if sprayed at 3 ml per leaf, the 1x rate increased to its highest deposition at 3 ml per leaf and then declined. The adjuvant rates of 1x and 2x with the Fighter as well as the phosphonate without the adjuvant, all performed the same on upper leave surfaces resulting in the same %FPC of 5% if sprayed at 2, 3, and 5 ml /leaf. Experimental adjuvant B can be recommended as a suitable adjuvant with Fighter (Fig. 4.6.5.6 (B and C)).

Rootmaster

Results showed that Rootmaster increased as the spray volume increased up to 3 ml/ leaf and decreased thereafter (Fig. 4.6.5.7 (A)). When Rootmaster was applied with experimental adjuvant A at the proposed registered rate of 50ml/100l a deposition peak (9.86 %FPC) was achieved at 4 ml per leaf, where after deposition receded. In combination with adjuvant A and B at all the other rates, Rootmaster performed worse than when sprayed on its own. When deposition quantity was separately observed on upper and lower leaf surfaces, adjuvant B at both rates performed consistent on the upper and lower leaf surfaces ranging between 1.93 and 4.29 %FPC (2-5ml). The exception being when 2ml were sprayed on the lower leaf surface a deposition quantity of 6.67 %FPC was achieved. The optimum rate to be effective is therefore 6 ml/leaf or if sprayed at 3 ml on the upper and lower sides of the leaves when Rootmaster is applied on its own.

Adjuvants

When the rate of experimental adjuvant A was doubled, all the rates showed a decrease in spray deposition showing that this rate will result in excessive run-off of spray material. On the other hand, experimental adjuvant B at the proposed registered rate of 7 ml/100 L water was initially better than the 1x or registered rated of 3.5 ml/leaf, but decreased at 10 ml/leaf, similar to 6 ml/leaf. The double rate of experimental adjuvant B (7 ml/100 L water) also showed an increase in deposition from 2 to 4 ml/leaf, but decreased if sprayed at 6 ml/leaf and stayed constant even at a rate of 10 ml/leaf. This shows that the saturation point for experimental adjuvant B will be at 4 ml/leaf (upper and lower sides).

b) Residue analyses

Unfortunately, Absolute Science could not provide any PO_3 residue analyses due to problems with the new analysis technique they had to master. As they are the only lab capable of this kind of analysis, no results could be recorded in this regard.

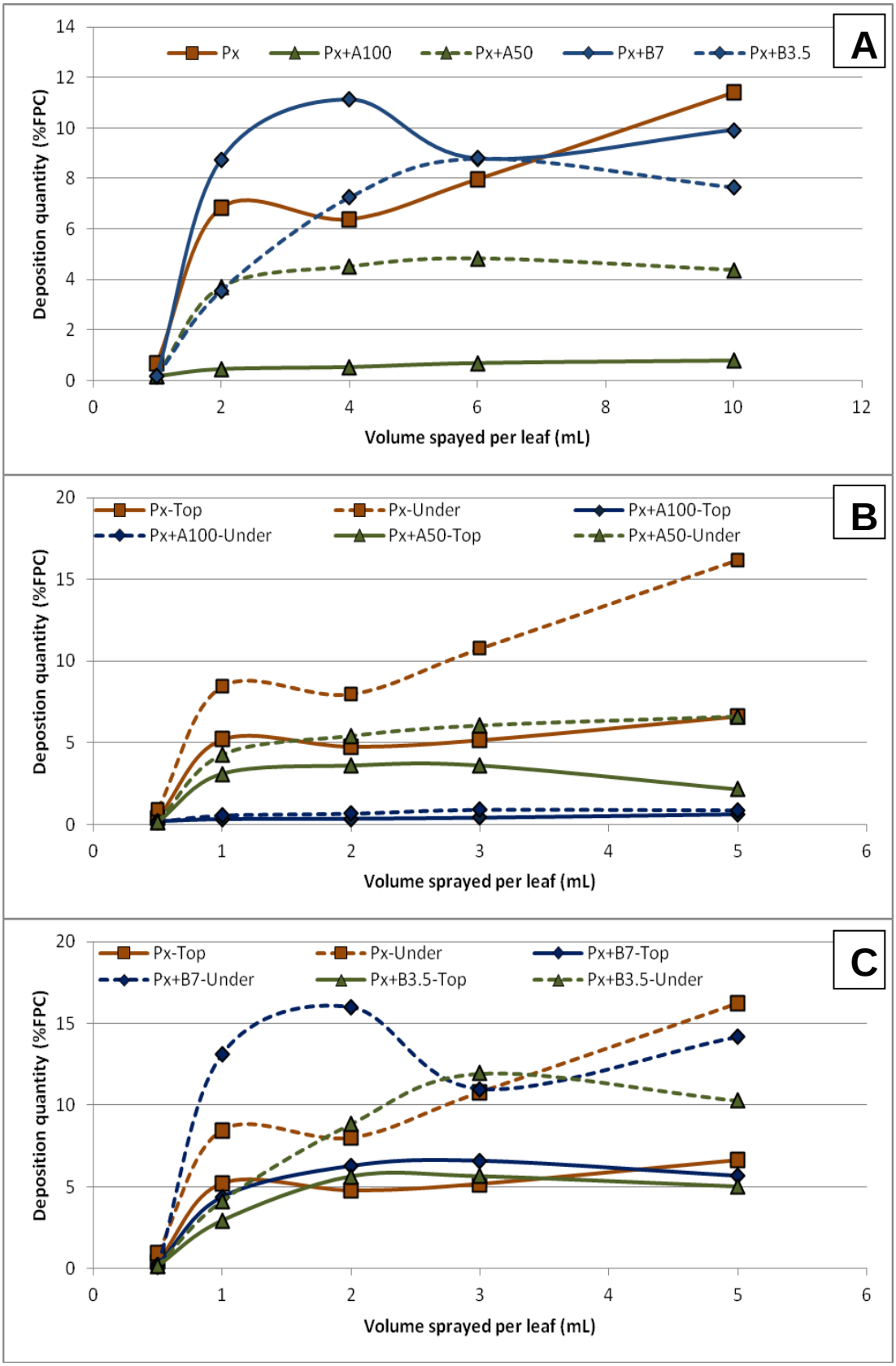

Figure 4.6.5.6. Mean deposition quantity of Fighter (Px) with different rates of adjuvants A and B, for upper and lower leaf surfaces combined (A), upper and lower leaf surfaces, respectively of Fighter with experimental adjuvant A (B) and Fighter with experimental adjuvant B (C), sprayed at predetermined spray volumes per leaf (n = 5) using a gravity fed mist spray gun.

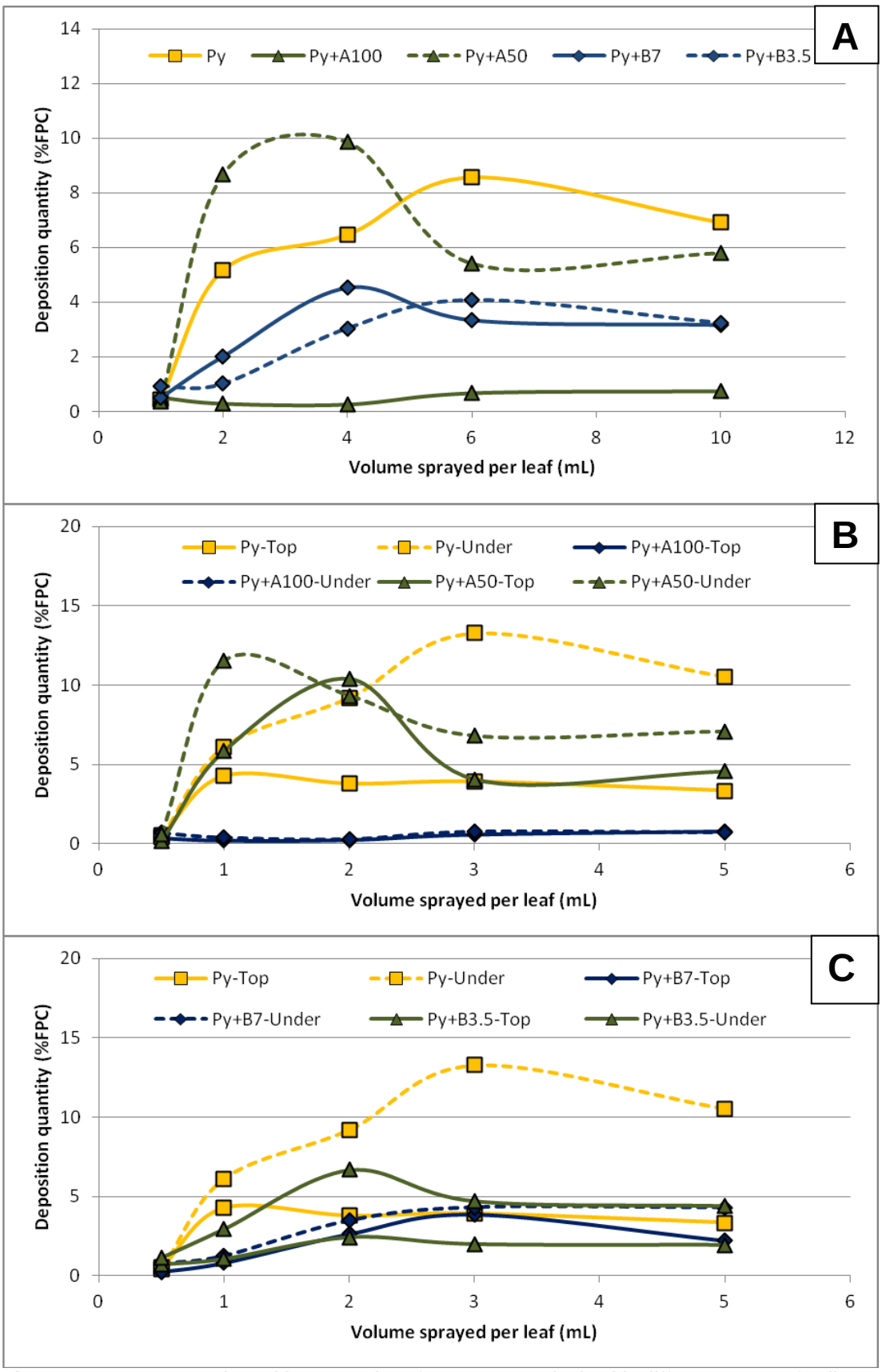

Figure 4.6.5.7. Mean deposition quantity of Rootmaster (Py) with different rates of adjuvants A and B, for upper and lower leaf surfaces combined (A), upper and lower leaf surfaces, respectively of Rootmaster with experimental Rootmaster A (B) and Rootmaster with experimental adjuvant B (C), sprayed at predetermined spray volumes per leaf (n = 5) using a gravity fed mist spray gun.

Laboratory trial 2

Methods to determine loss of spray drift

Both Figures 4.6.5.6 and 4.6.5.7 give an indication of the excess spray generated at low volumes of 0.5 ml and 1 ml per leaf. In a quest to determine the magnitude of excess, fluorescent pigment and fuschin was used as colouring when sprayed on black and white paper sheets, respectively. The fluorescent pigment was too difficult to distinguish from the black paper when observed under UV light (Fig. 4.6.5.8). Quantity analysis could therefore not be executed. The fuschin was therefore sprayed on white paper with and without a leaf placed in front thereof (Fig. 4.6.5.9). Unfortunately, through this method the amount of loss could not be quantified and was only used to show as an example.

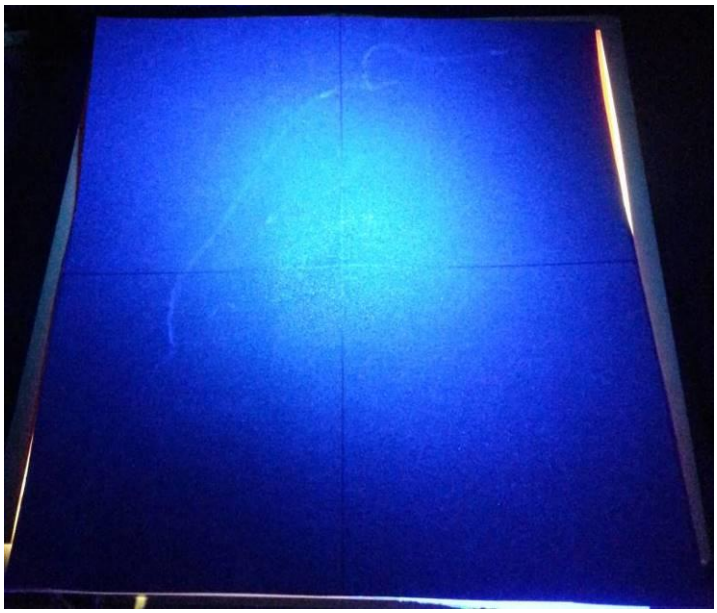

Figure 4.6.5.8. Black paper (24 x 24 cm) sprayed with 1 ml yellow pigment showing reflection of UV light making it difficult to photograph with some pigment spread over the borders of the square.

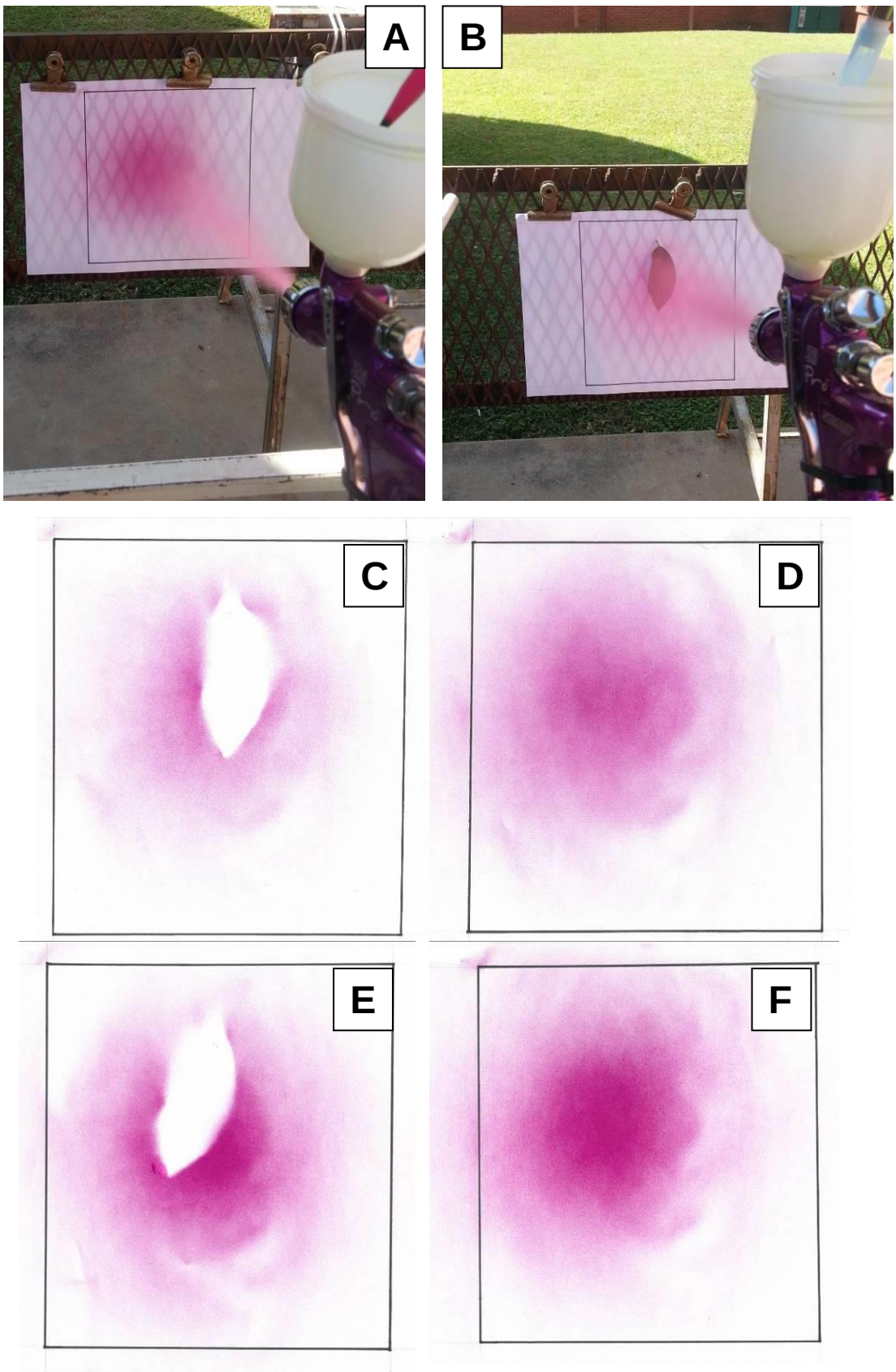

Fig. 4.6.5.9. Fuschin sprayed on white paper with (A) and without a leaf (B) using a Devilbiss spray gun at rates of 0.5 mℓ with (C) and without a mounted leaf (D) and 1 mℓ also with (E) and without a mounted leaf (F).

Field trials

2011-2012 season

Combined upper and lower deposition quantity analysis of fruit indicated that there was not a significant difference between the Rootmaster (5.48%), Fighter (6.01%) and water treatments (6.06%) (Table 4.6.5.1). However, when combined with Adjuvant A and B deposition quantity receded significantly, except in the case of Rootmaster sprayed with Adjuvant B (3.5ml/100L) (5.43%). When observing the combined leaf deposition quantity analysis, the water treatment is significant higher than the other treatments (6.46%). Furthermore, when Rootmaster was sprayed with Adjuvant B there was significantly less fluorescent pigment observed (2.69%). When separating the upper and lower deposition analysis' of respectively fruit and leaves, both incidences indicated a higher coverage on the upper than the lower surfaces. The only exception being the Rootmaster adjuvant B combination, 5.17% upper to 5.29% lower surface coverage. Concluding, in all cases Rootmaster in combination with Adjuvant A performed statistically the worst. While there was a clear tendency of upper surface coverage being higher than lower surface coverage, as would be expected.

Table 4.6.5.1. Mean quantitative deposition of Fighter and Rootmaster with different rates of experimental adjuvants A and B on leaf surfaces (A), upper and lower leaf surfaces and upper and lower fruit surfaces after field application with hand lances on Glen Ora trees at Joubert en Seuns on 18 January 2012.

Treatment	Mean quantitative deposition (% Fluo)[z]					
	Leaves (upper and lower)	Fruit (upper and lower)	Upper leaf surface	Lower leaf surface	Upper fruit surface	Lower fruit surface
Fighter	5.42 b	6.01 d	5.62 c	5.22 bc	7.15 h	4.89 cd
Fighter + Experimental adjuvant A	4.94 b	4.52 b	5.67 bc	4.21 b	5.17 de	3.88 ab
Fighter + Experimental adjuvant B	4.88 b	4.63 b	5.31 bc	4.46 b	5.53 de	3.73 ab
Rootmaster	5.28 b	5.48 cd	5.78 c	4.78 bc	7.06 gh	3.91 ab
Rootmaster + Experimental adjuvant A	2.69 a	3.93 a	2.88 a	2.51 a	4.37 bc	3.49 a
Rootmaster + Experimental adjuvant B	5.22 b	5.43 c	5.17 bc	5.29 bc	5.69 ef	5.18 de
Water	6.46 c	6.06 d	7.12 d	5.82 c	6.35 fg	5.78 ef

[z] Means (based on 3 replicates) followed by the same letter do not differ significantly ($P < 0.05$).

2013 and 2015 field trials

Results from both field trials showed that the medium and high volume phosphonate applications gave better control than the low volume applications from the day of application for two weeks. Good coverage in this case ensured good control of Phytophthora brown rot as the phosphonate acts as a contact fungicide. However, between week two and three, the low volume application also gave good control of Phytophthora brown rot and also performed well. This shows that the systemic action started to kick in and prevented further development of the disease under harsh conditions. This study confirms previous finding by Schutte et al. (1991) that with the ambimobility of phosphonates, it takes about 2 weeks to reach high residue levels in foliar parts of the tree. Therefore, growers must note that if they want to harvest within two weeks after application, a medium cover is required because the fungicides act as a contact fungicide. If they plan to harvest the fruit later on in the season (3+ weeks after application), then the low volume application will be effective as the ambimobility will kick in to allow effective control of the disease (Fig. 4.6.5.10 and Fig. 4.6.5.11).

Conclusion

The retention of Fighter and Rootmaster were tested on their own and in combinations with two adjuvants in laboratory and field trials using fluorometry. Leaf and fruit samples from a field trial showed that the water-only treatment resulted in higher deposition quantities of upper and lower leaves than phosphonates sprayed on their own. Phosphonates do not need adjuvants to improve their deposition on leaves. It was attempted to determine the amount of spray volume that gets lost after sprays with 0.5 and 1 ml with pigments on different

backgrounds without success. Low volume (½x) and normal (1x) spray applications of Fighter react like contact fungicides for the first two weeks after application after which the systemic action kicks in three weeks after application. Medium and high volume phosphonate applications gave better control than the low volume applications from the day of application for two weeks. Good coverage in this case ensured good control of Phytophthora brown rot as the phosphonate acts as a contact fungicide. However, between weeks two and three, the low volume application also gave good control of Phytophthora brown rot and also performed well. This shows that the systemic action started to kick in and prevented further development of the disease under harsh conditions.

Future research

None

Technology transfer

Talks were presented at the 2012 and 2014 Citrus symposia and various study groups and workshops.

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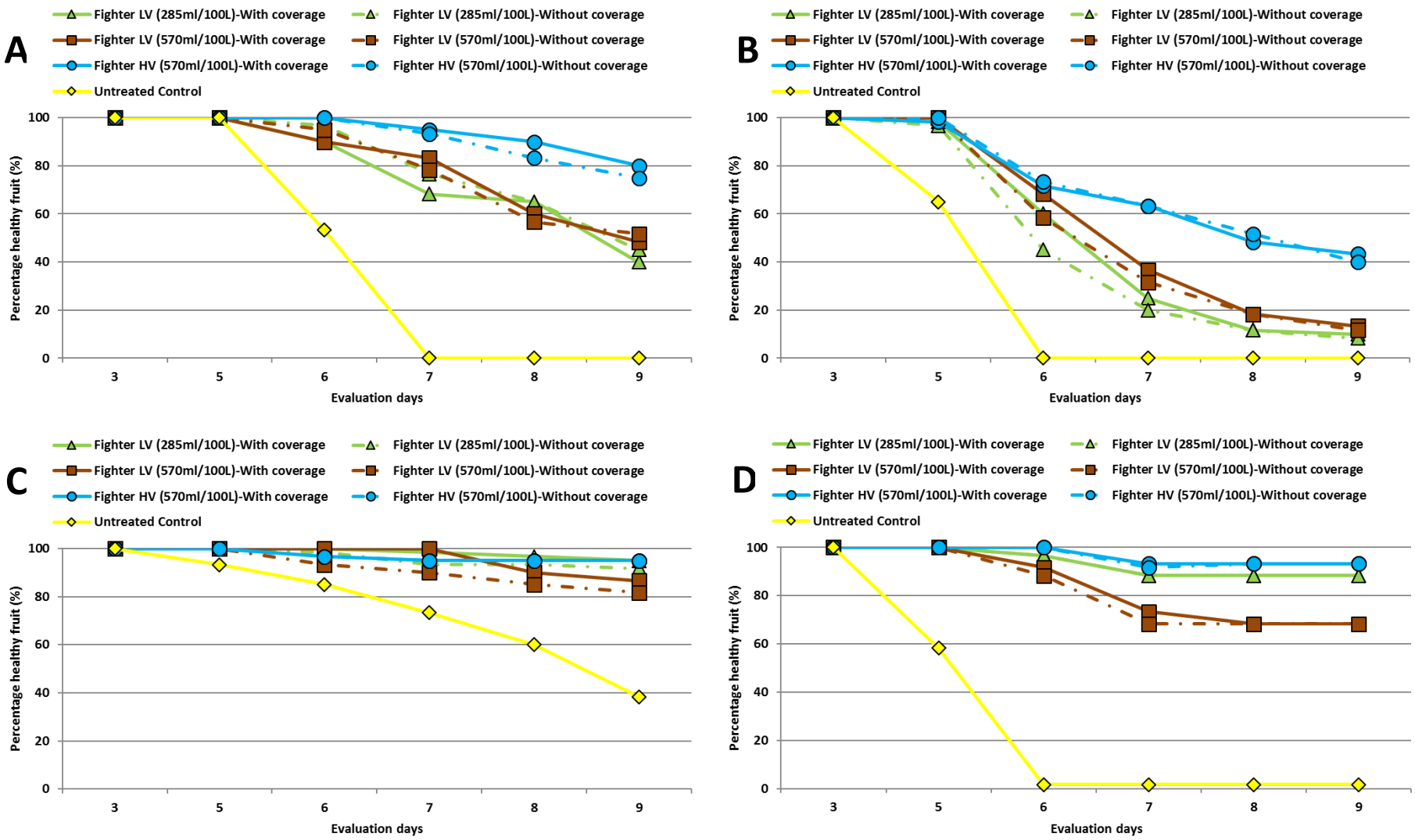

Fig. 4.6.5.10. Mean percentage navel fruit that remained healthy after 3, 5, 6, 7, 8 and 9 days' incubation under optimal growing conditions to determine the susceptibility to *Phytophthora nicotianae* var. *parasitica*, the cause of brown rot of citrus. Fruit were sampled over a period of 1(A), 2 (B), 3 (C) and 5 (D) weeks after a low (LV) and high volume (HV) application of Fighter at the registered (1x) and reduced (½ x) rates were applied in the orchard during 2013.

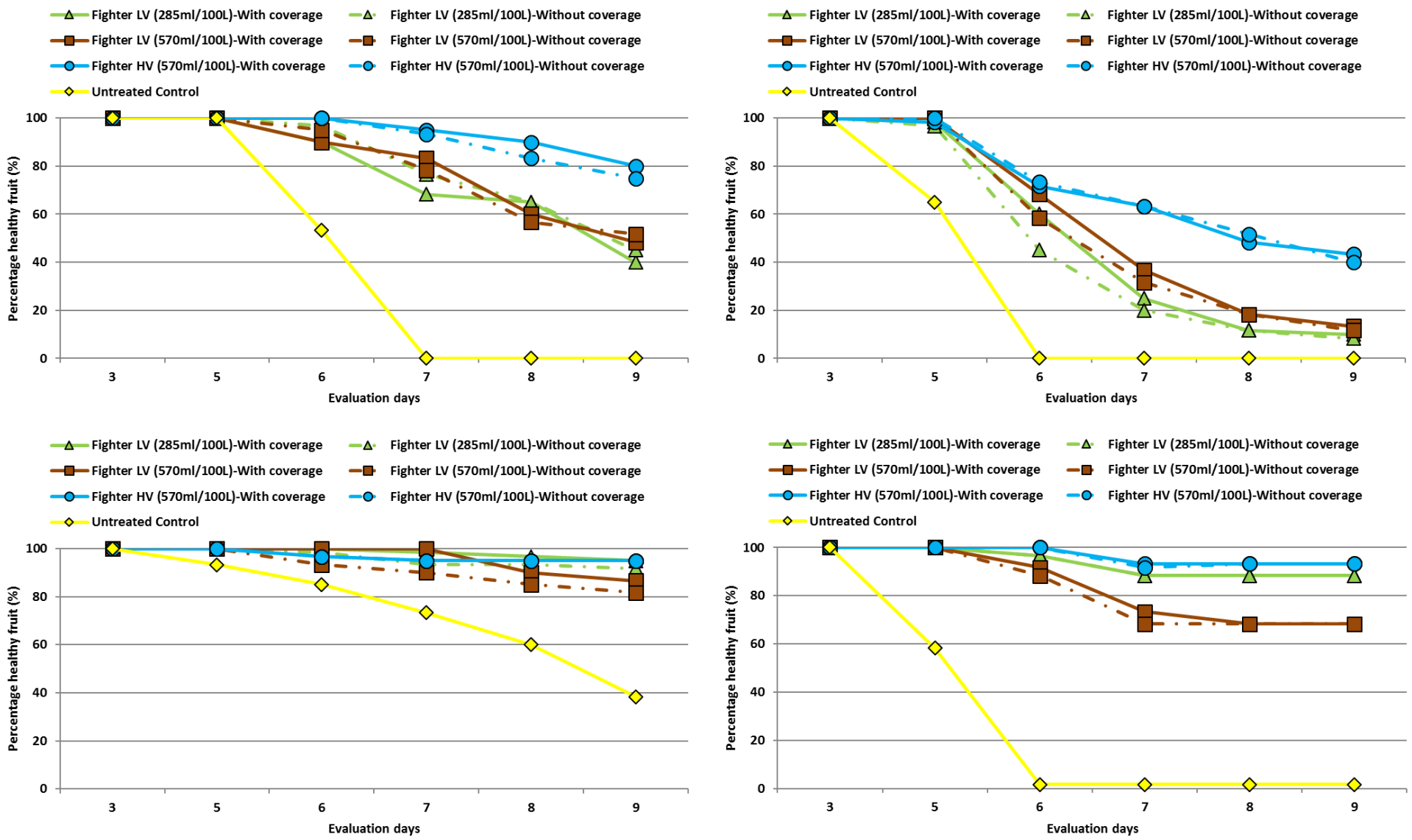

Fig. 4.6.5.11. Mean percentage navel fruit that remained healthy after 3, 5, 6, 7, 8 and 9 days' incubation under optimal growing conditions to determine the susceptibility to *Phytophthora nicotianae* var. *parasitica*, the cause of brown rot of citrus. Fruit were sampled over a period of 1(A), 2 (B), 3 (C) and 5 (D) weeks after a low (LV) and high volume (HV) application of Fighter at the registered (1x) and reduced (½ x) rates were applied in the orchard during 2015.

4.6.6 **FINAL REPORT: Identifying the fungi that cause CBS-like disease symptoms on citrus fruit**
Project 1088 (2014 – 2017) by GC Schutte, E Basson, C Kotze (CRI), T Jensen and S Coertze (SU)

Opsomming

Colletotrichum sp. en geen *Phyllosticta citricarpa* is van letsels soortgelyk aan sitrus swartvlek op pomelos geïsoleer. Hierdie isolate is toe onderwerp aan molekulêre identifikasie en twee spesies, genaamd *Colletotrichum gloeosporioides* en *C. boninense*, is hierdeur geïdentifiseer. Olinda Valencia vrugte is toe aan die bome met spoorsuspensies (1×10^7 spore/ml) van beide spesies geïnokuleer; een stel waar die vrugte gewond is en een stel waar vrugte ongewond gelaat is. Na 4 weke is die vrugte geoes, ondersoek vir letselvorming en isolasies uitgevoer is om Koch se postulate te voltooi. Die resultate wat hieruit verhaal is, was egter nie beslissend nie weens die rede dat geen van die isolate wat inokuleer is weer geïsoleer is nie. Gedurende die 2014-15 seisoen, is die proef op onvolwasse en volwasse Olinda Valencia vrugte herhaal; nes met die vorige seisoen is letsels slegs waargeneem op gewonde vrugte. Tydens hierdie seisoen is *C. gloeosporioides* vanuit letsels op onvolwasse vrugte geïsoleer. Dit was egter uit letsels (11-15%) wat deur elkeen van die isolate inokuleer is. Isolasies vanuit letsels op onvolwasse vrugte het geen van die geïnokuleerde isolate opgelewer nie wat aandui dat die vrugte se vatbaarheid dalk met ouderdom verander. In gevolgtrekking, kon sitrus swartvlek tipe letsels nie nageboots word deur die uitvoering van hierdie projek nie en bly die oorsaak van hierdie tipe letsels onbekend te wees.

Summary

Colletotrichum sp., and no *Phyllosticta citricarpa*, were isolated from grapefruit with symptoms similar to that of citrus black spot. These cultures were subjected to molecular identification and two predominant species were identified through this process, both belonging to the *Colletotrichum* genus, namely *C. gloeosporioides* and *C. boninense*. During the 2013-14 season mature Olinda Valencia fruit were inoculated on the trees with a spore suspension (1×10^7 spore/ml) of either species; one set of fruit wounded and the other set left unwounded. After 4 weeks the fruit were evaluated in regards to lesion formed and lesion size, where after isolations were made to fulfil Koch's postulates. The results were, however, inconclusive as the inoculated pathogens were not re-isolated. During the 2015-16 season the trial was duplicated on mature and immature fruit, and similar to the previous season lesions were only formed on wounded fruit. *C. gloeosporioides* was re-isolated from 11-15% of wounds on immature fruit inoculated with both isolates, respectively. The mature fruit yielded none of the inoculated isolates, suggesting a changing in susceptibility of fruit with maturity. In conclusion, citrus black spot like lesions could not be replicated during the course of the trial and the cause of these types of lesions remains unknown.

Introduction

During a recent study, fruit were inspected for citrus black spot (CBS) lesions. Out of the 136 lesions molecularly identified, only 6 were found to be *Phyllosticta citricarpa*, the causal pathogen of CBS. However, *Colletotrichum* spp. was isolated from several unidentified lesions. *Colletotrichum* spp. are known to cause three different anthracnose diseases of citrus: post bloom fruit drop and lime anthracnose caused by *C. acutatum* and post-harvest anthracnose caused by *C. gloeosporioides. C. gloeosporioides* is a primary coloniser of injured and senescent tissue and causes an important post-harvest disease but is incapable of invading healthy tissue.

By definition the term anthracnose is commonly applied to lesions containing acervuli and is in most cases associated with the bruised or injured rind of a citrus fruit. In the case of lime anthracnose the pathogen infects the juvenile tissue of Mexican lime and can in some cases cause localised necrotic lesions of variable sizes. It has further been documented that *Colletotrichum* spp. co-inhabits citrus black spot lesions, but could they be responsible for CBS type lesions? Therefore, the reason for the current study, to investigate the ability of *Colletotrichum* spp. to produce CBS like lesions.

Objectives

To collect isolates for identification and to construct pictorial sheets for the identification of CBS-like lesions on citrus fruit confused with CBS.

Materials and methods

Isolation and identification

In a joint CBS project between SA and the USA (project 1026) Star Ruby grapefruit were obtained from GFC at Komatipoort for two seasons. All the fruit was packed into 20 kg boxes and cold stored at CRI over a period of time. Inspections were done on the day of arrival and two more inspections were done two and four weeks later.

Fruit were inspected for lesions resembling citrus black spot (Fig. 4.6.6.1. A-D). All visible lesions were photographed, isolated from and identified using PCR to determine the percentage of CBS lesions that came directly from the orchard to the pack house, and lesions that were intercepted in the pack house. Isolations were made from the effected rind onto potato dextrose agar (PDA) and incubated at 25°C for 7 to 14 days. Isolated cultures were sub-cultured and sent to the University of Stellenbosch for identification. Cultures were identified molecularly by internal transcribed spacer region (ITS) sequence analyses. These sequences were subsequently blasted on Genbank for identification.

2013-14 Season

Inoculation of fruit

Untreated, lesion free fruit were selected from a 27-year-old Olinda Valencia orchard at Crocodile Valley Citrus Estate in Nelspruit, Mpumalanga, South Africa. A sealing strip was cut into 1 cm × 2 cm segments and a hole punched through the centre of each segment, using a cork borer. Holes made had a diameter of ±5 mm. Each segment was separately pasted onto 20 randomly selected fruit and inoculated with a 0.25 ml droplet containing a 1×10^7 spores/ml spore suspension of either *Colletotrichum gloeosporoides* or *C. boninense*. In each set of fruit 20 fruit were wounded with an alcohol sterilised dissect needle before inoculation, while the other half was left unwounded. Each set was repeated on four different trees. After inoculation, the sealing strip segments containing the spore concentration were sealed with Parafilm® and each fruit enclosed in a plastic bag as a humidity chamber (Fig. 4.6.6.2. A-F).

Re-isolation of inoculated fungi

Four weeks (28 days) after inoculation the fruit were collected from the orchard and inspected for lesions in the inoculation area (Fig. 4.6.6.2 A-F). Due to very little lesion formation, the fruit were stored at ambient temperature for an extra two weeks (14 days) whereafter a second inspection was conducted. Lesions were measured using a calliper, photographed and isolations made. From each lesion four 1 mm × 1 mm pieces of infected rind were isolated onto PDA and incubated at 25°C for 7 to 14 days. Cultures isolated were then initially identified morphologically to determine if molecular identification would be necessary.

2014-15 Season

Inoculation of fruit

Untreated lesion free fruit were selected from the same 28-year-old Olinda Valencia orchard at Crocodile Valley Citrus Estate in Nelspruit, Mpumalanga, South Africa. Two stages of fruit development were evaluated for susceptibility namely: immature (green) fruit and mature (coloured) fruit. The first stage was inoculated on 28 March 2015 and the second stage on 23 July 2015. A sealing strip was cut into 1 cm × 2 cm segments and a hole punched through the centre of each segment, using a cork borer. Holes made had a diameter of ±5mm. Each segment was separately pasted onto 10 randomly selected fruit and inoculated with a 0.25 ml droplet containing a 1×10^7 spores/ml spore suspension of either *Colletotrichum gloeosporoides* or *C. boninense*. Of each set of 10, half of the fruit were wounded with an alcohol sterilised dissect needle before inoculation, while the other half was left unwounded. As untreated control, 5 fruit per set were wounded with an alcohol sterilised dissect needle and left uncovered. Each set was repeated on four different trees. After inoculation, the sealing strip segments containing the spore concentration were sealed with Parafilm® and each fruit enclosed in a plastic bag as a humidity chamber.

Re-isolation of inoculated fungi

Four weeks (28 days) after each of the inoculation stages, the fruit were collected from the orchard and inspected for lesions in the inoculation area (Fig. 4.6.6.2. A-F). Lesions were defined as all wounds surrounded by a form of discoloration, the area was measured using an electronic calliper, photographed and isolations made. From each lesion four 1 mm × 1 mm pieces of infected rind were isolated onto PDA and incubated at

25°C for 7 to 14 days. Results were calculated as the percentage culture growth per lesion. Cultures isolated (Fig. 4.6.6.3 A and B), were initially visually identified where after these identifications were molecularly verified by internal transcribed spacer region (ITS) sequence analyses at the University of Stellenbosch.

Results and discussion

Objective / Milestone	Achievement
Apr –Jun 2014 1. Collect fruit samples from packhouses in Nelspruit, Karino and Komatipoort with CBS-like symptoms. 2. Isolate fungi for Koch's postulates, PCR and ITS sequencing.	1. Fruit were collected from Vergenoegd packhouse in Komatipoort and inspected for CBS-like symptoms. 2. *Colletotrichum* isolates were identified by the Plant Pathology department of the University of Stellenbosch.
Jul – Sept 2014 1. Collect more fruit samples from packing houses in Nelspruit, Karino and Komatipoort with CBS-like symptoms. 2. Isolate fungi for Koch's postulates, PCR and ITS sequencing.	1. Mature fruit were inoculated in an untreated orchard at Crocodile Valley estates in Nelspruit. 2. Fruit were collected from orchard and evaluated.
Oct – Dec 2014 1. Inoculate fruit with different isolates to fulfil Koch's postulates	1. Milestone already achieved.
Jan – Mar 2015 1. Inoculate fruit with different isolates to fulfil Koch's postulates	1. Green fruit were inoculated in an untreated orchard at Crocodile Valley Estates in Nelspruit.

2013-14 Season

Some of the lesions that were identified and subjected to isolations were smaller than 2 mm in diameter. Two species of *Colletotrichum* were isolated from these lesions and molecularly identified as *Colletotrichum gloeosporioides* and *C. boninense*. Both are known anthracnose pathogens, but also known to be endophytic in nature on a wide range of host plants. *C. boninense* is recently reported to occur on more and more hosts infecting twigs, leaves and berries (Damm et al., 2012) and also identified as one of the causal agents of anthracnose in avocado fruit in Mexico (Silva & Avila-Quezada, 2011). Inoculations induced lesions on the wounded fruit only and resulted in a 65% and 70% lesion forming success rate for *C. gloeosporioides* and *C. boninense*, respectively (Table 4.6.6.1).

Moreover, there was no statistical difference in the mean lesion size for each of the species with mean lesion sizes ranging between 3.1 mm and 3.2 mm. This could be a further indication that these fungi need a rind injury to induce lesions or to colonise CBS lesions after formation (Wager, 1952; Kotze, 1981). However, the back-isolations produced none of the inoculated fungi, and Koch's postulates could therefore not be fulfilled. The reason for poor isolation success might be the high temperature reached inside of the humid chambers or to the fact that the inoculation process has not been perfected yet. Another reason could be that the fruit were resistant to infection by the time of inoculation and future research would need to focus on inoculations at different maturity stages.

2014-15 Season

Lesions were formed on wounded inoculated fruit only, inducing a 100% and 90% (Table 4.6.6.2**)** success rate for *C. boninense* and *C. gloeosporioides*, respectively during the first inoculation phase. During the second inoculation phase a lesion forming success rate of 70% and 40% (Table 4.6.6.3) was achieved for *C. boninense* and *C. gloeosporioides*, respectively. The untreated controls did not form any kind of discoloration of the tissue around the created wounds. Furthermore, isolations from these resulting lesions yielded an array of different secondary infecting fungi, combining for a total incidence of 32.78% (Table 4.6.6.2**)** and 37.5% (Table 4.6.6.3) for the first and second inoculation period respectively.

During the first inoculation stage, *C. gloeosporioides* was isolated from both sets of inoculated wounds (15% and 11.11%; Table 4.6.6.2) while none of the isolations yielded any *C. boninense*. This suggests that either the inoculations were unsuccessful or that *C. gloeosporioides* is a stronger colonizer and that the subsequent inoculations were only responsible for ±4% incidence. From the second stage of inoculation none of the inoculated wounds yielded the inoculated pathogen. This could be attributed to the fruits' susceptibility changing with maturity. With regard to lesion size, there was no statistical difference in lesions formed by *C. boninense* or *C. gloeosporioides* during each of the inoculation periods. Average lesion size for the two periods ranged between 2.03 and 1.37 mm (Table 4.6.6.2; Table 4.6.6.3).

Conclusion

From the varying results it is difficult to make any conclusion, and the desired result of imitating citrus black spot lesions was not achieved. Ultimately both of these isolates can still only be seen as co-inhabitants of lesions and the true cause of the CBS-like lesions is still unknown.

Technology transfer

Data will be presented at the biennial CRI Symposium in August 2016.

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Table 4.6.6.15. Percentage lesions formed and average lesion size on Olinda Valencia fruit inoculated with *C. boninense* and *C. gloeosporioides* and inspected for lesion formation 42 days after inoculation.

Species	Wounded/Unwounded	Lesions formed (%)	Lesion size (mm)[x]
C. boninense	Wounded	70	3.2a
	Unwounded	0	-
C. gloeosporioides	Wounded	65	3.1a
	Unwounded	0	-

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

Table 4.6.6.2. Percentage lesions formed, average lesion size and re-isolation incidence of *C. boninense* and *C. gloeosporioides* observed from immature Olinda Valencia fruit 28 days after inoculation.

Species	Wounded/ Unwounded	Lesions formed (%)	Lesion size (mm)[X]	Incidence of fungi isolated (%)[Y]		
				C. gloeosporioides	*C. boninense*	Other
C. boninense	Wounded	100	1.90a	15.00a	0.00	5.00a
	Unwounded	0	-	-	-	-
C. gloeosporioides	Wounded	90	1.73a	11.11a	0.00	27.78a
	Unwounded	0	-	-	-	-

[X]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level (LSD = 0.4867)
[Y]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level (respective LSD values 26.66 and 23.74)

Table 4.6.6.3. Percentage lesions formed, average lesion size and re-isolation incidence of *C. boninense* and *C. gloeosporioides* observed from mature Olinda Valencia fruit 28 days after inoculation.

Species	Wounded/ Unwounded	Lesions formed (%)	Lesion size (mm)[X]	Incidence of fungi isolated (%)		
				C. gloeosporioides	*C. boninense*	Other[Y]
C. boninense	Wounded	70	2.03a	0.00	0.00	6.25a
	Unwounded	0	-	-	-	-
C. gloeosporioides	Wounded	40	1.37a	0.00	0.00	31.25a
	Unwounded	0	-	-	-	-

[X]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level. (LSD = 0.8835)
[Y]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level. (LSD =25.43

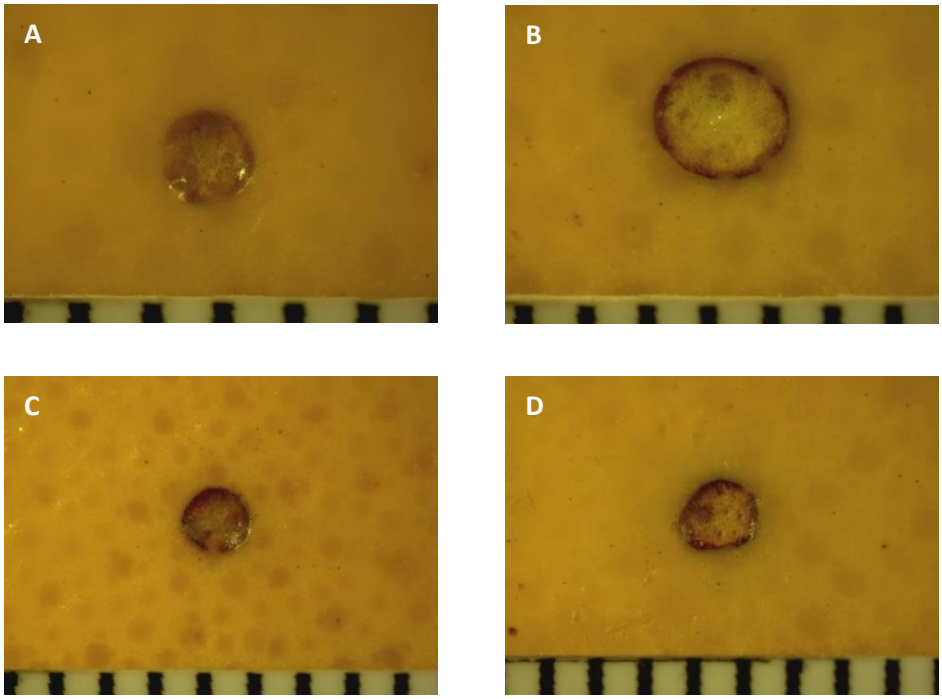

Fig. 4.6.6.1. Citrus-black-spot-like symptoms without pycnidia on grapefruit

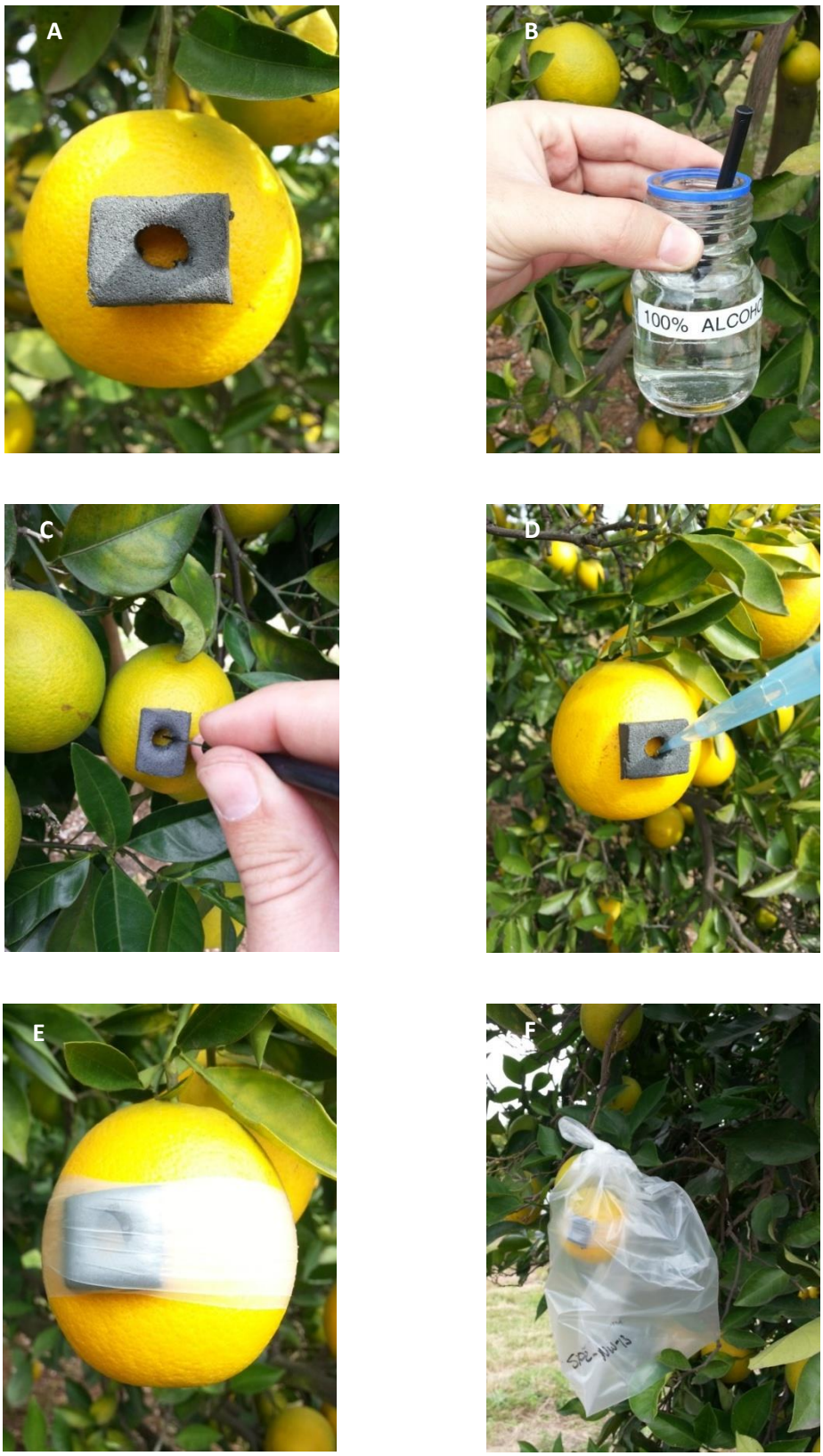

Fig 4.6.6.2. Preparation and inoculation of Valencia oranges with *Colletotrichum* isolates to fulfil Koch's postulates.

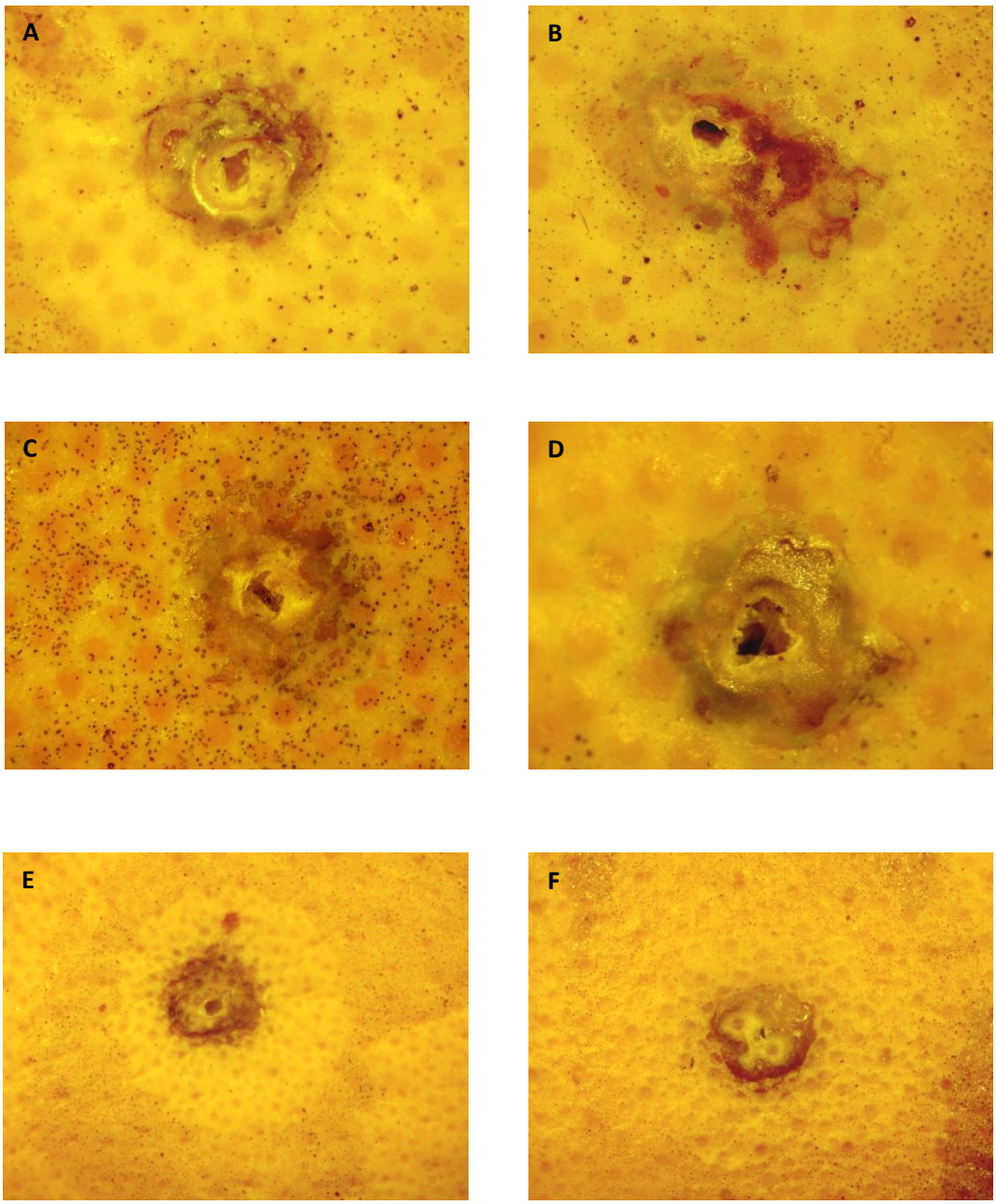

Fig. 4.6.6.3. Artificially infected fruit inoculated with *C. boninense* (A, C, E) and *C. gloeosporioides* (B, D, F) 42 days after inoculation.

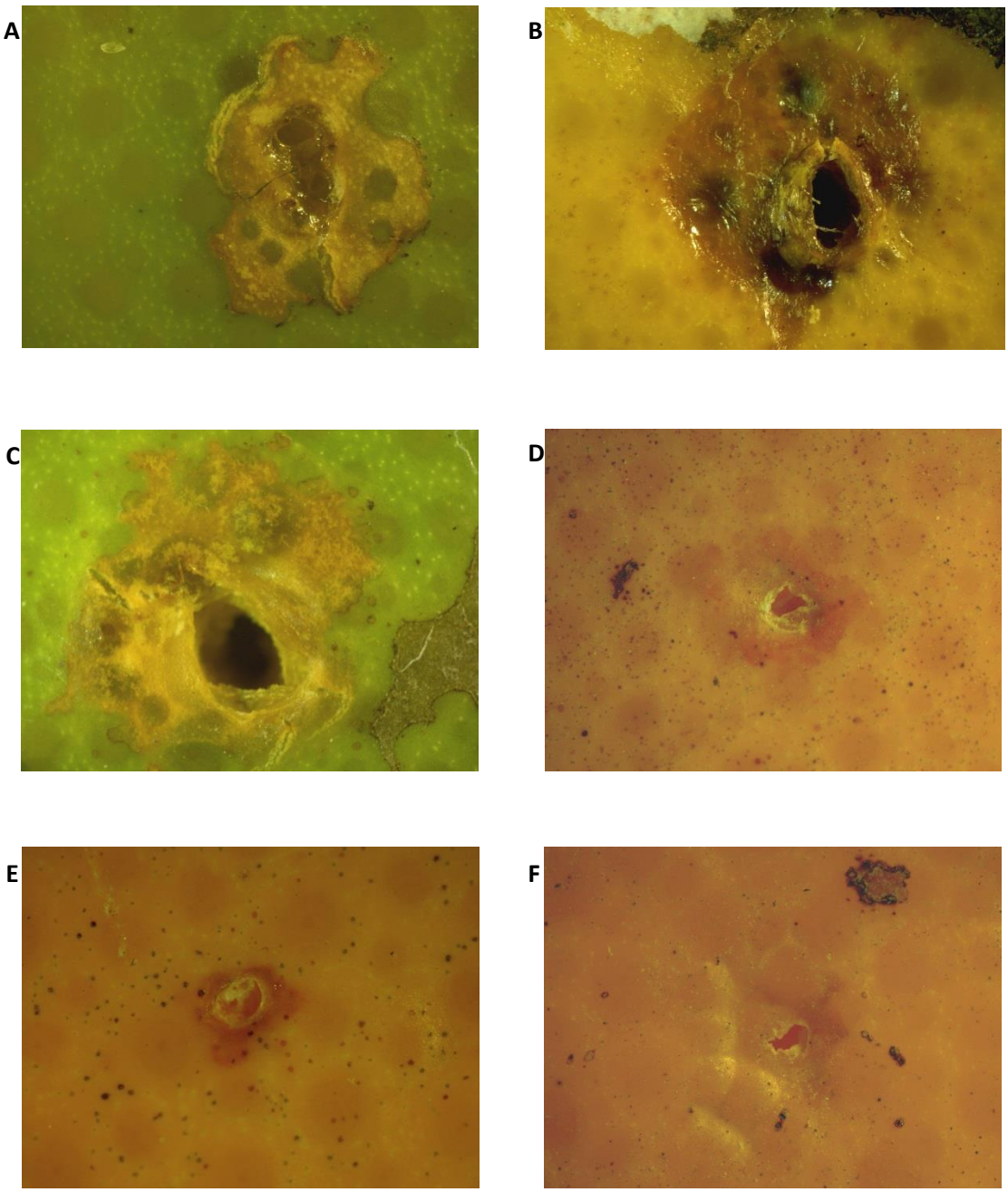

Fig. 4.6.6.4. Artificially infected fruit; *first* stage lesion formation (A-C) and *second* stage lesion formation (D-F) 28 days after inoculation

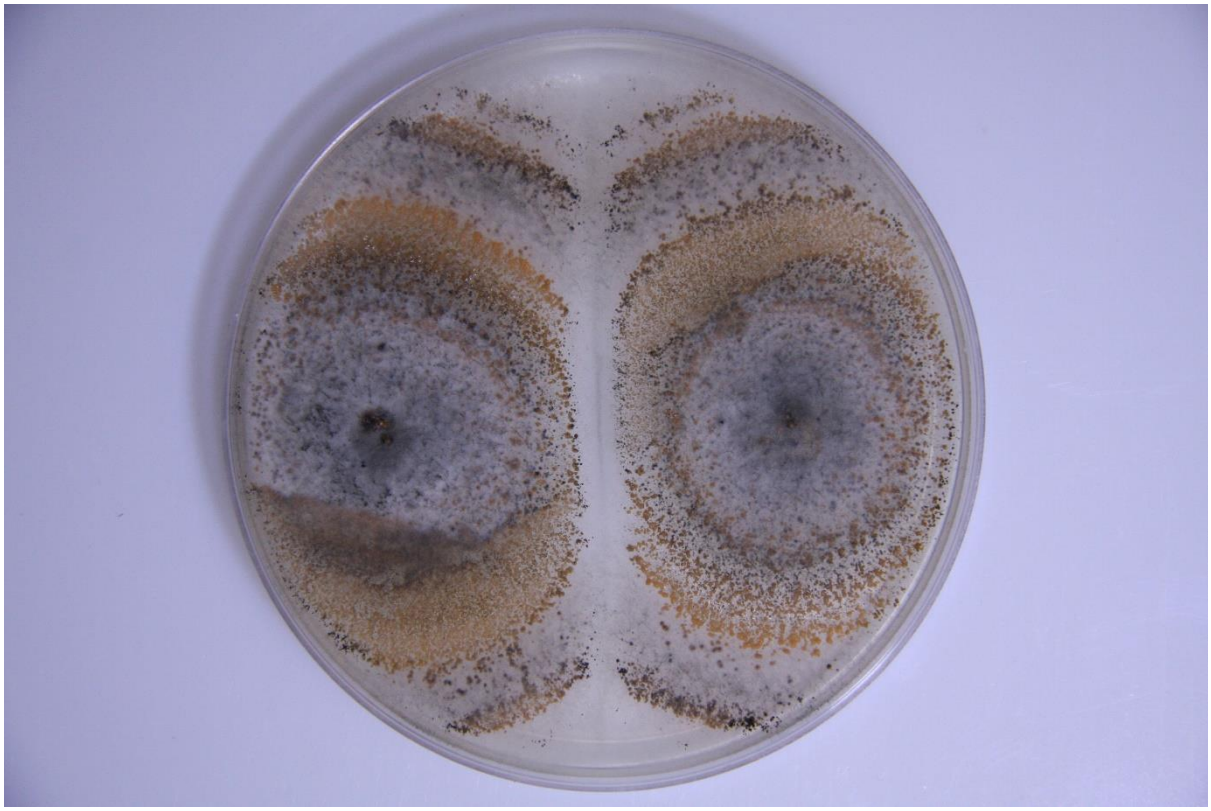

Fig. 4.6.6.5. Cultures isolated from inoculated Olinda Valencia fruit *C. gloeosporioides*

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

Project 1132 (2014/15 – 2016/17) by JG van Zyl and PH Fourie (CRI)

Summary

Spray application forms the backbone of pre-harvest pest and disease management strategies in South African citrus production. Due to zero tolerance status of Citrus black spot (CBS) (caused by *Phyllosticta citricarpa*) for export to the European Union, growers tend to use high application fungicide volumes ranging from 6000 to 12000 L ha^{-1}. However, high spray volumes are costly in terms of off-target losses (run-off and drift) and environmental pollution, amount and cost of water, fuel and plant protection product (PPP), the strain on equipment, and it is more labour intensive and therefore ultimately less cost-efficient than reduced volume applications, provided similar levels disease and pest control can be obtained. The potential of reduced volume applications has been shown in various previous studies. However, this potential must be proven through seasonal bio-efficacy trials. Various machines have been evaluated for this study. Reduced volume applications (1000 to 4000 L ha^{-1}) with the Martignani and Cima sprayers, but with increased pigment concentration (2× and 4× relative to a similar dose as 8000 L ha^{-1}), demonstrated deposition quantity and quality levels better than those of conventional high volume 1× sprays. Conventional machines did realise better deposition uniformity throughout the canopy (inside/outside canopy leaves; top/middle/bottom canopy leaves), indicating the importance of matching the sprayer profile to the canopy and also the role of proper canopy management in reduced volume application. Bio-efficacy sprays started in October 2015 in a Delta Valencia orchard outside Marble Hall (Mpumalanga). A modified spray programme consisting of four fungicide and pesticide applications have been made from October to January. A fifth and last spray was not applied due to the hot, dry weather experienced in the area the past season. Sprays were applied with a Cima and ATASA sprayer at 2000 L ha^{-1} (at 3.5× concentration) and a Jacto at 7000 L ha^{-1} at 1×. A spray deposition study on leaves was also done following sprays with these sprayers in the same orchard. The Cima at 2000 L ha^{-1} generally performed the best; often significantly better than the other treatments. No fruit could be evaluated in the deposition study due to fruit drop following the dry and hot weather and also hail damage on outer canopy fruit. No pest or disease has been noticed in the no spray control orchard to date, but a thorough disease and pest evaluation will be conducted prior to harvest.

Opsomming

Spuit toediening vorm die ruggraat van voor-oes pes en plaag beheer strategieë in Suid-Afrikaanse sitrus produksie. A.g.v. die zero toleransie status van sitrus swartvlek (veroorsaak deur *Phyllosticta citricarpa*) vir uitvoer na die Europese Unie, is produsente geneig om hoë swamdoder spuitvolumes te gebruik wat strek tussen 6000 tot 12000 L ha^{-1}. Hoë spuit volumes is duur in terme van produk verliese a.g.v. afloop en drif, omgewingsbesoedeling, die hoeveelheid water en koste daarvan, brandstof en plant beskermingsproduk wat gebruik word, asook die slytasie op masjinerie. Dit is ook baie arbeidsintensief en daarom op die einde minder koste-effektief as verlaagde volume toediening, mits siekte- en plaagbeheer suksesvol is. Die potensiaal van verlaagde volume toediening is in vorige studies bewys. Maar, of dit in praktyk sal werk kan slegs deur seisoenale bio-effektiwiteitsproewe getoets word. ʼn Reeks masjinerie is ondersoek vir die proefdoeleindes. Verlaagde volume toedienings (1000 tot 4000 L ha^{-1}) met die Martignani en Cima masjiene, maar met die pigment dosis verhoog (2× en 4×, relatief tot ʼn soortgelyke dosis teen 8000 L ha^{-1}), het beter deposisie kwantiteit en kwaliteit op blare getoon as met ʼn 1× hoë volume spuit. Konvensionele masjiene het beter deposisie uniformiteit deur die boom lower (binne/buite blare; bo/middel/onder blare) getoon, wat die belang van gepaste spuit profiel met die boom lower uitwys, asook die noodsaaklikheid van lowerbestuur vir laer-volume toediening. Bio-effektiwiteit-spuitproewe is in Oktober 2015 in ʼn Delta valencia boord buite Marblehall (Mpumalanga) begin. ʼn Program van vier fungisied en insekdoder toedienings is vanaf Oktober tot Januarie gespuit. Die 5de (laaste) toediening is nie gespuit nie a.g.v. die droë, warm weer die afgelope seisoen. ʼn Cima teen 2000 L ha^{-1} (3.5× produk konsentrasie), ATASA teen 2000 l ha^{-1} (3.5×) en ʼn Jacto teen 7000 L ha^{-1} (1×) is in die proef gebruik. ʼn Spuit deposisie proef is ook op blare gedoen en die Cima teen 2000 L ha^{-1} het deurgaans die beste gevaar; soms betekenisvol beter as die Jacto en ATASA. Geen vrugte kon evalueer word nie weens abnormale blom- en vrugval na die droë en warm klimaatstoestande die afgelope seisoen en haelskade in Oktober. Geen siekte of plaag is tot dusvêr in die kontrole blok waargeneem nie, maar ʼn volledige siekte- en plaag-evaluasie sal voor oes gedoen word.

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

Project RCE-6 (1128) (Apr 2015 – Jan 2018) by P.H. Fourie and M. Kellerman

Summary

Citrus black spot (CBS), caused by *Phyllosticta citricarpa,* is an A1 quarantine disease, and European importers currently have a zero tolerance for CBS on fresh citrus fruit imports. Parameters influencing germination and infection of pycnidiospores and ascospores have not been sufficiently studied for this disease. The influence of spore age (1 – 3 days), spore generation (1 – 5), germination media (water and Valencia juice) and incubation time (0 – 24 h) on pycnidiospore germination was studied using epifluorescence microscopy and confocal laser microscopy. Better germination results were obtained with spores older than 1 day and from the second and subsequent generations of spores oozing from pycnidia. Water was a sufficient germination medium for pycnidiospores on leaves, but Valencia juice was required for germination *in vitro.* The 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. Ascospores of *P. citricarpa* cannot be morphologically distinguished from ascospores of *P. capitalensis,* and therefore a method is being developed to quantify and distinguish between them. A qPCR protocol has been developed to quantify *P. citricarpa* pycnidiospores. Less than 1000 spores.mL^{-1} could not reliably be detected by the qPCR protocol, but spores ranging between 1×10^3 and 5×10^6 spores.mL^{-1} could be quantified. This method will eventually be used to quantify spores on spore traps from orchards, as well as to distinguish between *P. citricarpa* and *P. capitalensis*. Future work includes investigation of pycnidiospore infection, pycnidia maturation, ascospore germination parameters, and using qPCR to determine the ratio of *P. citricarpa* to *P. capitalensis* ascospores in orchard samples.

Opsomming

Sitrusswartvlek (SSV), wat veroorsaak word deur *Phyllosticta citricarpa,* is ʼn A1 kwarantyn siekte waarvoor Europese invoerders van vars sitrus geen toleransie het nie. Parameters wat die ontkieming en infeksie van piknidiospore en askospore beinvloed is nog nie breedvoerig bestudeer nie. Die invloed van spoor ouderdom (1 – 3 dae), spoor generasie (1 – 5), ontkiemingsmedia (water en Valencia sap) en inkubasie tydperk (0 tot 24 h) op ontkieming van piknidiospore was ondersoek met behulp van epifluoressensie mikroskopie en konfokale laser mikroskopie. Spore ouer as 1 dag en van 2de of later generasies het die beste ontkieming getoon. Spore het suksesvol in water op blare ontkiem, maar vir ontkieming *in vitro* was Valencia sap nodig. Die konfokale

laser mikroskopie beelde het soortgelyke ontkiemingspatrone van spore op suurlemoen en lemmetjie blare gewys, en dit wys dat lemmetjiebome se toleransie tot SSV nie met die ontkiemingsproses van spore te doen het nie. Askospore van *P. citricarpa* kan nie morfologies onderskei word van dié van *P. capitalensis* nie, en daarom moes ŉ metode ontwikkel word om dit te kan doen en terselfdertyd spore te kwantifiseer. ŉ qPCR protocol is ontwikkel om *P. citricarpa* piknidiospore te kwantifiseer. Minder as 1000 spore kon nie betroubaar met die qPCR protokol opgetel word nie, maar spore tussen 1×10^3 and 5×10^6 spore.mL^{-1} kon gekwantifiseer word. Hierdie metode sal mettertyd gebruik word om spore van spoorlokvalle in boorde te kwantifiseer, en om tussen spore van *P. citricarpa* and *P. capitalensis* te onderskei. Toekomstige werk behels die ondersoek van piknidiospoor infeksie, piknidia rypwording, askospoor ontkieming parameters en die gebruik van qPCR om die verhouding van *P. citricarpa* to *P. capitalensis* askospore in boordmonsters te bepaal.

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

Project RCE-7 (1129) (December 2014 – December 2018) by JG van Zyl and PH Fourie (CRI)

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa. Using proper spore dispersal and maturation models together with accurate weather data, integrated into easy to use web-based software, better decision making in terms of timing of spray application and choice of fungicides used can be made. CBS risk can also be determined more accurately from season to season. Various CBS disease prediction models have been identified and in order to determine which model works best, a model shootout have been proposed in a new CRI funded project. An IT company have been contracted for development of the web-based prediction system. Shapefiles have been created identifying citrus regions and weather reading points. The Centre for Geographical Analysis at Stellenbosch University has been contracted to build in accurate shapefile containing all citrus orchards in South Africa using SPOT6 satellite images. A web-based weather service has been identified with weather data recording starting in June 2016. A CBS model, including sub-models for CBS ascospore and pycnidiospore development, dispersal and infection, were formulated and presented to the IT company for programming. A good-to-spray model has also been developed and handed over to the development contractors. Alpha/Beta roll out is planned for September 2016.

Opsomming

Sitrus swartvlek is die belangrikste swamsiekte in sitrus in Suid-Afrika. Deur behoorlike spoor vrystelling en rypwording modelle te gebruik saam met akkurate weer data, geïntegreer in verbruikersvriendelike web-gebaseerde sagteware, kan die produsent help om beter besluite op plaasvlak te neem in terme van tydsberekening van spuit toediening en keuse van swamdoder. CBS risiko kan ook van seisoen tot seisoen bepaal word. Verskeie siekte voorspellingsmodelle is geïdentifiseer en ŉ evaluasie van beskikbare modelle is voorgestel in ŉ nuwe CRI-befondsde projek. ŉ Sagteware ontwikkelings maatskappy is gekontrakteur vir die ontwikkeling van die web-gebaseerde siekte voorspellingsisteem. “Shapefiles” wat sitrus produksie-areas sowel as weerpunte bevat is geskep. Die “Centre for Geographical Analysis” te Stellenbosch Universiteit is gekontrakteur om ŉ akkurate “shapefile” van alle sitrusboorde in Suid Afrika te skep deur die gebruik van SPOT6 satelliet fotos. ŉ Web-gebaseerde weerdiens is geïdentifiseer om weerdata te bekom vanaf Junie 2016. ŉ CBS voorspellingsmodel, met sub-modelle vir askospoor en pyknidiospoor ontwikkeling, vrystelling en infeksie, is geformuleer en aan die programeerders verduidelik. ŉ “good-to-spray” model is ook ontwikkel en oorgehandig aan die web ontwikkelaars. Die Alfa/Beta bekendstelling van die webtuiste is beplan vir September 2016.

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

Project RCE-8 (2014/12-2018/01) by Jacolene Meyer (QMS Laboratories)

Summary

This was the second season of volumetric ascospore trapping in some of the new areas in South Africa. For the second season in a row ascospores were released at all sites. In areas with few releases, trends were picked up between KIM data and months with high rainfall with many hours of high relative humidity. Using 1 July as biofix with the published DDTemp model, accurate (within 1-12 days in range) prediction of the date where mature ascospores should be readily available was verified in the new monitoring areas, following the same trend observed with data from Letsitele and Hoedspruit last season. Initial screening of ascospores using monoclonal antibodies specific to *Phyllosticta citricarpa* revealed potential of the technique to positively bind

with CBS ascospores. Packhouse data on CBS counts for four years of harvest has been obtained and will be examined against weather data from this area.

Opsomming

Hierdie was die tweede seisoen waar volumetriese spoorlokvalle in nuwe areas in Suid Afrika gebruik is. Vir die tweede seisoen in ŉ ry is askospore in alle areas vrygestel. In areas waar spoorvrystelling laag was, is daar ŉ korrelasie waargeneem tussen KIM data en maande met hoë reënval en periodes met hoë relatiewe humuditiet. Deur 1 Julie as 'biofix' tyd te gebruik in die gepubliseerde DDTemp model, was die datum wanneer askospore geredelik beskikbaar sou wees redelik akkuraat (tussen 1-12 dae) bepaal in die nuwe moniteringsareas wat saamstem met data van verlede seisoen vir Letsitele en Hoedspruit gegenereer is. Inisiële eksperimente met monoklonale teenliggaampies, spesifiek teen *Phyllosticta citricarpa*, lyk hoopvol om CBS askospore onder die mikroskoop te kan eien. Vier jaar se pakhuis data vir swartvlek is vanaf die Oos-Kaap verkry wat vergelyk sal word met weerdata vir die area.

4.6.11 **PROGRESS REPORT: Detection and spread of Citrus Black Spot pathogens**
Project RCE-9 (10/12/2014 – 31/03/2018) by Pedro Crous, (CBS-KNAW Fungal Biodiversity Centre (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 BCE. To investigate the putative incursion and persistence of *Phyllosticta* species on infected plant material, we explored the occurrence and the diversity of *Phyllosticta* spp. associated with *Citrus* spp. in European orchards, nurseries and gardens. We used a multi-locus DNA dataset consisting of the ITS, *actA, tef1, gapdh*, LSU and *rpb2* gene regions to investigate 99 isolates of *Phyllosticta*, of which 52 isolates were collected during extensive surveys performed in 2015 in Europe, two isolates were collected in Florida, USA, and three isolates in China. Based on the data generated here, we recovered several species associated with citrus plants in EU countries. In the EU, they were not found to be widespread, but symptoms of CBS were not observed during the surveys indicating that the fungi persisted but did not cause disease.

Opsomming

Die genus *Phyllosticta* wat sitrusswartvlek veroorsaak, kom wêreldwyd voor en bestaan uit ŉ verskeidenheid plantpatogeniese, endofitiese en saprofitiese spesies. *Phyllosticta citricarpa* veroorsaak (CBS). Die siekte kom wydverspreid in sitrusverbouingsareas voor, maar is afwesig in lande van die EU. *Phyllosticta citricarpa* word verwar met *P. capitalensis*, wat ŉ nie-patogeniese endofiet is wat algemeen van sitrusblare en vrugte geïsoleer word en ŉ verskeidenheid van ander gashere. Om die vermoedelike teenwoordigheid van *Phyllosticta* spesies op geïnfekteerde plantmateriaal te ondersoek, is die voorkoms en diversiteit van *Phyllosticta* spp. wat geassosieer word met *Citrus* spp. in Europese boorde, kwekerye en tuine ondersoek. ŉ Multi-locus DNA datastel bestaande uit ITS, *actA, tef1, gapdh*, LSU en *rpb2* geen-areas is ondersoek in 99 isolate van *Phyllosticta*, waarvan 52 isolate tydens 2015 in Europa versamel is. Twee isolate van Florida, VSA, en drie isolate van China is ook gebruik. Gebaseer op die data wat gegenereer is, is verskeie spesies geassosieer met sitrusplante in die EU lande. Simptome van SSV is nie waargeneem tydens die opnames nie wat daarop dui dat die swamme wat daar voorkom nie siekte veroorsaak nie.

4.7 **CRI DIAGNOSTIC CENTRE** (Elaine Basson, Jan van Niekerk, Aubrey Metane, and Bhekisisa Cele)

Table 4.7.1. Diagnostic samples analysed in the DC during the April 2015 – March 2016 report period.

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematode: Roots	11	464	3	1443
Nematode: Soil	1	14	24	1512
Phytophthora	4807[1]	507	71	2063
Water spore trap	159	2	10	0
Black spot identification (PCR)	0	149	0	30
Black spot benzimidazole resistance	0	67	0	10
Citrus greening (PCR)	0	8	0	1
Post-Harvest Resistance	0	28	0	0
Fruit & Foliar identification	0	20	27	327
Soil dilution plating	0	106	10	0
Internal Fruit Quality	0	3	0	0
TOTAL	**4978**	**1368**	**145**	**5386**

[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, 3598[2] nursery samples were received by the diagnostic centre for *Phytophthora* analyses (these include only quarterly samples from certified nurseries). Of these samples, 7.48% tested positive. In addition to soil and water samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans.* For the nematode root samples, 0% tested positive and for the nematode soil samples 0% tested positive.

Commercial samples

Samples were received from the following citrus growing areas: Eastern Cape, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Twenty-nine percent of the 464 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10 g of roots, and nematicide treatments were recommended. Fifty-six percent of the 507 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of Banana, Dry Beans, Ginger, Granadilla, Ornamentals, Peach, Pepper, Tobacco and Wheat. Nematodes found present on these crops included: *Scutellonema, Meloidogyne, Pratylenchus, Rotylenchulus, Tylenchorhynchus, Hoplolaimus, Helicotylenchulus,* and *Hemicycliophora. Phytophthora* and *Pythium* analyses were done on Avocado, Cashew, Granadilla, Macadamia, Peach and Pepper. The diagnostic centre analysed 48 soil samples from macadamia nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 5018 samples from experimental trials. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols.

CRI DIAGNOSTIESE SENTRUM (Elaine Basson, Jan van Niekerk, Aubrey Metane en Bhekisisa Cele)

Tabel 4.7.1. Diagnostiese monsters ontleed in die DC gedurende die April 2015 – March 2016 verslagperiode.

Ontleding	Sitrus kwekerye	Kommersiële monsters	Ander gewasse	Navorsings-monsters
Aalwurms: Wortels	11	464	3	1443
Aalwurms: Grond	1	14	24	1512
Phytophthora	4807[1]	507	71	2063
Water spoorlokval	159	2	10	0
Swartvlek (PKR)	0	149	0	30
Swartvlek benzimidazole bestandheid	0	67	0	10
Sitrusvergroeningsiekte (PKR)	0	8	0	1
Na-oes bestandheid (Imazalil)	0	28	0	0
Vrug- en blaar identifikasie	0	20	27	327
Grondverdunningsplate	0	106	10	0
Interne vrugkwaliteit	0	3	0	0
TOTAAL	**4978**	**1368**	**145**	**5386**

[1]Totale hoeveelheid monsters ontvang van gesertifiseerde kwekerye – sluit in kwartaal monsters, hertoets monsters en nie-gesertifiseerde kwekerye

Sitrus Gesertifiseerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaallikse monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal is 3598 monsters deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 7.48% positief getoets het (hierdie is slegs kwartaal monsters van gesertifiseerde kwekerye). Benewens die water en grondmonsters, moet kwekerye een keer per jaar ŉ wortelmonster instuur om vir *Tylenchulus semipenetrans* te toets. Van die wortelmonsters wat ontvang is, het 0.0% positief vir die teenwoordigheid van *T. penetrans* getoets en van die 3 grondmonsters het 0.0% positief getoets.

Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Oos-Kaap, Kwazulu-Natal, Limpopo, Mpumalanga, Noord-Kaap, Noord-Wes, en Wes-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Nege-en-twintig persent van die 464 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10g wortels gehad. Aalwurmdoder behandelings is in daardie gevalle aanbeveel. Ses-en-vyftig persent van die 507 monsters wat vir *Phytophthora* ontleed is, het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van Piesangs, Droë Bone, Gemmer, Granadilla, Ornamentele plante, Perske, Peper, Tabak en Koring gedoen. Aalwurms aangeteken op hierdie gewasse sluit in: *Scutellonema, Meloidogyne, Pratylenchus, Rotylenchulus, Tylenchorhynchus, Hoplolaimus, Helicotylenchulus,* en *Hemicycliophora.* Avokado's, Kasjoeneute, Granadillas, Makadamias, Perske en Peper monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 48 monsters vanaf makadamia kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 5018 monsters afkomstig uit navorsingsprojekte gedoen. Die Diagnostiese Sentrum het ook hulp verleen aan navorsingsprojekte in die identifikasie van moontlike sitrus swartvlek letsels deur middel van PKR.

5 PORTFOLIO: HORTICULTURE

5.1 PORTFOLIO SUMMARY

By Tim G Grout (Manager: Research and Technical)

With many of our citrus production areas experiencing drought during this report period, growers' focus has naturally turned to the basic requirements of citrus trees and how to maximise exports in less than ideal conditions. In this environment, our lack of understanding of some horticultural processes and critical resource levels is exposed and the importance of fundamental horticultural research is once-again appreciated. Dehydration between harvest and packing of Valencias was found to increase pitting but apart from trying to reduce moisture loss, pitting was reduced by preharvest applications of 2,4-D and s-ABA as well as postharvest TBZ application. Preharvest applications of ethylene synthesis enhancers were also found to reduce Peteca spot in lemons and should be commercialised. With the industry moving towards increased plantings of mandarins and in particular, late mandarins, more research is being focussed on these varieties and includes pruning, the timing of nitrogen applications, flowering and carbohydrate levels, growing mandarins under net and their susceptibility to chilling injury when undergoing cold sterilisation. The range of mandarin cultivars that now need to be evaluated has increased exponentially and the fact that the late mandarins can be grown in regions where soft citrus was not previously considered provides exciting new opportunities for these growers. Drought conditions raise the question of how much water citrus trees on different rootstocks in different soil types require and that is exactly what is being determined in a long-term project that is co-funded by the Water Research Commission. Earlier problems with instrumentation have been overcome and data are being generated that will be used in the development of models to predict water requirements for different citrus varieties and tree ages. These models will be critical in the future as water becomes an increasingly scarce resource. Other research that may only show its true value in the mid-term future is the use of NIR spectrophotometry in the packhouse for the detection of fruit with poor rind quality. Postharvest irradiation of fruit for insect disinfestation may also become an option for some markets in combination with a short cold treatment, so research is being conducted to determine the susceptibility of different citrus varieties and cultivars to damage from irradiation in order to establish a safe upper limit. With the increased use of shipping containers, there is growing interest in the possibilities of ambient loading into containers and research has been conducted to determine what temperatures can be reached and how long it will take to reach them. The scope of research in horticulture therefore extends from cellular physiology to cold-chain engineering for traditional and recently-released cultivars, and the results will hopefully all improve the citrus grower's bottom line.

PORTEFEULJE OPSOMMING

Met die droogte in baie van ons sitrusproduksie areas gedurende hierdie verslag tydperk, het produsente se fokus na die basiese vereistes van sitrusbome en hoe om uitvoere te verhoog in minder ideale omstandighede, verskuif. In hierdie omstandighede is ons gebrek aan kennis van sommige tuinboukundige prosesse en kritieke vlakke van hulpbronne blootgestel en word die belangrikheid van fundamentele tuinboukundige navorsing weereens waardeer. Dehidrasie tussen oes en pak van Valencias is gevind om gepokte skil te verhoog, maar behalwe om vogverlies te probeer verminder, het vooroes toediening van 2,4-D en s-ABA asook na-oes TBZ toediening dit ook verminder. Vooroes toedienings van etileen sintese versterkers is ook gevind om Peteka kol in suurlemoene te verminder en moet gekommersialiseer word. Met die bedryf wat beweeg na meer aanplantings van mandaryne en veral laat mandaryne, is meer navorsing gefokus op hierdie variëteite en sluit snoei, die tydsberekening van stikstofaanwendings, blom en koolhidraatvlakke, aanplant van mandaryne onder nette en hul vatbaarheid vir koueskade tydens koue sterilisasie in. Die reeks Mandaryn-kultivars wat nou geëvalueer moet word, het eksponensieel toegeneem en die feit dat die laat mandaryne nou verbou kan word in gebiede waar sagte sitrus voorheen nie oorweeg is nie, bied opwindende nuwe geleenthede aan hierdie produsente. Droogtetoestande het die vraag laat ontstaan van hoeveel water sitrusbome op verskillende onderstamme in verskillende grondtipes nodig het en dit is presies wat bepaal word in 'n langtermyn-projek met mede-finansiering deur die Waternavorsingskommissie. Vroeëre probleme met instrumentasie is oorkom en data word gegenereer wat gebruik sal word in die ontwikkeling van modelle om waterbehoeftes vir verskillende sitrusvariëteite en boom ouderdomme te voorspel. Hierdie modelle sal van kritieke belang in die toekoms wees soos water toenemend 'n skaarsste word. Ander navorsing wat eers sy werklike waarde later in die toekoms sal wys, is die gebruik van NIR spektrofotometrie in die pakhuis vir die opsporing van vrugte met 'n swak skilgehalte. Na-oes bestraling van vrugte om insekte te dood in 'n kombinasie met 'n kort koue behandeling kan ook 'n opsie vir sommige markte wees. Navorsing word nou gedoen om te bepaal hoe vatbaar die verskillende sitrusvariëteite en kultivars vir bestralingskade is, ten einde 'n veilige boonste waarde te stel. Met die toename in die gebruik van skeepshouers is daar groeiende belangstelling in die moontlikhede vir "ambient" laai in houers en navorsing is gedoen om te bepaal watter

temperature bereik kan word en hoe lank dit sal neem om dit te bereik. Die omvang van navorsing in hortologie strek dus van sellulêre fisiologie tot koue ketting ingenieurswese van ou en nuwe kultivars, en hopelik sal al die resultate die sitrusprodusente se “bottem-line” verbeter.

5.2 PROGRAMME: RIND CONDITION

Programme coordinator: Paul Cronjé (CRI-SU)

5.2.1 Programme summary

In this programme, research projects address various physiological disorders such as peteca of lemon, pitting of mandarin and Valencia orange as well as chilling injury. In general, these projects address the factors influencing susceptibility of the fruit to these disorders, and postharvest factors inducing the incidence of the disorders, as well as developing methodology to identify fruit with high susceptibility. In the final report on peteca spot (5.2.2) the conclusion is drawn that fruit maturity and the endogenous ethylene synthesis at harvest influence the susceptibility; and data collected over several seasons could indicate a protective action of ethylene in reducing rind sensitivity to peteca. It is hypothesised that if the internal ethylene synthesis is increased prior to harvest in sensitive fruit, i.e. immature fruit, a reduction in peteca can occur. By application of ethylene synthesis enhancers prior to harvest the incidence of peteca was reduced. Pitting of Valencia orange fruit was shown to be related to dehydration after harvest (5.2.3). The timeous application of TBZ after harvest reduced the incidence of pitting. The novel use of 2,4-D and s-ABA prior to harvest and the resulting reduction of pitting gives new opportunities to develop technologies to control this disorder. Late nitrogen (March) on mandarin – Nules and Nadorcott did not result in either a reduction in colour development or an increase in pitting and could indicate a possible change in how mandarins receive nitrogen in order to increase flower quality, specifically in late harvested cultivars (5.2.3). Irradiation of fruit in order to sterilise insect larvae could potentially be used to reduce the protocols of cold temperature treatment and duration (5.2.5). In this first year of this project the variation in sensitivity between cultivars become evident. In general, it seems that 300 Gray would be the highest dose all cultivars can tolerate, but this will be confirmed in 2016. Developing non-destructive technology such as VIS/NIR spectrophotometry could enable the early detection of fruit with low rind quality (5.2.6). The complex biological composition of the various citrus cultivars as well as the area interaction makes this a very challenging task. Breeding of new cultivars is essential to grow the citrus industry, however, an important component of the cultivar must be the ability to handle the South African citrus cold chain i.e. degreening as well as cold sterilisation. Of the various mandarin cultivars tested, the M37 cultivar showed very high chilling susceptibly, excluding it from any cold protocol shipments (5.2.4). Research on rind condition involves multi-season research projects to gain any information to control or understand these disorders. Significant progress has been made over the past 10 years, and must be built upon.

Programopsomming

Die skildefek projek spreek verskeie fisiologiese skildefekte aan soos peteka van suurlemoene, gepokteskil in mandaryne en lemoene as ook koueskade. In die algemeen adresseer die projekte eerstens die faktore wat vrugte vatbaar maak vir defekte en tweedens na-oes faktore wat die defek kan laat ontwikkel asook tegnologie om sensitiewe vrugte te identifiseer. In die finale verslag op peteka word die afleiding gemaak dat vrugouderdom en die vlak van interne etileen-sintese tydens oes die vatbaarheid beïnvloed en dat die aanwending van Ethephon voor-oes die etileen vlakke verhoog en die peteka verminder (5.2.2). Die hipotese was gestel dat interne etileen genoegsaam geproduseer moet word om die vrug af te-hard en peteka vatbaarheid verlaag. Gepokteskil van Valencia lemoene is bevind om negatief beïnvloed te word deur na-oes dehidrasie en rehidrasie asook dat die voortydige TBZ aanwending na-oes die voorkoms verlaag (5.2.3). Die gebruik van 2,4-D en s-ABA 2 weke voor oes en die vermindering van gepokteskil in die projek gee nuwe geleenthede vir die ontwikkeling van tegnieke op die defek te bekamp. Laat stikstof plasing in Maart op Nules en Nadorcott mandaryne het teen verwagting in geen negatiewe impak op kleurontwikkeling of voorkoms van skildefekte gehad nie. Dit resultaat kan ŉ moontlikheid bied om stikstof vlakke in laat mandaryne te verhoog tydens blom inisiasie om blom sodoende te verbeter (5.2.3). Bestraling van vrugte om inseklarwes te sterliseer bied ŉ geleentheid om die kouesterilisasie tempertuur en tydperk te verminder en so koueskade te vermy (5.2.5). In die eerste jaar se resultate is groot variasie tussen kultivars gedokumenteer. Oor die algemeen lyk 300 Gray na die dosis wat die meeste kultivars kan hanteer voor daar skildefekte ontstaan, dit moet egter in 2016 bevestig word. Om non-destruktiewe tegnologie te ontwikkel om ŉ skildefekte sensitiewe vrugte te identifiseer is n langtermyn doelwit in die program (5.2.6). Die kompleks interaksie tussen die biochemiese samestelling van die skil van kultivars en die omgewing maak dit erger ŉ baie uitdagende doelwit. Ontwikkeling van nuwe kultivars bly belangrik vir die bedryf maar die nuwe kultivars moet getoets word vir die vermoë om die SA koueketting te kan hanteer sonder kwaliteitsverlies. Verskeie mandaryn kultivars is getoets waarvan

slegs die M37 het hoë koueskade vlakke getoon (5.2.4). Navorsing in skildefekte strek oor multi-seisoen om genoegsame informasie in te win om die defekte te verstaan en te beheer. Daar was egter oor die laste 10 jaar betekenisvolle vordering in die program waarop voortgebou moet word.

5.2.2 FINAL REPORT: Effect of different chemical applications on development of Peteca spot in lemons

Project 833 (2006/7-2015/6) by P.J.R. Cronje (CRI at SU)

Summary

Peteca spot (PS) of lemon is a post-harvest physiological disorder resulting in the collapse of the oil gland. The oil subsequently leaks into the adjacent tissue and causes a darkened depression. The occurrence can be severe without any specific pre- or post-harvest practices to avoid or reduce the incidence. PS occurs in all citrus production areas of South Africa and is thought to be the result of the immature rind being subjected to postharvest stress associated with high CO_2 concentration in degreening, as well as the physical impact of the packing line and wax application. The incidence of PS was investigated over several seasons to identify factors that influence the incidence of the disorder. The first observation was the highly erratic incidence between seasons, as well as within an orchard in a season, with the early fruit being highly susceptible. In experiments to identify postharvest factors which influence the PS incidence, 3 ppm ethylene and 1% CO_2 were applied in a continuous flow-through system (20°C for 3 d). The CO_2 treatment resulted in significantly higher incidence compared to the other treatments. Following on from these results, postharvest Ethephon (2-Chloroethyl phosphoric acid) (200 mg/L and 400mg/L) and AVG (Aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L) applications to fruit resulted in a significant reduction in PS. In the subsequent season, the same treatments were applied in an orchard one week before harvest and a similar reduction in PS was recorded. Ethylene production from these fruit after harvest was measured and showed a transient spike in fruit from the Ethephon and AVG treatments. The results collected over several seasons could indicate a protective action of ethylene in reducing rind sensitivity to PS. It is hypothesised that if the internal ethylene synthesis is increased prior to harvest in sensitive fruit, i.e. immature fruit, a reduction in PS can occur.

Opsomming

Peteka is ʼn na-oes fisiologiese skildefek wat in suurlemoenvrugte voorkom en lei tot die verval van oliekliere in die skil. Die olie wat uit die kliere lek, beskadig die omliggende skilweefsel en lei tot die inval en verbruining van die beskadigde area. Hierdie onomkeerbare merk is die eerste sigbare simptoom van die defek. Die gebeurtenis is ʼn gereelde verskynsel. Daar is tans geen spesifieke voor- en no-oes bestuursmaatreëls in bestaan om dit te vermy of verminder. Gedurende die afgelope vier seisoene was navorsing gedoen om faktore wat vrug sensitiwiteit t.o.v. peteka te identifiseer. Die eerste waarneming was die uiters wisselvallige voorkoms per boom, boord, plaas of produksie area. Al konstante faktor was dat vrugte wat geoes was in die eerste pluk in die seisoen die mees sensitief was. In eksperimente wat gepoog het om na-oes toestande wat peteka kan beïnvloed te bepaal is vrugte aan skoon lug, 3 dpm etileen gas en 5% CO_2 gas blootgestel vir 3 dae teen 20°C. Die CO_2 behandeling het drastiese verhoogde vlakke getoon i.v.m. die ander behandelings, wat dui daarop dat CO_2 negatief is in die ontgroeningkamers. Die etileen behandeling het die voorkoms verminder en om daarby aan te sluit was in die daaropvolgende seisoen gepoog om die skil-etileenmetabolisme te manipuleer deur na-oes en later voor-oes Ethephon (2-Chloroethyl phosphoric acid) (200 mg/L and 400mg/L) and AVG (Aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L), ‘n etileen sintese inhibitor, toe te dien. Beide die behandelings het die voorkoms verminder van peteka in verskeie herhalings van die eksperimente. Daar was bevind dat die voor-oes gespuite vrugte n tydelike verhoging van interne etileenproduksie toon. In geheel dui die data op ʼn moontlike beskermende meganisme van etileen in die skil. Dit wil blyk indien vrugte nog fisiologiese onvolwasse is, al is die vrugte kommersieel reg vir oes, daar ʼn gebrek aan interne etileen sintese is wat benodig word om die vrug “af te hard” voor oes. Tans is die monsterneming en forsering van peteka in toe plastieksakkies die enigste praktyk wat gevolg kan word om hierdie sensitiwiteit van vrugte te bepaal.

Introduction

Peteca of lemon fruit is a post-harvest physiological disorder associated with the collapse of an oil gland, which generally occurs from harvest until cold storage. Peteca reduces the fruit quality due to the unsightly sunken light brown lesions that develop in the rind (Cronje, 2007). Incidence of peteca has been recorded as early as 1924 in the USA in lemon fruit shipped from Italy, from which it appears the name was adopted from *petecchia* as it was known in Italy (Fawcett, 1936).

Economically significant incidences of peteca have been recorded in most of the lemon producing regions viz. Tucuman, Argentina (Torres Leal, 2004), Chile (Undurraga *et al*., 2009), Australia (Wild, 1991), Lebanon (Khalidy et al., 1969) and California in the USA (Fawcett, 1936). In South Africa peteca has a negative impact on lemon producers every year in the inland warm and humid summer rainfall areas (Limpopo, KwaZulu-Natal and Mpumalanga provinces), as well as in the cooler coastal areas with summer (Eastern Cape) or winter (Western Cape) rainfall (Cronje, 2005).

The first symptom of peteca can be seen as early as 3-5 days after harvest and develops before the fruit has been put onto the packline. It consists of darkening of an individual oil gland and if the flavedo is removed the brown oil gland is visible with discoloured albedo tissue directly below. After a few days the rind tissue surrounding the collapsed oil gland sinks and adjacent oil glands can also collapse. This collapse of the oil gland can occur in green and yellow fruit but normally the sunken lesion is clearly seen in fruit with a yellow rind (Cronje, 2007). The sunken lesion develops without any physical or mechanical damage to the epidermal rind tissue, as seen in oleocellosis, which results in the leaking of the oil gland onto the rind epidermis and degeneration of the cortical cell contents.

Figure 5.2.2.1. Peteca of lemon fruit is associated with the collapse of the oil gland and results in phytotoxic damage to the rind.

The susceptibility of lemon fruit to this disorder has been associated with various pre and post-harvest factors. In South Africa low rainfall during the three months prior to harvest as well as cold/wet conditions (dramatic changes in day night temperature) close to harvest, have been seen to result in higher incidence of peteca, and concurs with the marked increase of peteca incidence reported in Tucumán after an abrupt temperature fall in the week prior to harvest (Torres Leal, 2004). The same conditions (low temperature and high relative humidity) was mentioned by Wild (1991) and Artes et al. (1983) to be deleterious and Undurraga et al. (2006) identified rainfall on lemon during harvest in Chile to be problematic in the development of peteca. Undurraga et al., (2002) reported that rind colour and therefore fruit rind maturity, play a role in fruit susceptibility and reported that yellow fruits have a greater incidence compared to fruits in the “silver” stage. The incidence of peteca in South Africa is generally associated with a higher occurrence during the initial weeks in the picking window, with a dramatic reduction in subsequent weeks (Fig. 5.2.2.2).

Peteca has been associated with calcium (Ca) imbalances in the rind (Khalidy et al., 1969) and the involvement of Ca-content in the rind as well as Ca-oxalate crystals has been investigated by Storey and Treeby (2002). In peteca affected areas of the rind they found 23% higher Ca and 30% lower Boron. However, they could not find any direct evidence of Ca-oxalate crystal growth being a causal factor of cell damage seen in peteca, a view supported by Undurraga et al., (2009).

Postharvest conditions can aggravate peteca in lemon fruit and Wild (1991) found the incidence increased after citrus wax application (commercial polyethylene based citrus wax with 16% solids) as well as fruit brushing. In contrast, storage for 3-5 days after harvest (no handling) as well as degreening, decreased the incidence of peteca. Cold storage at 3°C of yellow coloured lemons resulted in higher peteca incidence compared to “silver” coloured fruit storage at 3°C and 7°C (Undurraga et al., 2009).

Undurraga et al., (2009) hypothesised that there is a relationship between peteca and some physiological stress indicators such as hydrogen peroxide and peroxidase activity.

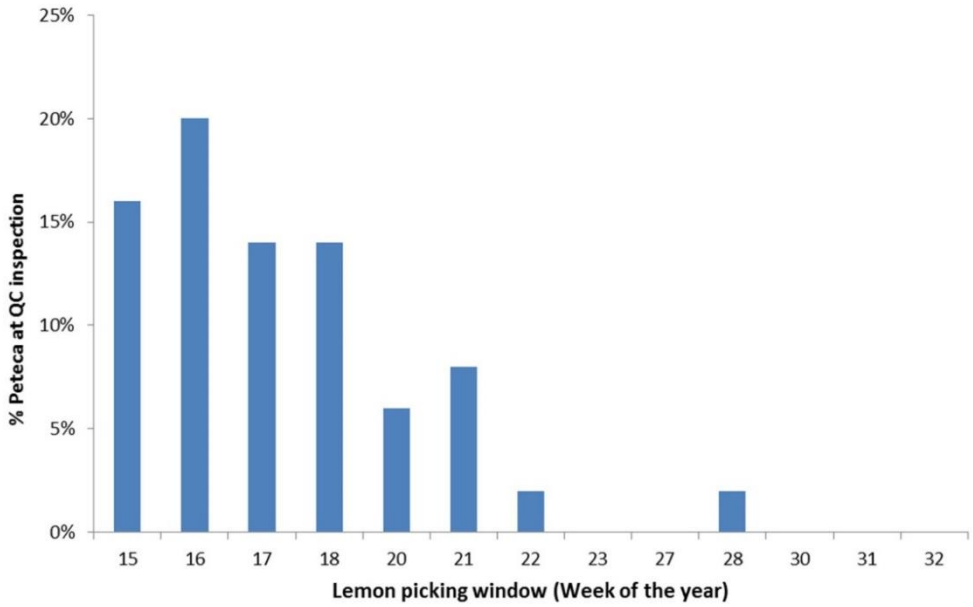

Figure 5.2.2.2. Incidence of peteca as reported at a citrus packhouse in Groblersdal, Mpumalanga, South Africa during 2012 (source Steve Turner, Core fruit).

Strategies to control peteca incidence are an important research area for the South African lemon producers. However, the erratic incidence of this disorder with large variations between seasons, orchards and within orchards in one season, have made it difficult to fully elucidate the impact of pre- and post-harvest factors on this physiological disorder. The influence of ethylene applied in degreening rooms is always suspected to affect citrus fruit quality negatively, however, contradictory reports were received from the South African lemon packhouses on the impact of ethylene (degreening) on peteca incidence. Therefore, the aim of this study was firstly to determine the influence of postharvest ethylene application and secondly the efficacy of pre-harvest manipulation of fruit ethylene metabolism on peteca incidence.

Material and methods

During four consecutive seasons, various experiments were conducted to determine the influence of ethylene on the incidence of peteca of lemon fruit. In 2009, fruit was harvested from a commercial 10-year-old 'Eureka' lemon (*Citrus limon* Burk.) orchard in Simondium, Western Cape. The fruit were of the same size and colour and were divided into 5 lots of 30 fruits. The 30 fruit were placed in closed plastic containers and connected to a continuous flow-through system, through which the gas treatments were applied i.e. normal air, 3 ppm ethylene or 5% CO_2 at 20°C for 3 days. In addition, 30 fruit were kept in a closed plastic container. After treatments fruits were evaluated for peteca incidence (% peteca per treatment). The experiment was repeated 3 weeks later with fruit from the same trees.

In the second experiment, pre-harvest manipulation of fruit ethylene production was done in the Eastern Cape (Addo) in 2010, 2011, 2012 and in Mpumalanga (Groblersdal) in 2012 in commercial 'Eureka' lemon orchards, 8-10 years old. In each area the orchards used were within a 10 km radius. Foliar applications of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) were applied to single tree replicates (n=8) in a complete randomised block design, one week before the first planed commercial harvest date. Buffer trees were left open between treatments which were applied with a motorised knapsack sprayer at 10 L/min. After one week the fruit were harvested and 10 fruit per replicate stored in closed plastic bags to "force" peteca incidence. This technique of forcing fruit at room temperature stems from the result in 2009, where significantly higher peteca developed in closed plastic containers (Table 5.2.2.1).

After storage for 14 days at ambient temperature (~20°C) in closed plastic bags, fruits were scored for peteca according to this index:

$$Peteca\ index\ (0-2) = \sum \frac{[Peteca\ (scale\ 0-2) \times number\ of\ fruit\ in\ each\ class]}{Total\ number\ of\ fruit\ in\ rep}$$

The ethylene production was measured in 2012 at harvest time and 1 week later from one orchard in the Eastern Cape. For each of the 8 replicates, 5 fruit were placed in sealed bottles for 5 hours. Samples were taken from the closed bottles with airtight syringes and ethylene concentrations were measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian Instrument Group, Palo Alto, California, USA). The volume of the lemons was determined as well as the weight to determine the ethylene production expressed as µl $C_2H_4 \cdot kg^{-1} \cdot h$. The data were analysed with SAS (data were analysed using a one-way ANOVA (SAS v. 6.12, SAS Institute, Carry, NC,) to test the significance ($P \leq 0.05$).

Results

The fruit that were closed in a plastic container had the highest incidence of peteca (41%) compared to the 21% observed in those treated with 5% CO_2 and the 18% in those treated with air. In contrast, the 3 ppm ethylene gas treatment resulted in no peteca development (Table 5.2.2.1). A repeating of the experiment three weeks later, from the same trees and with the same gaseous treatments resulted in no peteca development in the fruit. This difference in incidence between sampling dates indicates a possible influence of fruit maturity on sensitivity.

Table 5.2.2.1. The influence of continuous gas applied to lemon fruit from Simondium Western Cape in 2009, for 3 days at 20°C. Fruits were evaluated for peteca 1 day after treatments.

Treatment	% Peteca incidence	
	Sample 1	Sample 2 (3 weeks later)
Normal air (control)	18	0
5% CO_2	21	0
3 ppm Ethylene	0	0
Closed container	41	0

In the second part of the project, the pre-harvest foliar applications of Ethephon and AVG from 2010 to 2012 reduced the incidence of peteca of lemons in four out of the seven orchards (Fig 5.2.2.3A-C). However, variation in efficacy occurred between seasons, production areas and orchards. During 2012 in Groblersdal both of the orchards used showed no treatment effect (Fig. 5.2.2.3B). In addition, the difference in peteca incidence between the two orchards in this production area was pronounced, i.e. 0.25 - 0.35 in orchard 1 and ±0.05 in orchard 2 (Fig. 5.2.2.3B). This variation in incidence is also seen in Figure 5.2.2.3C with peteca incidence in the controls varying from 0.2 to 0.7.

The ethylene measurement of fruit after harvest and 1 week later indicate that the Ethephon treatment resulted in a significant spike in ethylene production (Fig. 5.2.2.4). The AVG treatment, known to reduce ethylene synthesis, resulted in measurable levels of ethylene, although similar to those of the control treatment.

Discussion

The incidence of peteca of lemon fruit has not previously been associated with ethylene metabolism in the fruit, but rather aspects such as Ca content in the rind (Khalidy et al. 1969) and post-harvest treatments (Wild, 1991). However, the change of peteca incidence in an orchard from one week to the next in 2009, could indicate that Ca is not the main factor determining peteca sensitivity, as the nutrient content in the rind does not change at that late stage of maturity. In addition, the reduction in peteca incidence after application of Ethephon and AVG, followed by a transient change in fruit ethylene synthesis, offers a new direction of research into elucidating the causal mechanism of this disorder.

The hormone ethylene is known to be involved in the senescence processes in fruit as well as the development of cellular degradation associated with stress conditions, albeit it can also protect fruit against stress conditions causing tissue damage (Yang and Hoffman, 1984; Lafuente and Sala, 2002).

Cajuste and Lafuente (2007) suggested that this endogenous hormone could induce protective properties of the citrus fruit rind against physiological disorders. In addition, ethylene metabolism has also been associated with the accumulation of defence properties against *Penicillium digitatum* infection (Marcos et al., 2005). The mechanisms underlying the reduced sensitivity to some physiological rind disorders of citrus after exposure to

ethylene has not been elucidated, but it is suspected that an increased protective action due to up-regulating of the rind phenolic metabolism may be involved, although additional defence mechanisms could also be induced by ethylene pre-treatment (Cajuste and Lafuente, 2007).

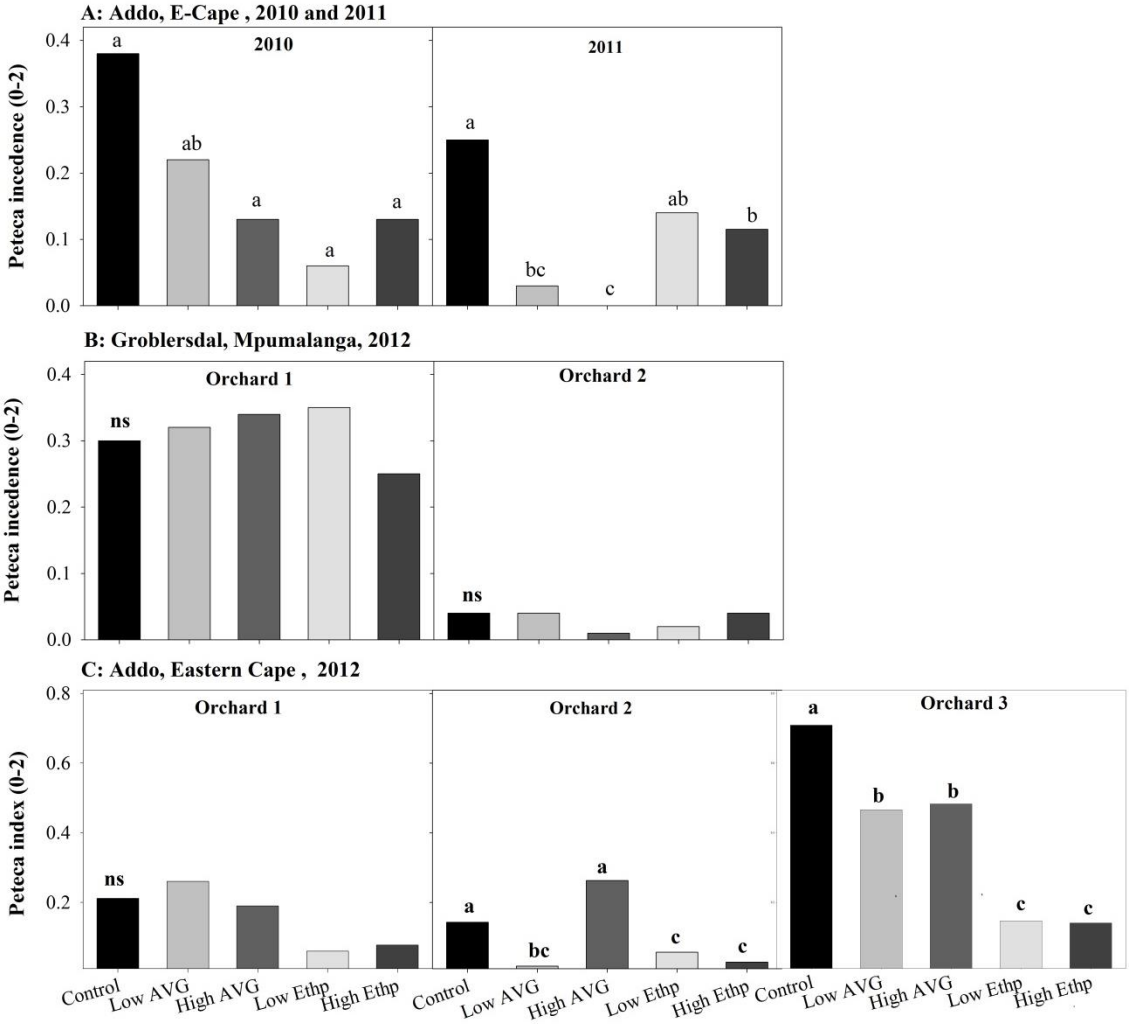

Figure 5.2.2.3. Incidence of peteca in lemon fruits in A: Addo, Eastern Cape (2010 and 2011), B: Groblersdal Mpumalanga (2012) and C: Addo, Eastern Cape (2012), after pre-harvest foliar application of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) 1 week before harvest (n =8). After harvest, fruits were stored at 20°C for 2 weeks in closed plastic bags prior to scoring for peteca incidence ($P \geq 0.05$).

The reduction in peteca incidence after both the pre-harvest manipulation of the fruit ethylene metabolism and the ethylene gas application, could indicate the possible induction of protective processes in the flavedo due to the higher ethylene prediction. It should be noted that the high and low Ethephon treatments resulted in leaf drop after application (±20% and ±10% respectively), but did not negatively affect the tree condition the following season. Currently the impact of peteca is managed by RSA lemon producers and packhouses with a strategy that includes the following three aspects. The fruit in the first part of the picking window are more susceptible compared to later in the season: therefore, moving harvest back two weeks can reduce the incidence dramatically even in the same orchard. Secondly, after a cold front, no fruit will be harvested for at least 3-5 days to reduce fruit rind turgor. The third aspect that has successfully reduced the incidence is the storing of fruits for 4-7 days in a shaded, well-ventilated area after being harvested, with minimum handling of the fruit. However, this strategy limits, but does not prevent, the incidence of peteca. Therefore, reducing the sensitivity of fruit to peteca by preharvest treatments would be a valuable tool to reduce the negative impact of peteca on lemon.

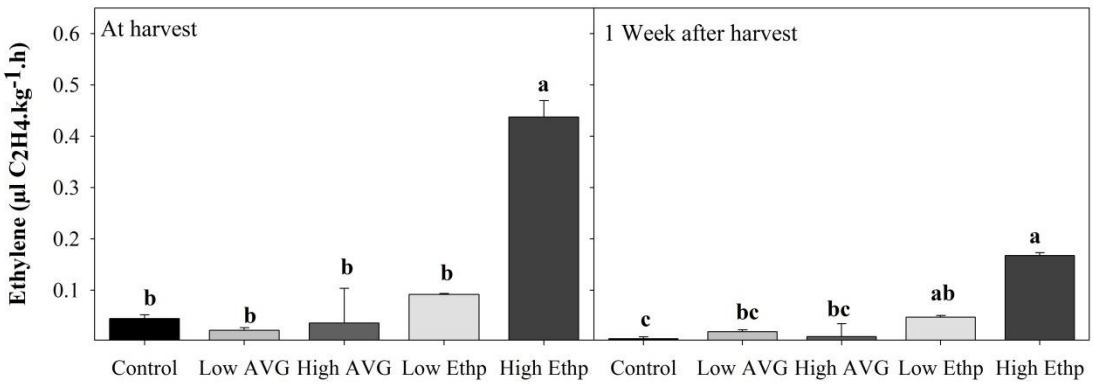

Figure 5.2.2.4. Ethylene of lemon fruit measured at harvest and 1 week later in Addo, Eastern Cape (2011), after pre-harvest foliar application of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (Aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) 1 week before harvest (n =8) ($P \geq 0.05$).

Summary of peteca risk management

- Test peteca sensitivity: 10 fruit in a closed transparent plastic bag a week prior to plan harvest. Store at ambient for 7-14 days and note peteca development without opening the bag. Delay packing fruit for another 2-3 days if high incidence is recorded.
- Do not harvest directly after cold front or rain and it is advised to delay harvest for at least 3 days.
- After harvest, place 10 fruit in a closed plastic bag from each batch of fruit being received at the packhouse. Store at ambient temperature and check every 1-2 days for peteca incidence. Delay packing of fruit if high incidence.
- After harvest fruit should be stored in a cool <20°C, well ventilated area to prevent build-up of CO_2 in fruit bins.
- Maintain good control over ALL factors in the degreening room. Low humidity and high temperature will result in high moisture loss, which negatively affects rind condition. High CO_2 delays the degreening process and aggravates peteca incidence.
- Start with the cold chain as soon as possible after packing.

Conclusion

Peteca of lemon is a physiological disorder associated with the collapse of the oil gland. The incidence of peteca varies significantly between seasons, orchards and within an orchard in one season. It is thought that immature fruit (first pick) as well as cold conditions prior to harvesting could negatively influence peteca incidence. The pre-harvest application of Ethephon and AVG, known to alter the rind ethylene metabolism, has resulted in a significant reduction of peteca over three seasons. However, the causal mechanisms as well as factors influencing fruit susceptibility to peteca are far from being elucidated. In addition, further research as to concentration and timing of application of these chemical needs to be undertaken.

Future research

The impact of pre-harvest climatic events such as cold fonts and changes in day night temperature should be quantified in addition to optimising the degreening process.

Technology transfer

- Could ethylene metabolism in lemon fruit influence peteca incidence? 19-23 Nov 2012. International Citrus Symposium, Valencia Spain.
- Management of Peteca spot in lemon fruit. 7th CRI research symposium. Aug 2012. Champagne Castle sport resort, UKZN.
- CRI packhouse meetings. 2012. Chilling and non-chilling rind disorder of citrus fruit.
- CRI packhouse. 2013. Strategies to reduce chilling injury and improve rind condition of citrus fruit.
- CRI packhouse meetings. 2014. Physiological rind disorders: Research update.
- Peteca spot of lemons. SA Fruit Journal April/May: 26-28.
- Progress in research on the control of peteca spot of lemon fruit: could ethylene metabolism influence susceptibility? SA Fruit Journal. April/May: 41-45.

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5.2.3 PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit

Project 958 (PHI-62) (2009/10 – 2016/7) by PJR Cronje, Jade North (CRI at SU), Jacques Ehlers, Jeanine Joubert and Helene Marias (SU-Horticulture)

Summary: Pitting of Mandarin citrus fruit

'Nules Clementine' and 'Nadorcott' mandarin are commercially important *Citrus* cultivars in South Africa. Both cultivars are prone to develop rind breakdown and pitting which are considered non-chilling related post-harvest physiological rind disorders. The progressive and erratic nature of these rind disorders results in high financial losses. The incidence of a rind disorder is thought to be associated firstly with an increased susceptibility, as influenced by pre-harvest aspects; and secondly with a trigger in the post-harvest environment. A study was conducted over two seasons to determine the effect of late nitrogen application (stage II and after summer flush), pre-harvest water stress and postharvest handling. The rind quality of 'Nules Clementine' and 'Nadorcott' mandarin fruit harvested from Citrusdal and Riebeeck Kasteel was evaluated. Soil applications of nitrogen at 20 $kg \cdot ha^{-1}$ and 40 $kg \cdot ha^{-1}$ were done on 21 January and 26 March 2014/2015, respectively. This was in addition to the standard 300 $kg \cdot ha^{-1}$ nitrogen provided by the producer. During 2015 a 1% urea foliar application was sprayed on 26 March. During the post-harvest period all fruit were dehydrated at 25 °C and 60 to 80% RH (0.7 to 1.1 kPa vapour pressure deficit) for two days, followed by rehydration at 100% RH for one day. Subsequently fruit were stored at either -0.6 °C or 4 °C for a 30-day period. There were no significant differences in fruit colour or size between the different nitrogen treatments. No increase in rind disorders or negative impacts on internal fruit quality were noted. To determine the impact of pre-harvest water stress, the soil below the trees was covered with plastic sheets three weeks prior to harvest to exclude rainfall or irrigation. The effect of postharvest stress was established by dehydrating and rehydrating fruit at 0.7-1.1 kPa vapour pressure deficit for different periods after harvest. Wax was applied on day 5 and thereafter fruit was stored at 4 °C for 30 days. The results indicated that pre-harvest water stress did not have a detrimental effect on fruit susceptibility to disorders. By early wax application, however, a decrease in moisture loss was

recorded, coinciding with lower incidences of rind disorders. The final part of the study was aimed at determining whether water loss during post-harvest handling, as induced by high vapour pressure, could increase disorders. From the results of the trials it can be concluded that exposing fruit to dehydration increased rind disorder susceptibility. Dehydration prior to wax application on day 5 also increased pitting and rind breakdown, whereas an early wax application, 2 to 3 days after harvest, reduced incidence. This study serves as a step to resolve the impact of factors predisposing the citrus fruit rind to progressive post-harvest disorders.

Opsomming: Gepokteskil van Mandaryn vrugte

'Nules Clementine' en 'Nadorcott' mandaryne is kommersiële belangrike *Citrus* kultivars in Suid-Afrika. Beide kultivars is geneig tot skilafbraak en gepokte skil wat as na-oes fisiologiese skildefekte beskou word en is onafhanklik van koueskade tydens opberging. Die progressiewe- en wisselvallige aard van hierdie skildefekte het groot finansiële verliese tot gevolg. Vermoedelik hou die voorkoms van 'n skildefek eerstens verband met 'n verhoogde vatbaarheid soos beïnvloed word deur voor-oes aspekte; en tweedens met 'n sneller vanuit die na-oes omgewing. Die invloed van 'n laat stikstof toediening (fase II en na die somer groei), voor-oes waterstres en na-oes hantering op skildefekte is oor twee seisoene bepaal. 'Nules Clementine' en 'Nadorcott' mandaryn vrugskilgehalte, geoes vanaf Citrusdal en Riebeeck Kasteel, is geëvalueer. Toediening van korrelkunsmis (stikstof) teen 20 kg·ha^{-1} en 40 kg·ha^{-1} is gedoen op 21 Januarie en 26 Maart 2014/2015, respektiewelik. Dié toedienings was bykomend tot die produsent se standaardbehandeling van 300 kg·ha^{-1} stikstof. 'n Blaartoediening van 1% ureum is addisioneel op 26 Maart 2015 aangewend. Alle vrugte was na-oes gedehidreer teen 25 °C en 60 tot 80% RH (0.7 tot 1.1 kPa dampdrukverskil) vir twee dae, gevolg deur 'n een dag rehidrasie periode van 100% RH. Vrugte is vervolgens opgeberg by óf -0.6 °C óf 4 °C vir 30 dae. Geen beduidende verskil in kleur en grootte van die vrugte tussen die verskillende stikstof behandelings of toename in skildefekte of negatiewe effek op interne vrugkwaliteit was waargeneem nie. Die impak van voor-oes waterstres op na-oes skilgehalte is bepaal deur grond onder die eksperimentele bome drie weke voor oes met plastiekseile te bedek om sodoende die invloed van reënval en besproeiing uit te skakel. Die effek van na-oes stres was geëvalueer in vrugte wat gedehidreer en re-hidreer was teen 0.7 tot 1.1 kPa dampdrukverskil. Die na-oes waks behandeling was op dag 5 toegedien, waarna vrugte opgeberg was by 4 °C vir 30 dae. Resultate dui daarop dat voor-oes waterstres geen negatiewe impak op die vrugte se vatbaarheid vir skildefekte gehad het nie. 'n Afname in vogverlies is wel gemeet met die wakstoediening wat ooreenstem met 'n laer voorkoms van skildefekte. Die finale gedeelte van die studie was daarop gemik om te bepaal of na-oes waterverlies, soos geïnduseer deur 'n hoë dampdruk verskil, die voorkoms van skildefekte kan induseer. Resultate dui aan dat die blootstelling van vrugte aan dehidrasie wel die vatbaarheid vir skildefekte verhoog het. Dehidrasie voor 'n wakstoediening op dag 5 het ook verhoogde skilafbraak en gepokte skil tot gevolg gehad, terwyl 'n vroeë waks, 2 tot 3 dae na oes, die voorkoms verlaag het. Hierdie studie dien as 'n skakel om die impak van faktore wat skil-vatbaarheid ten opsigte van fisiologiese defekte verhoog verder te ontrafel en dus sodoende aan te spreek.

Summary: Post-harvest rind pitting on 'Valencia' Oranges

Post-harvest rind pitting is a non-chilling related physiological rind disorder that affects various citrus cultivars and reduces fruit value. This disorder is characterised by the collapse of the flavedo sub-epidermal cells. Whilst the main cause of this disorder is unknown, it is aggravated by changes in relative humidity (RH) and rind water status. Studies were conducted on 'Turkey' and more susceptible 'Benny' Valencia oranges in Limpopo and Mpumalanga South Africa. The effect of fruit position, maturity and size on fruit susceptibility to this disorder was investigated. It was found that fruit from the outside of the canopy are more susceptible to this disorder probably due to greater exposure to variation in environmental conditions than fruit from the inside of the canopy. More mature fruit were also found to be slightly more susceptible, however, size did not influence incidence of this disorder. Various plant growth regulators were also evaluated to prevent pitting. The application of the synthetic auxins 2,4-dichlorophenoxy acetic acid (2,4-D) and 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) at 50 % petal drop (2,4-D) or after physiological fruit drop (2,4-D or 3,5,6-TPA) reduced the incidence of post-harvest pitting. Application of s-abscisic acid 1 week before harvest was also found to reduce incidence of this disorder, however, gibberellic acid applied in January did not reduce the incidence of post-harvest rind pitting. A systemic fungicide thiabendazole (TBZ) which reduces the incidence of chilling injury also reduced post-harvest pitting incidence when applied before fruit were subjected to stress inducing environmental conditions. Pre-harvest foliar application of TBZ 1 week before harvest and post-harvest dip treatments directly after harvest reduced post-harvest weight loss and incidence of this disorder. A citrus industry survey was conducted to estimate the financial impact of this disorder at foreign and local markets for producers. Markets generating higher prices had a lower tolerance for incidence of post-harvest rind pitting than lower priced markets and are therefore seen as high-risk. Due to this large reduction in market price for fruit with the disorder, treatments found during this study might be cost effective.

Opsomming: Na-oes gepokteskil van ‘Valencia’ lemoene

Gepokteskil is 'n na-oes fisiologiese skildefek wat nie met koueopberging by lae temperature gedurende geassosieer word nie en kan verskeie sitruskultivars affekteer en die waarde van vrugte verminder. Die defek word gekenmerk deur die ineenstorting van die sub-epidermale flavedo selle en alhoewel die hoof oorsaak van die defek nie bekend is nie word dit vererger deur variasie in relatiewe humiditeit (RH) en skil water status in die na-oes omgewing. Hierdie studie was op 'Turkey’ en die meer vatbaar ‘Bennie' Valencia lemoene in Limpopo en Mpumalanga Suid-Afrika gedoen. Die effek van voor-oes faktore soos vrug posisie, -volwassenheid en -grootte op die vatbaarheid van vrugte vir hierdie defek is ondersoek. Dit is bevind dat die vrugte aan die buitekant van die blaardak meer vatbaar is vir hierdie defek, waarskynlik as gevolg van groter blootstelling aan variasie in omgewings toestande as vrugte vanaf die binnekant van die blaardak. Daar is ook gevind dat meer volwasse vrugte ’n hoër vatbaarheid vir die defek het, maar dat vruggrootte dit nie beïnvloed nie. Die effektiwiteit van verskeie plant groei reguleerders om die voorkoms van gepokteskil te verminder is geëvalueer. Die toediening van sintetiese ouksiene 2,4-dichlorofenoksie asynsuur (2,4-D) en 3,5,6 trichloro-2-piridiloksi asynsuur (3,5,6 TPA) by 50 % blomblaarval (2,4-D) of na fisiologiese vrug val (2,4-D en 3,5,6-TPA) verminder die voorkoms van na-oes gepokteskil. So ook het die toediening van absisiensuur een week voor-oes die voorkoms van hierdie defek verlaag, maar daarteenoor het gibberelliensuur in Januarie geen effek op die voorkoms van na-oes gepokteskil gehad nie. Dit is al voorheen bewys dat 'n sistemiese swamdoder thiabendazole (TBZ) koueskade kan verminder, en TBZ het ook die voorkoms van na-oes gepokteskil verminder mits dit voor stres geïnduseerde omgewings toestande aangewend word. TBZ toediening een week voor-oes as ’n blaar bespuiting of as ’n doop behandeling direk na oes verminder gewig verlies en die voorkoms van die afwyking. ’n Sitrusbedryf opname was gedoen om die geskatte finansiële impak van gepokteskil in buitelandse en plaaslike markte op produsente te bepaal. Markte wat hoër pryse aanbied het ’n laer toleransie vir die voorkoms van na-oes gepokteskil as markte wat laer pryse aanbied en word gesien as hoë risiko. As gevolg van ’n drastiese afname in markprys vir vrugte met gepokteskil kan van die behandelings in die studie moontlik koste effektief wees.

From this project two MSc Agric theses were written and are available on the University of Stellenbosch library website for download.

Jacques Louis Ehlers, Post-harvest rind pitting on ‘Valencia’ Oranges:
http://hdl.handle.net/10019.1/98751

Jeanine Joubert: Influence of rind water content on mandarin citrus fruit quality:
http://hdl.handle.net/10019.1/98607

5.2.4 PROGRESS REPORT: Investigating cold storage potential of new mandarin citrus selections/cultivars and the effect of ethylene degreening on rind disorders

Project PHI-64 (Apr 2014 – Mar 2017) by N. Mathaba (ARC - ITSC)

Summary

The aim of this study was to investigate effect of ethylene de-greening and low temperature storage (-0.5, 2.0 and 4.5°C) on internal and external quality variables of new mandarin cultivars i.e./ ‘Sonet’, ‘Honey Gold’ (I22), ‘ARCCIT1614’ (B17) and ‘ARCCIT1519’ (B24), M37, Nova–ARC, ‘Nadorcott’ and ‘Nova’. Mandarin fruit were collected from the ARC-ITSC in Addo (Eastern Cape) during the 2014 and 2015 seasons. After harvesting, some fruits were de-greened while others were not de-greened, packed and transported to the Post-harvest Laboratory at ARC-ITSC in Nelspruit. Fruits were sorted into three replicates of 15 fruit each and subjected to cold storage temperatures (-0.5, 2.0 and 4.5°C) for 28 days. After 28 days of cold storage fruit were evaluated for weight loss, firmness, colour, total soluble solids (TSS), titratable acidity (TA), electrical conductivity (EC) and chilling injury (CI). Of these selections/cultivars only M37 showed significantly higher chilling injury incidence. However, there was significant increase in weight loss and electrical conductivity (EC) observed after 28 days of cold storage. Fruit lost firmness during storage and an increase in TSS and a reduction in TA were noted for all the selections. This observation was more pronounced in M37 and ‘Honey Gold’ (I22). All studied selections maintained their good external and internal qualities during the simulated cold disinfested protocol (-0.6°C for 22/24 days) with exception of M37 and ‘Honey Gold’ (I22).

A third season of cold sterilization evaluations, and concomitantly analysis of rind antioxidants, will be done during 2016. Furthermore, cold sterilization of other non-ARC selections and cultivars (Clemcott, Clemcott Seedless, Tango and Mandalate) will also be included in the study.

Opsomming

Die doel van hierdie studie was om die effek van etileen ontgroening en lae temperatuur opberging (-0,5, 2.0 and 4.5°C) op interne en eksterne gehalte veranderlikes van nuwe mandaryn kultivars (Sonet, 'Honey Gold' (I22), 'ARCCIT1614' (B17) and 'ARCCIT1519' (B24), M37, Nova–ARC, 'Nadorcott' en 'Nova') te ondersoek. Vrugte was verkry vanaf die LNR-ITSG se proefplaas op Addo (Oos-Kaap) gedurende die 2014 en 2015 seisoene. Na-oes was van die vrugte ontgroen terwyl ander nie die behandeling ontvang het nie, dit is verpak en vervoer na die na-oes laboratorium van die LNR-ITSG in Nelspruit. Daar is vrugte herverpak in kleiner kartonne, met 15 vrugte per karton. Elke behandeling het bestaan uit drie herhalings (kartonne) wat in koelopberging (-0.5, 2.0 and 4.5°C) geplaas was vir 28 dae. Na die 28 dae koelopbergingsperiode is die vrugte geëvalueer vir gewigsverlies, fermheid, kleur, totale oplosbare vastestowwe (TOV), titreerbare sure (TSS), elektriese geleiding (EG) en koueskade (CI). Van al die seleksies wat bestudeer was, het net seleksie M37 betekenisvol meer koue skade gehad. Na 28 dae koelopberging was daar 'n beduidende toename in gewigsverlies en elektriese geleiding (EG) waargeneem. 'n Toename in TSS en 'n afname in TA was waargeneem vir al die seleksies wat tot 'n toename in die Brix persentasie gelei het. Hierdie toename was meer prominent in M37 en 'Honey Gold' (I22). Alle seleksies, behalwe M37 en 'Honey Gold' (I22), het goeie eksterne en interne kwaliteit behou gedurende koelopberging.

'n Derde seisoen van lae temperatuur opberging tesame met bepaling van skil antioksidante sal gedurende die 2016 seisoen gedoen word. Verder sal addisionele seleksies soos Clemcott, Clemcott Seedless, Tango and Mandalate ingesluit word by die proewe.

5.2.5 PROGRESS REPORT: Effect of irradiation levels on internal and external citrus fruit quality

Project 1124 (PHI-63) (2014/5 – 2016/7) by P.J.R. Cronje and J. North (CRI)

Summary

Irradiation of citrus fruit to sterilize insect larvae such as false codling moth and various fruit fly species could develop into a technology that would enable the reduction of the cold sterilisation protocol. It has been determined that the minimum level of irradiation to sterilize FCM larvae is 60 Gy (+ 16 days @ 2.5°C) or a stand-alone treatment of 100 Gy. Due to the complexity of administering a specific target dose to all fruit in a carton or pallet, various problems would need to be addressed. The distribution of dosage through a package of fruit (box or pallet) is not uniform. An irradiation treatment specifies the minimum dosage required and inevitably many fruit in the treated package will therefore be exposed to higher dosages - generally in the order of 2 to 3 times the minimum required dosage, but this could be as high as 4 times. The min-max ratio is dependent on many factors including source of irradiation, installation, packaging and fruit type. Earlier exploratory trials indicated that citrus fruit is sensitive to irradiation dosages in the range of 200 to 500 Gy. In this experiment 7 cultivars were exposed to 200, 300, 400 or 500Gy and cold stored at either 2 or 7°C for either 42 or 62 days prior to evaluation of external and internal quality. There were significant differences in responses between cultivars. In general, at this very early stage of the project, 300 Gy seem to be the maximum level that most cultivars could tolerate. The full set of experiments will be reported in the 2016 season before a final conclusion will be made.

Opsomming

Bestraling van sitrusvrugte om inseklarwes daarin soos die false codling moth (FCM) asook vrugte vlieg spesie te sterliseer kan ontwikkel word in n tegnologie om die verlaging in die koue-sterilisasie protokol. Daar was bepaal dat die minimum bestralingsdosis om n FCM larwe te sterliseer 60 Gy plus 16 dae @2.5°C is of n bestraling van 100 Gy sonder koelopberging. As gevolg van verskeie komplekse aspekte om die verlangende dosis toe te dien in n karton of pallet basis moet seker probleem opgelos word. Eerstes is die verspreiding van die dosis deur n pallet nie uniform nie. Oor die algemeen spesifiseer bestralings dosis die minimum waaraan alle vrugte blootgestel moet word en dit lei daarna dat ander vrugte in die pallet/karton teen heelwat hoër dosis (2 tot 3 en soms tot 4 keer) bestraal word. Hierdie min-mak ratio is afhanklik van verskeie faktore soos die bestralings aanleg, karton asook vrugtipe. Voorlopige proewe het getoon sitrus vrugte sensitief is tussen 200-500 Gy. In hierdie seisoene se eksperimente was 7 kultivars aan bestraling van 200, 300, 400 en 500Gy blootgestel voor opberging teen of 2°C of 7°C vir 40 of 60 dae waarna vrugte geëvalueer was vir skil en interne

kwaliteit. In die algemeen blyk 300 Gy die maksimum bestralings vlak wat die vrugte kan hanteer. Hierdie hele reeks proewe sal in 2016 herhaal word voor finale afleidings gemaak kan word.

5.2.6 PROGRESS REPORT: Non-destructive prediction and monitoring of post-harvest rind quality of citrus fruit using Vis/NIR spectroscopy

Project PHI 2/2014 (01/01/2015 - 31/12/2016) by Lembe Magwaza, University of KwaZulu-Natal

Summary

The development of various types of post-harvest physiological rind disorders, including chilling injury, rind pitting of oranges and grapefruit, rind breakdown disorder (RBD) of 'Nules Clementine' mandarins and peteca spot of lemons limits post-harvest storage potential and causes commercial losses. The overall aim of the study is to develop non-destructive methods to predict quality of citrus fruit. Experimental sites were identified in 4 provinces. 'Nules Clementine' mandarins fruit were harvested from 2 orchards in the Eastern and Western Cape provinces. 'Benny Valencia' fruit were harvested from 2 orchards in Mpumalanga and Limpopo provinces, while Marsh grapefruit were harvested from 1 orchard in KwaZulu-Natal, 2 in Limpopo and 1 in Mpumalanga provinces. Fruit were harvested from mid-May until mid-July 2015 whereafter internal quality measurements were done. The fruit were cold stored to determine the effect of pre-harvest factors (spatial location of fruit on tree, maturity status) on susceptibility to physiological rind disorders. The Vis/NIR spectral evolution of the fruit during refrigerated storage, including the development of rind pitting was recorded. Preliminary research studies were conducted to assess the use of Vis/NIRS to monitor and predict internal fruit quality. Rind biochemical analysis and chemometric analysis of spectral data will be performed at the end of laboratory experiments.

Opsomming

Die voorkoms van verskeie na-oes fisiologies-skildefekte soos gepokteskil, koueskade, skilafbraak en peteka bly problematies en beperk die na-oes potensiaal van die sitrusvrugte en verminder die waarde. Die oorkoepelende doel van die studie is gemik om n nie-destruktiewe metodes te ontwikkel waarmee kwaliteit van die sitrusvrug voorspel kan word. Daarvoor is proefpersele in 4 provinsies gebruik waar 'Nules Clementine' mandaryn vrugte van 2 boorde in die Wes en Oos-Kaap geoes is. Daar was ook 'Benny' valencia lemoene in twee boorde in Mpumalanga en Limpopo elk ge-oes en so ook 'Marsh' pomelo's in 1 boord in Mpumalanga, KZN en Limpopo elk. Die vrugte was geoes tussen middel Mei tot middel Julie in 2015 waarna die interne kwaliteit bepaal was. Die vrugte is koud gestoor om die impak van voor-oes faktore soos posisies in die boom en oes-rypheid te bepaal op die voorkoms van skildefekte. Die Vis/NIR spektrale evolusie van die vrugte gedurende koelopberging, insluitend die ontwikkeling van skildefekte was bepaal. Voorlopige navorsing was gedoen om die gebruik van Vis/NIR te assesseer en te monitor om die interne kwaliteit te voorspel. Skilbiochemiese analises asook chemometriese analises sal tesame met die spektrale data gebruik vir dataontleding aan die einde van die navorsing.

5.3 PROGRAMME: FRUIT PRODUCTION AND QUALITY

Programme coordinator (Acting): Tim G Grout (CRI)

5.3.1 Programme summary

Research on fruit production and quality requires a long-term approach due to the multiple factors that are involved in determining these attributes. The research to quantify citrus' water requirements in different parts of the country that is cofunded by the Water Research Commission has had to be extended due to difficulties experienced with instrumentation in citrus trunks. However, large volumes of high-quality data are now being generated that can be used in developing irrigation models (5.3.2). A project investigating flower induction in early and late mandarins and various means of manipulating this is underway, but it is too soon to draw any conclusions (5.3.3). A related project having a more in-depth look at the importance of carbohydrates, hormones and photosynthesis at different times of the season in mandarins has just started with a view to learning how to maintain a consistent yield (5.3.7). The increasing trend to grow citrus under net is being investigated in two projects. One of these involves a comparison between adjacent blocks of mature trees that were either under nets or in the open (5.3.4). Early results are showing that the net does improve size but results in more vegetative growth and poorer fruit colour. The second project involves a randomised trial on young trees that will monitor many more parameters including pest infestations (5.3.8). A multi-year project on the pruning of mandarins is underway but differences between pruning methods will only become apparent after a few seasons (5.3.5). 2,4-D has been shown to reduce the size of the stylar opening in navel oranges

but it may also reduce the incidence of Alternaria black core rot and this is under investigation (5.3.6). Research on nitrogen release from organic soil amendments has shown that manure-based composts have a higher initial NO_3^- contribution to the soil profile but that organic soil amendments have no impact on the NH_4^+ contribution to the soil profile (5.3.9). The quest for more consistent production and fruit quality will continue in this research programme.

Programopsomming

Navorsing op vrugproduksie en kwaliteit vereis 'n langtermyn-benadering as gevolg van die verskeie faktore wat betrokke is by die bepaling van hierdie eienskappe. Die navorsing om waterbehoeftes van sitrus in verskillende dele van die land te bepaal en wat ook deur die Waternavorsingskommissie befonds word, moes verleng word as gevolg van probleme wat ondervind is met instrumentasie in sitrusstamme. Groot volumes hoë gehalte data word egter nou gegenereer wat gebruik kan word in die ontwikkeling van besproeiingsmodelle (5.3.2). 'n Projek wat blominduksie in vroeë en laat mandaryne ondersoek en verskeie maniere van manipulering hiervan is aan die gang, maar dit is te vroeg om enige gevolgtrekkings te maak (5.3.3). 'n Verwante projek met 'n meer in-diepte ondersoek na die belangrikheid van koolhidrate, hormone en fotosintese op verskillende tye in die seisoen in mandaryne het begin met die oog daarop om te leer hoe om 'n konsekwente opbrengs te bewerkstellig (5.3.7). Die toenemende tendens om sitrus onder nette aan te plant word in twee projekte ondersoek. Een van hierdie projekte behels 'n vergelyking tussen aangrensende blokke van volwasse bome wat óf onder nette of in die oopte aangeplant is (5.3.4). Vroeë resultate toon dat die nette die grootte verbeter, maar lei tot meer vegetatiewe groei en swakker vrugkleur. Die tweede projek behels 'n gerandomiseerde proef op jong bome wat baie meer parameters sal insluit soos monitering van plaag infestasies (5.3.5). 'n Langtermyn jaar projek op die snoei van mandaryne is aan die gang, maar verskille tussen snoeimetodes sal eers na 'n paar seisoene beskikbaar wees (5.3.5). 2,4-D het getoon om die grootte van die nawel opening in nawels te verminder, maar dit kan ook die voorkoms van Alternaria swart kernvrot verminder en dit word nou ondersoek (5.3.6). Navorsing oor die vrylating van stikstof uit organiese grondtoedienings het getoon dat kompos (met mis) 'n hoër aanvanklike NO_3^- bydrae tot die grondprofiel het maar dat organiese grondtoedienings geen invloed op die NH_4^+ bydrae van die grondprofiel het nie (5.3.9). Die soeke na meer konsekwente produksie en vrugkwaliteit sal in hierdie navorsingsprogram voortgesit word.

5.3.2 PROGRESS REPORT: A novel approach to water and nutrient management in citrus

Project 986 (August 2010 – Mar 2018) by J.T. Vahrmeijer (CRI at UP), N.J. Taylor (UP), K. Küther, M. Sam (UP) and M. Banda (UP), A. Bresler (UP), N. Neethling (UP)

Summary

Accurate estimates of evapotranspiration (ET) are fundamental for water management practices, design of irrigation systems and irrigation scheduling. Evaporation (E), which is a non-beneficial water loss, can be a significant portion of ET due to the large areas of bare soil between tree rows in citrus orchards. Transpiration on the other hand relates to tree assimilation and water productivity that influence water use efficiency. Therefore, it is important to partition ET into its E and T components and to evaluate them separately. Nine months of good data that included measurements of T volumes, soil evaporation, ET, stomatal conductance, photosynthesis, interception of photosynthetically active radiation (PAR) and stem and leaf water potentials was collected from a number of citrus orchards in Citrusdal. Results indicated that T did not follow the increase in reference evapotranspiration (ET_o) during the hot dry summer months, while in winter T values were much closer to ET_o. Transpiration in a 9 year-old 'Washington' Navel orchard varied between 0.06 and 1.29 mm day^{-1} (average of 0.76 mm day^{-1}) and in a 7 year-old 'Midknight' Valencia orchard between 1.42 and 2.62 mm day^{-1} (average of 2.11 mm day^{-1}), with the higher T values reflecting a bigger canopy and larger leaf area index (LAI). Both solar radiation (R_s) and vapour pressure deficit (VPD) influence stomatal conductance and it is believed that in the mornings R_s control changes in stomatal conductance, but as the day progresses VPD increases and stomata start to close. Transpiration was modelled with a crop coefficient approach and a simplified canopy conductance approach. Preliminary results for the crop coefficient approach indicate an adequate estimate of seasonal water use for a 7 year-old 'Midknight' Valencia orchard, but overestimated water use in a 9 year-old 'Washington' Navel orchard. The simplified canopy conductance model performed reasonably well on a monthly basis, but poor estimates of T were obtained at the start of the season.

Opsomming

Die betroubare bepaling van evapotranspirasie (ET) is fundamenteel vir waterbestuurspraktyke, ontwerp van beproeiingsisteme en besproeiingskedulering. Verdamping, wat 'n nie-voordelige waterverlies is, kan 'n

wesenlike fraksie van ET vorm as gevolg van die groot oop grondoppervlaktes tussen die boomrye in sitrusboorde. Transpirasie (T) aan die ander kant hou verband met koolstofassimilasie in bome asook waterproduktiwiteit wat weer die doeltreffendheid van watergebruik bepaal. Daarom is dit belangrik om ET in sy E en T komponente te verdeel en afsonderlik te evalueer. Hoë kwaliteit data wat metings van transpirasie, grondverdamping, ET, huidmondjiegeleiding, fotosintese, fotosintetiese aktiewe straling (PAR) en stam- en blaarwaterpotensiale insluit, is vir nege maande in verskillende sitrusboorde in Citrusdal versamel. Resultate dui daarop dat T nie die verhoging in verwysingsevapotranspirasie (ET_o) gedurende warm droë somers volg nie, terwyl T waardes gedurende die wintermaande nader aan ET_o is. Transpirasie in ŉ boord met 9 jaar oud 'Washington' nawels het tussen 0.06 en 1.29 mm dag^{-1} (gemiddeld van 0.76 mm dag^{-1}) gewissel, terwyl vir 7 jaar oue 'Midknight' Valencia's T tussen 1.42 en 2.62 mm dag^{-1} (gemiddeld van 0.76 mm dag^{-1}) gewissel het. Die hoër T waardes in die 'Midknight' Valencia's word verklaar deur ŉ groter blaardak en blaaroppervlakindeks (BOI) as in die geval van die 'Washington' nawels. Beide sonstraling (R_s) en dampdrukverskil (VPD) beïnvloed huidmondjiegeleiding. Dit wil voorkom of R_s gedurende die oggende huidmondjiegeleiding beheer, maar soos wat VPD verhoog begin die huidmondjies sluit. Transpirasie is gemodelleer met ŉ gewaskoeffisientmodel en met ŉ vereenvoudige blaardakgeleidingsmodel. Voorlopige resultate vir die gewaskoeffisient modelbenadering dui daarop dat die seisoenale watergebruik vir die 7 jaar oue 'Midknight' Valencia's realisties voorspel is, maar vir die 9 jaar oud 'Washington' nawels is die watergebruik oorskat. Die vereenvoudige blaardakgeleidingsmodel het T op ŉ maandelikse basis realisties voorspel, maar T is swak gemodelleer aan die begin van die seisoen.

5.3.3 PROGRESS REPORT: Determining the time and duration of flower induction in early vs late mandarin cultivars and evaluating the effect of hand thinning, pruning and girdling on leaf and root carbohydrate levels, fruit size, vegetative regrowth and alternate bearing in Nadorcott mandarin

Project 1106 (Apr 2014-Mar 2018) by Jakkie Stander and Paul Cronje (CRI at SU)

Summary

The objective of this project is to pinpoint and compare the time and duration of flower induction in mandarins, by measuring the flowering inhibition response to GA_3 applications at different times throughout the expected flower induction period. Thereafter the project will evaluate manipulations of vegetative and reproductive growth from January to April to change carbohydrate allocation and/or restore carbohydrate levels and reduce the effect of endogenous gibberellins on flower induction in May to August. With this in mind, 'Nadorcott' mandarin trees will be used in experiments to establish whether there are significant treatment effects on carbohydrate availability in the leaves and possibly correlating it with the following season's fruit load and quality. In addition, treatment effects on problems such as small fruit size and vigorous vegetative regrowth will be quantified throughout.

Opsomming

Die doel van hierdie projek is om die tyd en durasie van blominduksie in mandaryne te bepaal en te vergelyk, deur die blomreaksie op verskillende GA_3 toedienings tydens die verwagde blominduksie periode te meet. Daarna sal verskillende manipulasies van vegetatiewe, sowel as reproduktiewe groei vanaf Januarie tot April evalueer word, met die doel om koolhidraat allokasie tussen sinkorgane te manipuleer en/of om koolhidraatvlakke te herstel en die inhiberende effek van interne gibereliene op blominduksie vanaf Mei tot Augustus te verminder. 'Nadorcott' mandaryn bome sal in eksperimente gebruik word om vas te stel of daar enige betekenisvolle effek van behandelings op blaar- en wortel koolhidraat-vlakke is en dit moontlik korreleer met vruglading en kwaliteit. Behandelingseffekte sal ook addisioneel evalueer word op probleme soos klein vrugte en aggresiewe vegetatiewe groei.

5.3.4 PROGRESS REPORT: Effect of shade net on fruit production and pruning requirements of mandarin citrus

Project 522020 (2014/5 – 2016/7) by N.J.R. Roets, R.B. Cronje (ARC-ITSC) and I.F. Ngwamba (UKZN)

Summary

During the 2015/2016 season the effect of shade net on production and growth of 'Nadorcott' mandarin was investigated for the second consecutive season. It was found that the use of low intensity shade net resulted in improved fruit size (~6 mm increase in fruit diameter) and quality (higher TSS/TA-ratio). The yield for the 2015 season was not affected, as well as fruit set after the flowering period at the end of 2015 (2016 crop).

The higher dew points obtained under the net, especially during winter nights (up to 5°C higher), pointed towards a potential of nets providing protection against possible frost and associated damage, in addition to the protection it offers against hail and sunburn damage to fruit. However, some disadvantages found using net were more vigorous vegetative flush, in which case vegetative flushes of trees under the net were approximately 9% longer than trees not under the shade net. Secondly, fruit under the net was found to colour poorer under the net, with a less intense orange colour. More vigorous vegetative growth would mean higher input costs to control growth. In general, leaves of trees under the net were found to be larger with a higher chlorophyll content. The trial should continue to confirm the results of the 2015/2016 season to enable a final recommendation after the 2016/2017 season.

Opsomming

Die effek van skadunet op groei en produksie van 'Nadorcott' manderyne was verder ondersoek gedurende die 2015/2016 seisoen. Daar was gevind dat skadunet vruggrootte (~6 mm toename in vrugomtrek) en kwaliteit beduidend (hoër TOV/TS-verhouding) verbeter het. Die opbrengs vir die 2015 seisoen en vrugset na blom gedurende die 2015 seisoen was egter nie geaffekteer deur die net nie. Doupunt was hoër onder die net, veral gedurende winter aande (tot 5°C hoër) en dit wys dat nette, behalwe vir beskerming teen hael en sonbrand van vrugte, verdere beskerming kan bied teen ryp en die skade geassosieer daarmee. Die gebruik van skadunet hou egter ook sekere nadele in. Daar was gevind dat bome onder skadunet vegetatief baie meer groeikragtig is, waar in hierdie geval takke van bome onder die net ongeveer 9% langer was as takke van bome wat nie onder die net was nie. Vrugte van bome onder die net het gedurende rypwording nie so goed gekleur soos vrugte van bome wat nie onder die net was nie en was mider oranje in kleur. Die meer groeikragtige bome onder net kan moontlik aanleiding gee tot hoër insetkoste om die groei te beheer. Die proef word gedurende die 2016/2017 seisoen herhaal om die resultate van die 2015/2016 seisoen te bevestig voordat 'n finale aanbeveling gedoen word.

5.3.5 PROGRESS REPORT: Effect of pruning on fruit production of Nadorcott mandarin

Project 522019 (2014/05 – 2016/17) by R.B. Cronje, C.F. Human and I.M. Ratlapane (ARC-ITSC)

Summary

A trial aimed at developing pruning strategies for 'Nadorcott' mandarin, both young and old trees, was initiated in 2014. A new trial on one (1) year old trees, aimed at forming tree structure from the beginning, was included in August 2015. The trial on older trees (10 years old) consists 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). The trial on young trees (4- and 1-year old, respectively) includes three (3) treatments, namely two selective hand pruning treatments (pyramid and open vase shape) and a control (untreated until trees touch each other). Pruning was carried out in July and August 2015 and January 2016 (shoot control). The intended pruning in November 2015 for the selective pruning after fruit drop could not be done as the pruning contractors used by the farm pruned these trees in August 2015 although they were clearly marked. All removed branches were weighed at all pruning times to determine the amount of plant material removed from each treatment and pruning time. Tree height was measured before and after pruning. Leaf samples were taken at fruit set (Sept), after fruit drop (Nov) and at flower initiation (April) to determine changes in starch levels of the trees.

In the 10-year old trees, yield after the first year's pruning was highest in the selective pruning after fruit drop (26.6 kg/tree), followed by the control (21.6 kg/tree), and lowest in the severe selective pruning and selective and mechanical pruning combination (14.6 and 14.2 kg/tree, respectively). Yield data corresponded with the amount of cut-off branches of the previous pruning. Severe pruning reduced yield more than light pruning. There were no big differences in fruit size. However, the treatments with higher crop (selective pruning after fruit drop and light selective pruning) tended to have more fruit in the size categories 69-72 mm, whereas lower crop, as in mechanical pruning after harvest, gave higher percentages fruit in size category 73-78 mm. After pruning in August 2015, tree height was reduced by up to 30 cm on average (1-12% of initial height). Removed plant material of all pruning times combined, was lowest in the mechanical pruning treatment (7.1 kg/tree) and the highest in the severe selective pruning treatment (22.2 kg/tree). At flowering, light and severe selective pruning had the overall highest percentage flowering. Mechanical pruning had the highest percentage white flower, severe selective pruning had the highest percentage green flower and flush. Fruit set was highest in selective pruning after fruit drop and mechanical pruning, and lowest in light and severe selective pruning, and in the control. Alternate bearing patterns as a result of the previous year's pruning are clearly visible. Starch content for the new season is still being analysed.

Yield in the four (4) year-old trees was highest in the control (45.4 kg/tree), which was not pruned at all in July 2015, followed by the open vase pruning (30 kg/tree) and selective (pyramid) pruning (29.2 kg/tree). There were big differences in fruit size between the treatments. The control had smaller fruit (30% in category 60-64 mm) and the other two treatments bigger fruit (> 50% in categories 65-68, 69-72 and 73-78 mm). After harvest, tree height was reduced in all treatments by about 30 cm (18% reduction), but cut-off branches of all pruning times combined were 3.8 kg for the selective and open vase pruning and 2.8 kg for the control. Flower intensity during September 2015 was highest in the open vase treatment (79%), followed by the selective pruning (75%), and was lowest in the control (32%). As was also seen in the big trees, high yields in the previous season caused low flowering in the following season in the small trees, but the trend was much stronger. This was also confirmed by starch content data that showed highest values at harvest for the lower cropping selective and open vase treatments (7.6 and 6.5%, respectively) and low value for the high yielding control treatment (3.7%). High yield uses high amounts of tree reserves. There was, however, little difference in fruit set.

The one (1) year old trees were only lightly pruned to correct branching angles and shape them into the specific tree structures. No data for cut-off branches or starch were taken at this stage. First yield data for these trees will be collected in June 2016.

The trials will continue with the second trial harvest data being collected in June 2016 and the third season's pruning done in July/August 2016.

Opsomming

'n Proef vir die ontwikkeling van 'n snoeistrategie vir 'Nadorcott' mandarin op ouer sowel as jong bome, het in 2014 begin. Om die vorming van die boomstruktuur vanaf die begin te bepaal is 'n proef op eenjarige bome in Augustus 2015 ingesluit. Die proef op ouer bome (10 jaar oud) bestaan uit ses behandelings wat selektiewe snoei met die hand (lig en hard na oes, of net na vrugval), meganiese snoei na oes, 'n kombinasie van hand en meganiese snoei in alternatiewe jare en 'n kontrole (plaaspraktyk) insluit. Die proef op jong bome (4 en 1 jaar oud, respektiewelik), sluit drie behandelings in, naamlik twee selektiewe hand snoei behandelings (piramiede en oop kelk) en 'n kontrole (onbehandeld tot bome aan mekaar raak). Die snoei behandelings is in Julie en Augustus 2015 (na vrugval, slegs een behandeling) en Januarie 2016 (waterloot beheer) gedoen. Die snoeiaksie vir selektiewe snoei na vrugval wat in November 2015 sou plaasvind, is nie gedoen nie omdat die snoeikontrakteurs wat die nie-proefblokke moes snoei, die proefgedeeltes wat duidelik gemerk was ook gesnoei het. Alle afgesnyde takke is geweeg op al die snoeidatums om die hoeveelheid plantmateriaal wat van elke behandeling en snoeityd verwyder is te bepaal. Boomhoogte is voor en na snoei gemeet. Blaarmonsters is met vrugset (Sept), na vrugval (Nov), en tydens blominisiasie (Apr) versamel om veranderings in styselwaardes te bepaal.

In die 10 jaar oue bome was die oes na die eerste jaar se snoei die hoogste in die selektiewe snoei na vrugval (26.6 kg/boom) gevolg deur die kontrole (21.6 kg/boom). Die laagste oes was by die harde selektiwe snoei en meganiese snoei kombinasie (14.6 kg en 14.2 kg/boom, onderskeidelik). Oesdata kom ooreen met die hoeveelheid plantmateriaal verwyderd van die vorige snoei. Daar was nie noemenswaardige verskille in vruggrootte by die verskillende snoeibehandelings nie. Die behandelings met die hoogste oes (selektiewe snoei na vrugval en ligte selektiewe snoei) het meer vrugte in die katogorie grootte 69 tot 72 mm, terwyl die kleiner oes (meganiese snoei na oes) 'n hoër persentasie in die katogorie 73- 78 mm het. Na die snoei in Augustus 2015 was boomhoogte gemiddeld 30 cm kleiner in al die behandelings. Die verwyderde plantmateriaal van alle snoeitye gekombineer was die laagste in die meganiese snoei behandeling (7.1 kg/boom) en die hoogste in die harde selektiewe snoeibehandeling (22.2 kg/boom). Die ligte en harde selektiewe snoei het die hoogste persentasie blomme/boom gehad. Meganiese snoei het die hoogste persentasie wit blomme gelewer terwyl die harde selektiewe snoei die hoogste persentasie groen blomme en nuwe lootgroei gelewer het. Vrugset was die hoogste in selektiewe snoei na vrugval en meganiese snoei, en die laagste in ligte en harde selektiewe snoei asook die kontrole. Alternatiewe drag patrone as gevolg van die vorige jaar se snoei was duidelik sigbaar. Stysel data vir die nuwe seisoen is nog nie beskikbaar nie.

Oes in die vier (4) jaar oue bome was die hoogste in die kontrole (45.4 kg/boom) wat nie gesnoei was in Julie 2015 nie, gevolg deur die oop kelk snoei (30 kg/boom) en selektiewe (piramied) snoei (29.2 kg). Groot verskille in vruggroote tussen behandelings is waargeneem. Die kontrole het kleiner vrugte gelewer (30% in kategorie 60-64 mm) en die ander twee behandelings groter vrugte (meer as 50% in katogorie 65-68, 69-72 en 73-78 mm). In al die behandelings was boomhoogte na oes kleiner met omtrent 30 cm (18% vermindering). Die totale verwyderde plantmateriaal van alle snoeitye gekombineer was 3.8 kg vir die selektiewe en oop kelk snoei en 2.8 kg vir die kontrole. Die blom intensiteit gedurende September 2015 was die hoogste in die oop kelk behandeling (79%) gevolg deur die selektiewe snoei (75%) en die laagste in die kontrole (32%). Dieselfde

verskynsel soos waargeneem in die 10 jaar oue bome waar ‘n groot oes opgevolg word deur ‘n verminderde blomset in die volgende seisoen het meer prominent voorgekom in die jonger (4 jaar) bome. Hierdie neiging is ook bevestig deur die stysel inhoud data. Die stysel inhoud was die hoogste by die selektiewe en oop kelk behandelings (7.6 en 6.5%, onderskeidelik) wat die laagste oes gelewer het. Die stysel inhoud was die laagste by die kontrole (3.7%) wat die hoogste oes opbrengs gelewer het. Groter oeste gebruik meer boom reserwes. Daar was nie groot verskille in die vrugset nie.

Die een (1) jaar oue bome is slegs lig gesnoei om takgroei en vorm te beheer. Geen data vir die verwyderde plantmateriaal of stysel toetse is geneem nie. Die eerste oesdata sal versamel word in Junie 2016.

Die proef gaan voort met die tweede oesdata wat in Junie 2016 versamel word en die derde seisoen se snoeiaksie in Julie/Augustus 2016.

5.3.6 PROGRESS REPORT: Potential of 2,4-D as commercial solution to Alternaria black core rot (ABCR) in Navel oranges

Project 1130 (Apr 2015-Mar 2017) by Jakkie Stander and Dr. G.C. Schutte (CRI)

Summary

Alternaria black core rot (ABCR) is a pre-harvest disease of Navel oranges. It is caused by the fungus *Alternaria citri* and causes infected fruit to colour and drop prematurely. The rupturing of the fruit style provides lesions through which the fungus can penetrate to remain quiescent in the space between the secondary and primary fruit until fungal growth is stimulated under favourable conditions and eventually causes fruit drop.The effect of 2,4-D on the structural integrity of the fruit style during blossom period, has the potential of creating a physical barrier to the natural entry point of the fungus and could be another option as control agent of ABCR in Navel oranges. The aim of this study is to evaluate the effect of full bloom application of 2,4-D on ABCR of Navel oranges, as well as the effect when combined with Folicur®, a systemic control agent of ABCR. Results could increase potential of registering 2,4-D as a commercial solution to problems associated with open navel-ends.

Opsomming

Alternaria kernvrot is ’n groot voor-oes problem in Nawel lemoene. Dit word veroorsaak deur die fungus *Alternaria citri* en veroorsaak die voortydige verkleuring en val van vrugte wat lei tot oesverliese. The absisie van die blom-/vrugstyl voorsien ’n ingangspunt vir die swam om die vrug te penetreer en te ontwikkel onder gunstige omgewingskondisies. Die effek van 2,4-D om blom-/vrugstyl absisie te inhibeer, het die potensiaal om die penetrasie van die fungus te verhoed en kan moontlik as alternatiewe oplossing vir Alternaria kernvrot dien. Die doel van hierdie studie is om die die effek van volblom-toediening van 2,4-D te evalueer vir effektiwiteit om Alternaria kernvrot te verminder en te vergelyk met huidige beheermiddel, Folicur®. Resultate kan bydrae tot die moontlike registrasie van 2,4-D vir oplossing van probleme geassosieer met oop Nawel-ente.

5.3.7 PROGRESS REPORT: Studies on the reproductive development of ‘Nadorcott’ mandarin (*C. reticulata* Blanco)

Project 1131 (Apr 2015-Mar 2018) by Jakkie Stander, P.J.R. Cronje (CRI), and G.H. Barry (XLnT Citrus)

Summary

The objective of this PhD research project is to study the reproductive development of “on” and “off” ‘Nadorcott’ mandarin (*C. reticulata* Blanco) trees throughout fruit development. During the first year of the project, data will be collected throughout the production season 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 is to identify, measure and integrate the various aspects that could influence yield, i.e. carbohydrates, phytohormones and photosynthesis capacity, into a crop model. Such a model would potentially be used to identify opportune timing of a horticulture manipulation technique during the various tree phenological stages to obtain a consistent yield.

Opsomming

Die objektief van hierdie PhD navorsingsprojek is om die reproduktiewe ontwikkeling van 'Nadorcott' mandaryn (*C. reticulata* Blanco) te bestudeer. Gedurende die eerste jaar van die projek, sal data deurgans versamel word om moontlike korrelasies te vind tussen verskeie hortologiese verskynsels soos wortelgroei, blom-ontwikkeling, vrugset, vegetatiewe groei, vruglading en –kwaliteit, en fisiologiese parameters soos blaar- en wortel koolhidraatvlakke, mineraal-nutrient vlakke, interne planthormone en fotosintese tempo. Die oorhoofse doel van hierdie multi-seisoenale projek is om na die eerste seisoen, die verskeie faktore wat vruglading beinvloed te identifiseer, kwantifiseer en evensieel te manipuleer om konstante oeste van goeie kwaliteit vrugte te verseker.

5.3.8 PROGRESS REPORT: The benefits of shade netting for citrus fruit quality

Project RCE4 (Project 1123) (2015/6 – 2017/8) by Paul Cronje, Jakkie Stander, Teunis Vahrmeijer, Jade North, Martin Gilbert, Jan van Niekerk (CRI at SU), Graham Barry (XClnt citrus) Remy Rosalie, Robert Brown, Johane Botes and Du Toit Prins (SU)

Summary

This project was in its development phase during 2015, with the first data collection season starting at fruit set in 2015. All aspects regarding the development of the experimental site were successfully completed and a 2 ha Nadorcott orchard (3-year old) was covered with 20% shade netting. After completion of the structure and redesign of the irrigation system, various instrumentations were installed at the site to measure environmental and soil conditions. From 2016 until the end of 2017, the following students will be involved full time with different aspects of this project: one postdoctoral student (US-HortSci), three MSc (US-HortSci), one PhD (UP-Plant and Soil) as well as a postdoctoral candidate. The initial data captured in Oct-Dec 2015 will serve as a standard over the next three seasons to determine how the fruit, tree, soil biology and insect profile change due to shading.

Opsomming

Gedurende 2015 was hierdie projek in die ontwikkelings fase waarna die eerste data seisoen in aanvang geneem het in 2015 na vrug set. Gedurende die ontwikkeling fase was dit krities om eerstens 'n ideale boord te identifiseer met 'n hoë waarde jong kultivar en wat nie teen n steil helling geleë is nie. Daarna was dit belangrik om gepaste kontrakteurs te kry om die struktuur te bou op industrie spesifikasies. Al die aspekte was suksesvol afgehandel. Na die oprigting van die struktuur is die besproeiingstelsel aangepas en is verskeie metings instrumente in die boord geïnstalleer. Vanaf 2016 tot einde 2017 sal daar drie MSc studente voltyds op verskei aspekte van die projek navorsing uitvoer. Daar is ook vanaf November 2015 'n na doktorale genoot betrokke wat op die impak van skadunette op karoteen en koolhidraat vlakke in die vrug fokus.

5.3.9 PROGRESS REPORT: Nitrogen release from organic soil amendments

Project 1113 (August 2014 – Mar 2015) by J.T. Vahrmeijer (CRI at UP), M.C. Pretorius (CRI), C.M. van Heerden (UP) and E. Tesfamarium (UP)

Summary

Soil organic matter is a major source of plant nutrients and improves the physical properties of soils, such as the water holding capacity, aggregate stability and soil porosity. However, there is some concern that nitrogen (N) may be released from the organic material late during the season and this will influence the colouring and quality of citrus fruits. Incubation studies to determine the type of organic N fractions and the release patterns from three different organic materials are currently underway. Results from field trails at Letaba Estates, that commenced in September 2015, indicated a significant initial increase in NO_3^- in the soil and total N percentage in the leaves after fertiliser and organic material application. Cattle manure (CM) resulted in the highest increase in total N percentage in the leaves, while the control and other composts resulted in similar increases in the total leaf N. NO_3^- content of vermicompost (VC) and CM was higher than citrus-waste compost (CWC), suggesting that manure-based composts have a higher initial NO_3^- contribution to the soil profile. NH_4^+ in the soil profile had the same trend as the control and all other organic soil amendments suggesting that organic soil amendments have no impact on the NH_4^+ contribution to the soil profile. For the duration of the field trials, an increase in the soluble carbon percentage in the soil profile was observed in all applications, except for the CWC.

Opsomming

Die organiese materiaal van grond is 'n belangrike bron van plantvoedingstowwe en verbeter die fisiese eienskappe van grond soos waterhouvermoë, aggregaatstabiliteit en grond porositeit. Daar is egter 'n mate van kommer dat stikstof (N), laat deur die seisoen uit die organiese materiaal vrygestel kan word, wat die kleur en kwaliteit van sitrusvrugte sal beïnvloed. Inkubasie studies om die tipe organiese N-fraksies en die vrystellingstendens uit drie verskillende tipes organiese komposte te bepaal, word tans uitgevoer. Resultate van veldproewe by Letaba Estates, wat in September 2015 begin het, dui op ʼn betekenisvolle aanvanklik toename in NO_3^- in die grond en totale N van die blare, na die toeding van kunsmis en kompos. Gekomposteerde beesmis (BM) het die grootste toename in totale N van die blare veroorsaak, terwyl die kontrole en ander komposbehandelings soortgelyke toenames in totale blaar N tot gevolg gehad het. Die NO_3^- konsentrasie in vermikompos (VK) en BM was hoër as in die kontrole en sitrusafvalkompos (SAK), wat daarop dui dat mis-gebaseerde kompos aanvanklik ʼn groter NO_3^- bydrae tot die grondprofiel maak. Die NH_4^+ konsentrasie in die grondprofiel het dieselfde tendens as die kontrole en die ander kompostipes, wat daarop dui dat kompos geen invloed op die NH_4^+ konsentrasie in die grondprofiel het nie. 'n Toename in die oplosbare koolstof (C) in die grondprofiel is waargeneem vir al die behandelings, behalwe in die geval van SAK.

5.4 PROGRAMME: COLD CHAIN & PACKAGING

Programme coordinator: Paul Cronjé (CRI at SU)

5.4.1 Programme summary

No programme summary is provided due to there only being one project.

5.4.2 PROGRESS REPORT: Pre-cooling: ambient loading and forced air cooling of citrus fruit for cold sterilization markets

Project 1125 (2014/15 – 2017/8) by P.J.R. Cronje and Jade North (CRI), 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 2014/15 season, experiments were completed to test the viability of an increase in the scope of ambient loading of citrus fruit. In the first experiment, the impact of ambient loading on Mandarin fruit (soft citrus) quality was compared with normal precooling. No negative quality implications were recorded. The second experiment determined the possibility of ambient loading and cooling in order to induce the fruit into cold sterilisation protocols i.e. reaching a pulp temperature of -0.6°C. In addition to the normal stowing, manipulations of the air flow by closure of sections of the T-bar were tested for impact on cooling rate and uniformity. The results indicate a variation in cooling at various positions in the container from 3-7 days to reach -0.6°C at all probe positions. However, one probe in each container never reached -0.6°C. Overall the results give an indication that this technology does not offer an immediate option to induce fruit into cold sterilisation protocols from ambient temperature without precooling. In the third experiment the performance of the standard ambient loading practice was compared to two novel airflow strategies: the channelling configuration, which reduced airflow bypass between pallets; and the horizontal configuration, which forced air horizontally through the pallets. Standard ambient loading was able to cool the produce within about 3 d to the seven-eighths cooling time. The horizontal configuration performed worst on all aspects related to cooling. The cooling performance of the container clearly depended on the way in which it was stowed and convectively cooled, and stowage methods 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 ʼn vermindering in druk op die verkoelingsfasiliteite in die hawes. Gedurende 2014/5 was eksperimente afgehandel wat die wesenlikheid van uitbreiding van die warm-laai protokol getoets het. In die eerste eksperiment op sagtesitrus warm gelaai was geen negatiewe impak waargeneem nie (2°C vir 18 dae na EU). In die tweede eksperiment is gepoog om via warm-laai vrugte in koue-sterilisasie protokol te induseer (-0.6°C). Bykomende tot die gewone lugvloei patroon is daar ook eksperimentele bedekking van die T-bar vloer getoets om verkoelingtempo en uniformiteit te verbeter. Die resultate dui op ʼn groot variasie in eenvormige verkoeling tussen posisie in die houer wat strek

van 3-7 dae om -0.6°C te haal. Daar was egte in elke houer ŉ posisie wat nie die verlange temperatuur bereik het nie. Die data dui daarop dat die tegnologie van warm-laai tans nie gebruik kan word om sitrusvrugte in koueprotokol van -0.6°C te induseer nie. Die afsperring van die vloer het positiewe resultate getoon en verkoeling verbeter. In die derde eksperimente was die gewone houer vergelyk met ŉ verder eksperimentele lugvloei manipulasie asook die "Horizontal air flow kit". Die standaard konfigurasie, geen bedekking van die T-Bar vloer, het die beste resultate behaal en in 3 dae was die 7/8ste tempertuur bereik in kontras met die "Horizontal air flow kit" wat verkoeling drasties vertraag het. Die verkoeling in ŉ 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 verkoelingstempo en eenvormigheid in houers te verbeter.

5.5 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator: Johan Joubert (CRI)

5.5.1 PROGRAMME SUMMARY

Lemons (Eureka) remain the mainstream competitor in the citrus industry with regard to consumer demands for various reasons, including health benefits etc. Lemons are suitable for most citrus production areas, including the cold areas (5.5.13, 5.5.28). The lemon demand remains very high and as a result there are high 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.

The cool and intermediate citrus areas remain the best mandarin producing options (5.5.4, 5.5.7, 5.5.8, 5.5.9, 5.5.10, 5.5.19, 5.5.20, 5.5.21, 5.5.22, 5.5.27) due to specific climatic requirements. Mandarins follow lemons with high consumer demand and 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.

Due to the poor grapefruit (Star Ruby) prices in 2014, many producers removed their trees or topworked them to either lemons or mandarins, depending on the climatic region and specific rootstock choice. Prices recovered well in the 2015 season due to better export quantity control that prevented the international markets from being flooded. Marsh performed well in niche markets, resulting in some growers planting Marsh trees again. The new Valencia selections performed well (5.5.2, 5.5.3, 5.5.5, 5.5.6, 5.5.25) in the suitable citrus production areas (Letsitele) where demand for low seeded or seedless Valencias with good crop production increased. 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 (5.5.11, 5.5.12, 5.5.23, 5.5.24, 5.5.26). Producers located in the hot production areas 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 (5.5.14, 5.5.15, 5.5.16). 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.

PROGRAMOPSOMMING

Suurlemoene (Eureka) 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 (5.5.13, 5.5.28). Die aanvraag na suurlemoene bly baie hoog, met die gevolg dat nuwe aanplantings steeds toeneem. 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.

Die koel en intermediere sitrus areas is steeds die beste opsie vir mandaryn produksie (5.5.4, 5.5.7, 5.5.8, 5.5.9, 5.5.10, 5.5.19, 5.5.20, 5.5.21, 5.5.22, 5.5.27) wat meer beperk word deur spesifieke klimaats vereistes. Mandaryne volg na suurlemoene met 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.

Met die swakker pomelo (Star Ruby) pryse van 2014 het heelwat produsente hulle bome verwyder of oorgewerk na suurlemoene of mandaryne, afhangend van die klimaat streek en spesifieke onderstam keuse. Pryse het goed herstel gedurende die 2015 seisoen wat grootliks toegekryf kan word aan beter volume beheer om te voorkom dat internasionale markte gevloed word. Marsh het goed presteer in nis markte wat tot gevolg gehad het dat sekere produsente weer Marsh bome aanplant. Die nuwe Valencia seleksies presteer goed (5.5.2, 5.5.3, 5.5.5, 5.5.6, 5.5.25) in die geskikte sitrus produksie areas (Letsitele), waar aanvraag vir lae saadinhoud of saadlose Valencia's met goeie opbrengste toeneem. 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 (5.5.11, 5.5.12, 5.5.23, 5.5.24, 5.5.26). 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 (5.5.14, 5.5.15, 5.5.16). 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.

5.5.2 PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)

Project 75A by J. Joubert and M.P. Cele (CRI)

Opsomming

Weipe het wel 'n ligte oes op die bome geproduseer, wat Limpopo SL as vroegste eksperimentele Valencia seleksie vervang het. Seleksies wat hierdie seisoen, volgens optimum rypheid van vroeg tot laat goed presteer het vir hierdie vogtige warm produksie area, is soos volg. Weipe kan eerste geoes word en kom op 'n jong boom ouderdom in drag, wat vinniger kontant vloei kan verseker. Valearly is een van die nuwe vroeë Valencia opsies wat voor Turkey inpas. 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.

Benny 2 kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer. Alpha en Midknight, aangevul deur Gusocora, Jassie en McClean SL verteenwoordig die middel van die Valencia seisoen vir hierdie area. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Henrietta (lae saadinhoud), Louisa en Lavalle 2. En dan laastens sal Skilderkrans volg, wat 1.7 sade per vrug produseer.

Weipe, Valearly, Jassie, Henrietta, Louisa, en Skilderkrans 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

Weipe produced a light crop on the trees this season, which replaced Limpopo SL as the earliest maturing experimental Valencia selection. Selections that performed well in this season, in this hot, humid production area, according to optimal maturity from early to late, are as follows. Weipe bears fruit precociously and will start producing good yields on young trees, generating returns for your investment sooner. Valearly, one of the new early maturing varieties matures before Turkey. 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.

Benny 2 would follow, with good internal quality, production and fruit size. Alpha and Midknight, with the addition of Gusocora, Jassie and McClean SL represent the middle of the Valencia season for this area. The later selections can broaden the list of choices to extend the season, commencing with Henrietta (low seeded), Louisa and Lavalle 2 followed by Skilderkrans, producing 1.7 seeds per fruit.

Weipe, Valearly, Jassie, Henrietta, Louisa and Skilderkrans remain experimental/semi-commercial selections that performed well. These selections could be included in future plantings when more and better information becomes available.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Benny 2, Delta (control), Gusocora, Henrietta, Jassie, Lavalle 2, Louisa, McClean SL, Midknight 1, Moosrivier Late 1, Mouton Early, Skilderkrans, Turkey (control) and Weipe at Esselen Nursery and Riverside, Malelane, Mpumalanga.

Table 5.5.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.5.2.2. List of Valencia selections evaluated at Esselen Nursery (Malelane) during 2015.

Selection	Rootstock	Year Planted	No. of trees
Alpha	CC	1996	1
Benny 2	C35	2003	1
Gusocora	SC	2008 (Topwork)	1
Henrietta	MxT	2006	1
Lavalle 2	CC	2010 (Topwork)	2
Louisa	MxT	2006	1
Midknight 1	MxT	2006	1
Skilderkrans	MxT	2006	1
Turkey	CC	1996	1
Weipe	TB	2010 (Topwork)	1

Table 5.5.2.3. List of Valencia selections evaluated at Riverside (Malelane) during 2015.

Selection	Rootstock	Year Planted	No. of trees
Delta	C35/SC	2012	5/5
Jassie	C35/SC	2012	5/5
McClean SL	C35/CC/SC	2012	5/5/5
Moosrivier Late 1	C35	2012	5
Turkey	C35/CC/SC	2012	5/5/5
Valearly	C35	2012	5

Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Esselen Nursery (Malelane) during the 2015 season.

<u>Alpha</u>
Alpha produced bigger fruit size this season (compared to 2014) ranging from medium to large/xlarge (count 72-48) on the trees due to a lighter crop (alternate bearing). The tree condition remained very good on Carrizo rootstock, a well known rootstock with good replant qualities. There was an increase from 0.3 to 0.7 seeds per fruit this season, still qualifying as a seedless Valencia. The fruit shape remained fairly round with smooth skin

texture and thin rind thickness; no navel end was visible and there were no thorns on the bearing branches. There was a good crop on the trees. The external colour improved to T1 with exceptional internal quality. Brix was above 11, juice content was 58% and acid levels remained above 1.0%, indicating good shelf life for the fruit (Table 5.5.2.4) and complying with minimum export standards. Based on the internal quality results in Table 5.5.2.4, estimated maturity will be end of June to end of July.

Benny 2

The fibre strength (rag) remains soft compared to the other Valencia selections, except for Turkey being softer. The fruit size peaked from count 88 to 56 this season, optimal Valencia export fruit size. Tree size remains one third smaller on Citrange 35 compared to the other combinations, resulting in easier spray and harvesting practices. Benny 2 matures after Turkey and before Midknight or Delta so fits in well in the harvesting and packing programme. The fruit hangs well on the trees to harvest at a later time, resulting in fewer rind problems (pitting). Seed count was lower this season and peaked at 2.1 seeds per fruit (4.4 to 6.1 for 2014). There was no delay in external colour this season and colour development was between T1 and T3. Brix: acid ratio was 9.8 and Brix 10.7, complying with export standards. Based on the internal quality results in Table 5.5.2.4, maturity is estimated as end of June to middle of July.

Gusocora

The external colour development improved this season from between T3 and T5 (2014) to between T1 and T2 for 2015. There was no delay in external colour when compared to the internal quality maturity, with average Brix of 10.1 and acid of 0.81%. Swingle as rootstock will delay external colour development and result in higher acid levels on the fruit. Gusocora was completely seedless compared to the 0.3 seeds per fruit last season, and will be regarded as a seedless selection. The internal quality complied with the export standards with improved Brix content (10.1) and Brix: acid ratio above 12. Based on the internal quality results in Table 5.5.2.4, maturity is estimated as mid to end of July.

Henrietta

Fruit shape remained round, rind texture smooth and small thorns were visible on the bearing branches. Rind thickness was fairly thin; fruit peeled easily and contained a medium amount of rind oil. The seed count remained above 4.0 seeds per fruit, similar to the 2012 and 2014 season. The tree condition on MxT was very good and might be a possibility for a future scion: rootstock combination. Fruit size remained the same this season and peaked from medium to large (count 72 – 56). This specific size range was more favourable for Valencia exports. Internal quality indicated that Henrietta matures late in the Valencia season, with an acid content of 1.4% and external colour T1-2. Based on the internal quality results in Table 5.5.2.4, maturity is estimated as mid July to mid August.

Jassie

Jassie produced an average crop on the young trees at Riverside, bearing fruit for the first time. The fruit size varied from medium to large, count 72 to 56. Due to the young tree age the fruit developed a coarse pebbly rind that will become smoother with time. Seed count per fruit varied from 1.0 to 1.5 seeds per fruit. Internal quality was average with high juice levels (above 50), low Brix (below 10) and fairly high acids (above 1.1). External colour peaked from T1 to T2 with the final evaluations. Maturity seems to be mid to end of July based on the results in Table 5.5.2.4.

Lavalle 2

Yield remained good to excellent; one of the qualities of the Lavalle 2 selection. Another quality is good fruit size for a Valencia selection. The fruit size varied from count 64 to 48, excellent for Valencia production. The higher acid level (1.2%) duringindicated that this selection was late maturing internally. There were no seeds in the fruit evaluated this season; a decrease from last season when the count was 1.8 seeds per fruit. The internal quality complied with the export standards, producing Brix levels of 11 and juice of 58%. Lavalle 2 evaluated was planted on CC developing a medium tree size with good internal quality. Based on the internal quality results in Table 5.5.2.4, maturity is estimated as mid to end August.

Louisa

The seed count increased from 0.9 to 1.1 seeds per fruit this season, and at Group 91 the fruit remained completely seedless. The fruit size tended to be erratic this season (count 88-40), possibly due to the lighter crop. Fruit shape was round, rind texture medium to fairly smooth and the rind was medium to fairly thick. The fruit peeled easily and the internal colour remained yellow. There was a lighter crop compared to the other selection of the same age. With the evaluation the acid levels were higher compared to Henrietta, still indicating a late to ultra-late Valencia selection, with external colour already at T1. Based on the internal quality results in Table 5.5.2.4, maturity is estimated as mid to end of August.

McClean SL
The standard McClean will be included in future trials as a control to compare the SL selection's performance. McClean 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 72-48). The internal quality was good with high juice levels for the trial site of 58%, Brix 10.8 and acceptable acid levels (1.0%). There was a slight delay in external colour ranging from T1-2. Based on the internal quality results in Table 5.5.2.4, maturity will be mid to end July.

Midknight 1
MxT in combination with Midknight resulted in a medium-large sized tree; slightly smaller compared to the tree size of Swingle. The bud union looks very good; smooth with no signs of incompatibility.

The fruit size was similar to the previous season and the tree produced medium to large fruit (count 64-56). Fruit shape is fairly round, rind texture medium-coarse, fibre strength fairly soft and the fruit peels easily. Internally the flavour varied from good to very good, with juice levels around 54% and Brix 12. The acid level was similar this season (1.0%), but complied with the export requirements (Max 1.8 to min 0.85). Based on the internal quality results in Table 5.5.2.4, estimated maturity will be end of June to middle of July.

Moosrivier Late 1
Moos Late 1 developed a high acid level (1.1%) when the juice (52%) and Brix (8) content were ready for harvesting at Riverside (first crop), and the external colour was delayed up to T1-5. Moos Late 1 was completely seedless this season compared with 0.8 to 2.1 seeds per fruit last season. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag. Based on the internal quality results in Table 5.5.2.4 estimated maturity for Moos Late 1 will be from the end of July to the middle of August.

Valearly
Valearly, bearing an average crop for the first time on the trees, was completely seedless this season. The internal quality of the fruit was average with fairly low juice (47%), Brix of 9.4 and acid of 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 as well as optimum external colour development. Estimated maturity according to Table 5.5.2.4, seems to be 2nd week of May to mid-June.

Skilderkrans
Skilderkrans developed medium to large fruit size (count 72-48) on the trees, due to the lighter crop. The internal quality was good, with a high acid content, similar to the previous season of 1.6% at external colour T1-2, above the export maximum of 1.4% for Middle East and below the 1.8% for Europe by the time of the evaluation. The fruit peeled fairly easily, rind thickness was thin, rag was medium tough (raggy/strong), fruit shape was round and the rind texture medium-rough. The average seed count increased this season from 1.5 to 1.7 seeds per fruit compared to 2014. Based on the internal quality results in Table 5.5.2.4, estimated maturity will be the end of July to middle August.

Turkey (Control)
Fruit size remained similar to last season from count 88 and 48, medium to large fruit size for this season. 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 of 1.9 on average. 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 rind disorders developing. Based on the internal quality results in Table 5.5.2.4, estimated maturity will be the end of May to mid-June.

Weipe
There was a light crop on the Weipe trees for the 2015 season; compared to the previous season with no crop. Internal quality was average with low juice levels (40%), Brix of 8 and acid content of 0.85%. The fruit was completely seedless and developed a deep orange external colour (T1-4). Maturity is estimated to be middle of May to the beginning of June based on the results in Table 5.5.2.4.

Conclusions

Weipe developed low juice and did not comply with the export standards. The internal quality for this season for the remaining selections evaluated, complied with the export standards, with the exception of the late maturing Louisa and Skilderkrans, where the acid levels were above 1.4% (ME). Skilderkrans remained a late maturing Valencia with high acid levels (1.6%) at both Esselen and Group 91's trial sites. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selection. There was no Brix: acid ratio below 7:1 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. The average seed count for this season was fairly low with the exception of Henrietta (4.1 seeds per fruit), indicating less cross pollination in the mixed trial block. McClean SL remained completely seedless. Jassie will be one of the future possibilities to include on a smaller scale in new Valencia plantings (optimum Valencia fruit size, high juice levels, low seed counts). Fruit size increased on the trees, between count 88 and up to count 40 on selections with lighter yields.

Table 5.5.2.4. Internal fruit quality data for Valencia and late orange selections at Esselen Nursery and Riverside (Malelane) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	2015/07/28	Esselen	74-87	72-48	58.0	11.3	1.12	10.1	0.7	T1
Benny	C35	2015/06/02	Esselen	72-83	88-56	53.0	10.7	1.09	9.8	2.1	T1-3
Delta	C35	2015/06/02	Riverside	73-84	72-56	53.5	8.6	1.07	8.0	0.0	T2-5
Delta	C35	2015/07/28	Riverside	76-84	72-56	52.1	9.6	0.97	9.9	0.0	T1-2
Delta	SC	2015/07/28	Riverside	75-84	72-56	50.7	10.0	0.93	10.8	0.0	T1
Gusocora	SC	2015/07/28	Esselen	78-86	64-48	54.5	10.1	0.81	12.5	0.0	T1-2
Henrietta	MxT	2015/07/28	Esselen	79-85	64-56	56.6	10.6	1.38	7.7	4.0	T1-2
Henrietta	MxT	2015/08/13	Esselen	74-78	72-64	62.1	11.0	0.94	11.7	4.3	T1
Jassie	C35	2015/06/02	Riverside	78-85	64-56	47.2	8.7	1.22	7.1	0.0	T4-7
Jassie	C35	2015/07/28	Riverside	75-84	72-56	55.4	8.8	1.09	8.1	1.5	T1-2
Jassie	SC	2015/07/28	Riverside	80-85	64-56	51.1	9.6	1.18	8.1	1.0	T1-3
Lavalle 2	CC	2015/07/28	Esselen	78-88	64-48	57.5	10.9	1.20	9.1	0.0	T1-3
Louisa	MxT	2015/07/28	Esselen	75-89	72-48	51.4	11.0	1.48	7.4	0.0	T1-2
Louisa	MxT	2015/08/13	Esselen	71-90	88-40	55.9	11.5	1.55	7.4	1.1	T1
McClean SL	CC	2015/06/02	Riverside	75-84	72-56	48.7	8.7	1.06	8.2	0.0	T4-7
McClean SL	C35	2015/06/02	Riverside	73-83	72-56	49.3	8.9	1.25	7.1	0.0	T4-6
McClean SL	C35	2015/07/28	Riverside	75-86	72-48	53.4	10.8	1.12	11.9	0.0	T1
McClean SL	CC	2015/07/28	Riverside	80-85	64-56	53.3	10.6	0.87	12.2	0.0	T1-3
McClean SL	SC	2015/06/02	Riverside	75-85	72-56	47.7	8.9	1.08	8.2	0.0	T4-6
McClean SL	SC	2015/07/28	Riverside	79-89	64-48	58.6	9.6	0.93	10.3	0.0	T1-4
Midknight 1	MxT	2015/07/28	Esselen	79-82	64-56	54.2	11.9	1.00	9.6	0.0	T1
Moos Late 1	C35	2015/07/28	Riverside	80-90	64-40	51.6	8.5	1.06	8.0	0.0	T1-5
Skilderkrans	MxT	2015/07/28	Esselen	73-89	72-48	54.7	11.2	1.49	7.5	1.1	T1-2
Skilderkrans	MxT	2015/08/13	Esselen	73-89	72-48	57.2	11.7	1.66	7.0	2.3	T3-6
Turkey	CC	2015/06/02	Esselen	74-84	72-56	53.2	11.7	1.07	10.9	0.0	T1-4
Turkey	CC	2015/06/02	Riverside	79-88	64-48	49.6	8.6	0.98	8.8	1.6	T3-5
Turkey	C35	2015/06/02	Riverside	76-87	72-48	50.3	7.7	0.83	9.3	1.8	T3-5
Turkey	SC	2015/06/02	Riverside	72-83	88-56	51.8	9.6	1.05	9.1	2.3	T3-5
Valearly	C35	2015/06/02	Riverside	75-88	72-48	47.1	9.4	0.85	11.1	0.0	T1
Weipe	TB	2015/05/26	Esselen	78-89	64-48	39.2	8.1	0.84	9.6	0.0	T3-6
Weipe	TB	2015/06/02	Esselen	80-85	64-56	42.1	8.1	0.89	9.1	0.0	T1-4

5.5.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele & Hoedspruit)

Project 75B by J. Joubert and M.P. Cele (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. Weipe SL kan die seisoen begin, hierdie seleksie het Limpopo SL vervang 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. Benny 1 en 2 volg na Turkey met goeie produksie en medium tot groot vuggrootte. Delta as kontrole pas in voor Gusocora. Gusocora volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings. Midknight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vruggrootte, gladde skille en lae saadtellings per vrug. Du Roi is volgende met uitstekende oeste op die bome en medium tot medium/groot vrugte (telling 88 tot 56). Lavalle is huidiglik die laatste rypwordende Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vruggrootte en produksie.

Daar is ŉ reeks experimentele/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. Bend 8A1&2 volg, hierdie seleksies kan ook as aanvulling saam met Benny 1 en 2 gebruik word. Die middel van die Valencia seisoen word gekomplimenteer deur Jassie en Henrietta, wat goeie produksie en interne kwaliteit vrugte lewer. Louisa en Du Toit Laat word meer aan die einde van die Valencia seisoen ryp met medium tot groot vruggroote, gevolg deur Ruby en Skilderkrans. Ruby is die enigste rooi gepigmenteerde Valencia huidiglik beskikbaar met uitstekende produksie op die bome, kleiner vruggrootte met tot 2.5 sade per vrug. Laat in die seisoen kan aangevul word met Moosrivier Late 1, soos meer inligting beskikbaar word uit verdere evaluasies.

Summary

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production areas. Recommendations have therefore been made accordingly. Weipe SL will start the season, replacing Limpopo SL 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. Benny 1 and 2 follow after Turkey with good production and medium to large fruit size. Delta as a control fits in before Gusocora. Gusocora follows next with completely seedless fruit and very good Brix: acid ratios. Midknight 1 and 2 cover the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Du Roi follows with excellent crop on the trees and medium to medium-large fruit size (count 88 to 56). Valencia Late and Lavalle are currently the latest maturing Valencia selections that are being planted commercially, developing excellent fruit size and yield.

A series of experimental/semi-commercial selections has also been included in the hot production areas. The selection range follows from early, mid-, to late-maturing options. The season starts with Valearly, competing with Turkey to be the earliest maturing Valencia. Bend 8A1&2 follows; these will be additional selections available to fill in with Benny 1 and 2. The middle of the Valencia season will be complimented by Jassie and Henrietta, delivering good production and internal quality fruit. Louisa and Du Toit late mature more towards the end of the Valencia season with medium to large fruit size; followed by Ruby and Skilderkrans. Ruby is the only red pigmented Valencia available with excellent yield production, smaller fruit size and up to 2.5 seeds per fruit. Late in the season 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, Bend 8A1&2, Benny 1&2, Delta, Du Roi, Du Toit Late, Gusocora, Henrietta, Jassie, Lavalle, Louisa, McClean SL, Midknight 1, 2 & F17, Moosrivier Late 1 & 2, Valearly, Ruby, Skilderkrans, Turkey, Val Late and Weipe at Moriah Citrus (Hoedspruit), Bosveld Citrus (Letsitele) and Group 91 (Letsitele).

Table 5.5.3.1. List of Valencia selections evaluated at Group 91 (Letsitele) during 2015.

Selection	Rootstock	Tree Age	No. of trees
Bend 8A 1	CC/SC	2005	10/10
Bend 8A 2	CC/SC	2005	10/10
Benny 1	CC/SC	2005	10/10
Benny 2	CC/SC	2005	10/10
Henrietta	CC/RL	2006	Semi-Com
Jassie	CC/SC	2005	10/7
Louisa (Letaba Oranje)	CC	2007	Semi-Com
Midknight 1	CC/SC	2005	10/10
Moosrivier Late 1	CC/SC	2005	10/10
Moosrivier Late 2	CC/SC	2005	10/10
Ruby	CC/SC	2005	10/10
Skilderkrans	CC/SC	2005	10/6
Turkey	C35	2005	2
Turkey	CC	2005	4
Turkey	SC	2005	10

Table 5.5.3.2. List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2015 season.

Selection	Rootstock	Planted
Alpha	C35/SC	2009
Benny	C35/SC	2009
Benny 2	C35/SC	2009
Delta (control)	SC	2009
Du Roi	C35/SC	2009
Du Toit Late	CC/SC	2011
Gusocora	SC	2009
Jassie	CC/SC	2011
Lavalle	C35/SC	2009
McClean SL	C35/SC	2009
Midknight 1	C35/SC	2009
Midknight 2	C35/SC	2009
Turkey	C35/CC/SC	2011
Valearly	C35/CC	2011
Val Late	C35/SC	2009

Table 5.5.3.3. List of Valencia selections evaluated at Moriah Citrus (Hoedspruit) during the 2015 season.

Selection	Rootstock	Top-worked
Benny 2	MxT	2011
Gusocora	MxT	2011
Lavalle	MxT	2011
Midknight 1	MxT	2011
Midknight F17	MxT	2011

Results and discussion

Alpha

Alpha was planted on C35 and Swingle at the Bosveld trial site to compare tree development (vigour) and yield production. The internal quality was good, juice levels peaked at 50%, Brix was above 10 and acids were fairly high between 1.3 and 1.7%. Fruit size varied from count 88 to 56, excellent for Valencia production and export. External colour peaked from T1 to T2. Maturity seems to be end of June to middle of July (Table 5.5.3.4).

Bend 8A1

Bend 8A1 on Carrizo and Swingle produced a similar crop, and the tree condition on both combinations improved this season. Fruit size peaked from medium to large, count 88 to 56. The internal quality improved

with better juice levels on both rootstocks (above 50%); Brix ranged from 11.2 up to 11.9; acids were lower (1.4%) after the second evaluation and Brix: acid ratios were above 8. Fruit characteristics of Bend 8A1 were a fairly round fruit shape, fairly smooth rind, easy fruit peeling, the fibre strength is fairly soft and the internal colour light yellow. The fruit was seedless this season. Maturity seems to be mid July to beginning of August (Table 5.5.3.4).

Bend 8A2

Bend 8A2 performed well and internally produced better juice content (54%) than 8A1, slightly higher acids (1.5%) and average Brix: acid ratios (8.5:1). Fruit size varied from small to medium (count 105 to 64) on both Carrizo and Swingle. Fruit shape was round, fairly smooth rind texture, and deep yellow internal colour; it peeled easily with fairly thin rind. The seed count per fruit increased from average 1.3 to 2.2 seeds per fruit. External colour was advanced compared to Bend 8A1 (T1 with second evaluation). Fruit maturity is estimated at mid July to the end of July (Table 5.5.3.4).

Benny 1 and 2

Benny was evaluated at all three trial sites: Bosveld, Moriah and Group 91. There was a good crop on both selections and fruit size peaked between count 105 and 56 (very good for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, high rag content and medium rind thickness. Benny 1 and 2 internally produced similar juice levels (average 53%), Brix (average 11.4) acid (1.5%) and seed counts (average 2 seeds per fruit). External colour on both selections by the time of harvest varied between T1 and T2. Based on ratios, Benny 1 and 2 mature end of June to beginning of July (Table 5.5.3.4).

Delta (control)

Delta, as control variety, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 125 and 72 with good internal quality, reaching juice levels of 58%, Brix of 10.5 and acid content of 0.9%. The external colour of the fruit was between T3 and T4. Maturity is end of June to middle of July (Table 5.5.3.4).

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. There was a good yield on both combinations and fruit size peaked between count 88 and 56. The external colour peaked at T1and the average seed count was 2 seeds per fruit. Swingle developed a juice content of 56.4%, Brix of 10.5 and acids of 1.4%. Maturity is middle to end of July (Table 5.5.3.4).

Du Toit Late

Du Toit Late produced medium fruit size (count 88 to 72) on the trees, with 3.5 seeds. The internal quality was good, juice levels above 50%, Brix up to 12, and higher acids for the later maturing selection. External colour peaked from T1 to 3. Maturity seems to be middle to end of July according to Table 5.5.3.4.

Gusocora

Gusocora was evaluated at Moriah and Bosveld Citrus this year. The fruit was completely seedless and developed a good internal quality where juice (48%), Brix (11) and acid (1.1) complied with export requirements. The external colour varied from T1 to T2, correlating with the internal quality and Brix:acid ratio of 10. Fruit size 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 5.5.3.4).

Henrietta

Fruit size decreased slightly and peaked at count 125 to 88; and count 125 was small for Valencia export. The fruit shape was slightly oblong with a smooth rind texture, deep yellow internal colour and very good flavour. Fibre strength was medium, with a medium thick rind, and the fruit peeled easily with fairly low rind oil. The internal quality on Carrizo was good with average juice (53%), Brix (12) and acid (1.6%) levels. Henrietta on Carrizo (rootstock inducing high internal quality) indicated the shelf life potential of the selection. There were 1.7 seeds per fruit counted on average, lower compared to the 2014 season count of 3.4 seeds per fruit. The external colour of the fruit developed into a deep orange, very favourable for export markets. Maturity is end of July to beginning of August (Table 5.5.3.4).

Jassie

Fruit size at Bosveld was bigger (peaked between count 88 and 56) compared to Group 91 with count 125 and 88. Production was good on both rootstock combinations. Internal quality was good with juice levels of 53%, Brix above 12 and average acid levels of 1.4%. Seed count varied from 2.3 to 4.0 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, rind was smooth and the fruit peeled easily. Jassie bore high numbers of fruit inside the tree. Maturity is end of July to beginning of August in this area (Table 5.5.3.4).

Lavalle

There was a decrease in seed production this season and Lavalle produced 0.3 seeds per fruit compared to last year's 0.6. The internal quality complied with export requirements and acid level declined above 1.1% at the second evaluation at the end of July. Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August/September. 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 end of September (Table 5.5.3.4).

Louisa

The fruit set remained lighter compared to Henrietta and fruit size (counts 88 and 64) was smaller compared to 2014. Internal quality did not comply with the requirements, with juice content of 46.7%; but Brix 12.4 and acid level of 1.3% indicate that Louisa qualifies as one of the later maturing Valencia selections available currently. Louisa remained seedless; the internal colour of the fruit was light yellow, fruit shape round, medium smooth rind texture and fairly thick rind. There were small thorns on the bearing branches and the tree height on Carrizo measured 2.5 m (compact selection). Acid levels and ratios indicate that this cultivar matures end of July to middle of August (Table 5.5.3.4).

McClean SL

McClean SL was planted on Swingle at the Bosveld trial site, with a crop production of 100 kg/tree (good to very good). McClean SL remained completely seedless similar to all the other trial sites where the selection was included. Fruit size peaked from count 88 to 48. External colour varied from T1-2, Juice was above 51%, Brix on the lower side (8.3 to 10.8) and acids remain fairly high towards the end of the season, resulting in low Brix:acid ratios (5.5:1 to 8.5:1). Maturity seems to be middle of July to end of July (Table 5.5.3.4).

Midknight 1 & 2

Midknight 1 and 2 bore an average to good yield of between 70 and 90 kg per tree on the three rootstock combinations where CC was the smallest. The fruit size varied between count 125 and 40, juice content was around 52%, Brix levels around 11 and acids at 1.2%. Midknight 1 outperformed Midknight 2 with a better Brix level. Midknight 1 and 2 developed low seed numbers in the fruit, ranging from 0.2 up to 0.8 seeds per fruit. Fruit shape was round, rind texture was fairly smooth, fruit was raggy with a medium rind thickness and peeled moderately. Maturity seems to be middle of July to the end of July (Table 5.5.3.4).

Moosrivier Late 1 and 2

This season Moos Late 2 developed a more favourable fruit size (small to medium size count 105-64) compared to Moos Late 1 (small to large/extra-large size count 125-56) on both rootstocks. Crop production for Moos Late 1 was better compared to Moos Late 2. The tree canopy on Moos Late 2 was very dense with limited light inside the tree for proper fruit set (additional window pruning required). Moos Late 1 performed well, developing internal qualities that met export standards and high acids (up to 1.6%) indicating a late maturing Valencia selection. The seed count per fruit varied from 0.8 to 3.0 (a decrease from 2014). Moos Late 2 developed slightly lower Brix and fairly lower acid levels and was completely seedless. When internal quality was taken into consideration, Moosrivier Late 1 was the earlier maturing selection this season; estimated maturity end of July to middle August. Moosrivier Late 2 with delayed external colour (T2-5) seemed later maturing (Table 5.5.3.4).

Ruby

Ruby performed similarly on both rootstocks with Brix content of 11.2 to 12.5, juice levels of 55% and acids around 1.5%. External colour on the fruit was between T1 and T4. Fruit size was similar to 2013 (count 125-88) and produced a good to very good crop on the trees (70-80 kg/tree), bearing in mind the relatively small tree size (compact tree). Seeds per fruit decreased more this season and varied from 1.9 up to 2.5. Fruit shape was round, with fairly smooth rind texture, medium strong fibre internally, medium rind thickness and fruit peeled easily. Internal colour was dark red and well developed. Ruby's estimated maturity time will be end of July to mid-August (Table 5.5.3.4).

Skilderkrans

Skilderkrans at Group 91 cropped lighter this season but still produced an average yield on the trees. Fruit size varied from small to large (count 105-48). Internally the Brix content was good (12.1) and the acid level of 1.3 to 1.7% indicated a later maturing Valencia selection. Juice level increased to average 55%; above the minimum required export figure. There was no delay in external colour on Carrizo or Swingle with T1 at all evaluations. 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 mid-August on both rootstocks (Table 5.5.3.4).

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 small, to medium and large (count 88-48), high Brix content (above 12); fairly high acid levels and better Brix:acid ratio. The average seed count per fruit peaked at 7 seeds. The external colour (between T2 and T3) at the beginning of June was similar on all three rootstocks. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 developed the smallest tree size (2.5 m) in combination with Turkey. Based on the ratios, maturity will be end of May to middle June (Table 5.5.3.4).

Valearly

Valearly, bearing an average crop for the first time on the trees, was completely seedless this season. The internal quality of the fruit was good with juice levels above 49%, good Brix of 11.9 and acid of 1.0. In comparison with the other early maturing selections (Turkey and Weipe), Valearly seems to be at least two weeks earlier, similar to Weipe, with optimum external colour development. Estimated maturity according to Table 5.5.3.4 seems to be 2nd week of May to middle June.

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 and fruit size peaked from medium to large (count 88 to 64), optimal Valencia export quality. Acid levels were above 1.6% when the second evaluation was completed, indicating the late maturity qualities of the selection. The juice content was below 50% and Brix averaged at 11. Seed count varied from 0.5 to 0.6 seeds per fruit. Maturity will be late in the season and according to Table 5.5.3.4, peak middle to end of August.

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 Moriah trial site and was top-worked on MxT (older trees) as well as planted on C35 (young trees). There was limited fruit on the trees due to the smaller sized trees and light crop. Fruit size was small to large (count 105-56), internal quality was fair (juice 40%, acid 0.75%%) with higher Brix level (9.5). Colour development ranged from T1 to T3. Maturity is estimated to be end of May to the middle of June (Table 5.5.3.4).

Conclusion

Alpha performed similar to the 2014 evaluation, developing a good crop on the trees. The internal quality was good and fruit size peaked between counts 88 to 56.

Bend 8A2 produced fruit with higher seed counts per fruit. The internal quality on Bend 8A 2 improved and was better compared to Bend 8A1. The fruit size, this season, was smaller on 8A1 (counts 105-64). Benny 1 and 2 produced similar fruit qualities this season, as well as yield production and fruit size (peaked from count 105 to 56). Du Roi was evaluated on Swingle this season with fruit size ranging from count 88 to 64 (similar to 2014 season).

Gusocora performed well on Swingle (and average on MxT), meeting the export standards (acid on the lower side). The fruit size of Henrietta decreased even more in 2015 and peaked between count 125 and 88. The internal quality of the fruit was excellent on Carrizo and developed high Brix (53%) and fairly high acids with peak maturity. There was an average seed count in the fruit of 1.7.

Jassie produced an excellent internal quality on Carrizo as well as Swingle, and smaller fruit size (count 125-56) due to the cropload on the trees. Lavalle 1 was ultra-late, maturing in August/September (acid above 1.3%) on both rootstocks (Swingle and MxT).

Louisa was completely seedless, and developed medium fruit size (count 105-88) for Valencias, setting a better crop on the trees, with a more compact tree development (2.5 m on Carrizo). McClean SL remained completely seedless at the trial site with good internal quality and optimum fruit size (count 88-48).

Fruit quality on Midknight 1 was better, with higher Brix than Midknight 2. External colour was delayed on Midknight 2 this season. Normally, Carrizo produces lower acids and develops better external colour compared to Swingle, but with the Midknight 1 selection the opposite seems to be true. In the first evaluations Carrizo's acid levels were higher compared to Swingle.

Moosrivier Late 2 is later maturing when compared to Moosrivier Late 1, with higher acid percentages on Carrizo and delayed external fruit colour. Moosrivier Late 2 remained completely seedless.

Ruby produced good quality fruit, higher acids and good yields on the compact tree size and bigger fruit size. Skilderkrans performed well this season, bearing a light crop with good internal quality and similar seed count (0.3 per fruit) as 2014.

Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered. Weipe cropped a light yield on the MxT rootstocks at the trial site, with poor to fair internal quality and low acid levels by the end of May. Future evaluations will determine the value of this cultivar for the citrus industry.

This was the second evaluation of Alpha, Benny 2, Delta (control), Du Roi, Gusocora, Lavalle 1, Midknight 2 and Valencia Late at the Bosveld trial site, and the first for Benny 2, Gusocora, Lavalle 1 and Weipe at the Moriah trial site, so information is limited and future evaluations will improve recommendations on these varieties.

Table 5.5.3.4. Internal fruit quality data for Valencia orange selections at Moriah Citrus (Hoedspruit), Groep 91 and Bosveld Citrus (Letsitele) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Alpha	SC	2015/07/01	Bosveld	70-84	88-56	49.3	10.2	1.28	8.0	0.8	T3-5
Alpha	SC	2015/07/29	Bosveld	71-80	88-64	50.5	10.4	1.70	6.1	0.8	T1-2
Bend 8A1	CC	2015/06/11	Groep 91	69-79	88-64	52.1	11.6	1.39	8.3	0.0	T1-3
Bend 8A1	SC	2015/06/11	Groep 91	70-80	88-64	50.8	11.2	1.45	7.7	0.0	T1-5
Bend 8A1	CC	2015/07/01	Groep 91	69-81	105-64	51.0	11.9	1.33	8.9	0.0	T1-2
Bend 8A1	SC	2015/07/01	Groep 91	70-77	88-72	52.4	11.5	1.46	7.9	0.0	T1
Bend 8A2	CC	2015/06/11	Groep 91	70-78	88-64	54.3	11.0	1.48	7.4	2.1	T1-4
Bend 8A2	SC	2015/06/11	Groep 91	62-78	125-64	54.4	11.1	1.55	7.2	2.5	T4-5
Bend 8A2	CC	2015/07/01	Groep 91	71-75	88-72	54.9	11.4	1.50	7.6	2.9	T1
Bend 8A2	SC	2015/07/01	Groep 91	62-72	125-88	54.1	11.5	1.36	8.5	1.1	T1
Benny	SC	2015/06/10	Bosveld	68-81	88-64	54.4	10.1	1.45	7.0	3.1	T2-5
Benny 1	CC	2015/06/11	Groep 91	71-89	88-48	51.7	11.4	1.39	8.2	2.5	T1-2
Benny 1	SC	2015/06/11	Groep 91	68-77	88-72	53.7	11.3	1.73	6.5	3.2	T1-3
Benny 1	CC	2015/07/01	Groep 91	62-72	125-88	53.9	12.6	1.23	10.2	2.5	T1
Benny 1	SC	2015/07/01	Groep 91	68-77	88-72	47.3	11.7	1.55	7.5	2.3	T1
Benny 2	CC	2015/06/11	Groep 91	68-79	88-64	54.0	11.3	1.60	7.1	1.3	T1-3
Benny 2	SC	2015/06/11	Groep 91	65-76	105-72	52.1	10.7	1.58	6.8	1.7	T1-3
Benny 2	CC	2015/07/01	Groep 91	71-76	88-72	55.7	12.1	1.57	7.7	1.3	T1
Benny 2	SC	2015/07/01	Groep 91	65-76	105-72	54.7	12.6	1.64	7.7	2.3	T1
Benny 2	MxT	2015/05/28	Moriah	74-84	72-56	60.3	10.9	1.68	6.5	1.3	T1-3
Benny 2	MxT	2015/07/02	Moriah	72-80	88-64	48.2	11.2	1.00	11.2	0.0	T1
Delta	SC	2015/07/01	Bosveld	63-73	125-72	50.7	10.5	1.23	8.5	0.0	T2-4

Delta	SC	2015/07/29	Bosveld	70-76	88-72	65.5	10.6	1.12	9.5	0.0	T1-2
Du Roi	SC	2015/06/10	Bosveld	71-85	88-56	53.2	10.3	1.50	6.9	3.0	T1-4
Du Roi	SC	2015/07/01	Bosveld	70-79	88-64	54.8	10.2	1.39	7.3	1.0	T1-5
Du Roi	SC	2015/07/29	Bosveld	74-83	72-56	61.2	10.9	1.35	8.1	1.7	T1
Du Toit Late	CC	2015/07/01	Bosveld	70-77	88-72	52.5	11.3	1.30	8.7	3.2	T1-2
Du Toit Late	SC	2015/07/01	Bosveld	69-71	88	50.6	12.0	1.45	8.3	3.7	T 2-3
Gusocora	SC	2015/07/01	Bosveld	70-75	88-72	50.2	10.3	1.11	9.3	0.0	T1-4
Gusocora	SC	2015/07/29	Bosveld	70-80	88-64	52.3	11.3	1.09	10.4	0.0	T1-2
Gusocora	MxT	2015/07/02	Moriah	72-81	88-64	47.1	10.7	1.03	10.4	0.0	T1-2
Gusocora	MxT	2015/07/30	Moriah	78-87	64-48	44.0	11.1	1.13	9.8	0.0	T1
Henrietta	CC	2015/07/01	Groep 91	63-72	125-88	52.8	12.2	1.55	7.9	1.7	T1-2
Jassie	CC	2015/07/29	Bosveld	75-82	72-56	52.1	11.3	1.12	10.1	2.3	T1
Jassie	SC	2015/07/29	Bosveld	72-79	88-64	51.1	10.9	1.17	9.3	4.0	T1-4
Jassie	CC	2015/07/01	Groep 91	64-71	125-88	54.7	12.4	1.57	7.9	2.5	T1
Jassie	SC	2015/07/01	Groep 91	60-69	125-88	53.1	12.3	1.59	7.7	3.6	T1-2
Jassie	CC	2015/07/29	Groep 91	60-70	88	47.5	14.2	1.53	9.3	3.2	T1-2
Jassie	SC	2015/07/29	Groep 91	65-73	105-72	54.0	13.1	1.63	8.0	3.3	T1
Lavalle	SC	2015/07/01	Bosveld	74-83	72-56	53.7	10.4	1.56	6.7	0.0	T2-4
Lavalle	SC	2015/07/29	Bosveld	79-89	64-48	54.4	10.0	1.12	8.9	0.3	T1-4
Lavalle	MxT	2015/07/02	Moriah	72-78	88-64	53.6	10.3	1.47	7.0	0.0	T2-5
Lavalle	MxT	2015/07/30	Moriah	75-80	72-64	54.7	10.7	1.13	9.5	0.0	T1-2
Louisa	CC	2015/07/01	Groep 91	65-71	105-88	46.7	12.4	1.3	9.5	0.0	T3-4
McClean SL	SC	2015/06/10	Bosveld	71-88	88-48	52.9	9.4	1.12	8.4	0.0	T4-6
McClean SL	SC	2015/07/01	Bosveld	70-79	88-64	51.8	10.8	1.27	8.5	0.0	T1-2
McClean SL	SC	2015/07/29	Bosveld	75-88	72-48	52.7	8.3	1.51	5.5	0.0	T1-2
Midknight 1	SC	2015/06/10	Bosveld	80-89	64-48	52.5	10.2	1.26	8.1	0.0	T1-4
Midknight 1	SC	2015/07/01	Bosveld	80-85	65-56	51.4	10.3	1.12	9.2	0.0	T1-2
Midknight 1	SC	2015/07/29	Bosveld	84-91	56-40	52.1	10.5	1.42	7.4	0.2	T1-2
Midknight 1	CC	2015/06/11	Groep 91	66-76	105-72	53.2	11.4	1.18	9.7	0.0	T1-3
Midknight 1	SC	2015/06/11	Groep 91	68-77	88-72	52.6	11.2	1.07	10.5	0.0	T1-4
Midknight 1	CC	2015/07/01	Groep 91	65-72	105-88	56.6	11.9	1.10	10.8	0.8	T1
Midknight 1	SC	2015/07/01	Groep 91	64-70	125-88	53.7	12.1	1.05	11.5	0.0	T1
Midknight 1	CC	2015/07/29	Groep 91	66-72	105-88	54.8	11.8	1.20	9.8	0.0	T1
Midknight 1	SC	2015/07/29	Groep 91	63-75	125-72	52.9	11.9	1.18	10.1	0.5	T1
Midknight 1	MxT	2015/07/02	Moriah	77-85	72-56	49.7	11.2	1.09	10.3	0.0	T1
Midknight 1	MxT	2015/07/30	Moriah	79-82	64-56	49.3	10.5	0.96	10.9	0.0	T1-2
Midknight 2	SC	2015/06/10	Bosveld	80-85	64-56	51.2	9.2	1.23	7.5	0.3	T4-6
Midknight 2	SC	2015/07/01	Bosveld	79-85	64-56	51.7	9.6	1.33	7.2	0.0	T1-2
Midknight 2	SC	2015/07/29	Bosveld	80-93	64-40	43.5	10.4	1.60	6.5	0.0	T1-4
Midknight F17	MxT	2015/07/02	Moriah	63-72	125-88	47.2	8.7	1.31	6.6	0.0	T2-4
Moos Late 1	CC	2015/07/29	Bosveld	70-85	88-56	58.1	12.1	1.39	8.7	1.9	T1
Moos Late 1	SC	2015/07/29	Bosveld	71-85	88-56	48.3	12.3	1.09	11.3	1.1	T1-2
Moos Late 1	CC	2015/07/01	Groep 91	65-69	105-88	53.7	11.2	1.59	7.0	2.6	T1
Moos Late 1	SC	2015/07/01	Groep 91	69-76	88-72	56.9	11.1	1.61	6.9	3.0	T1-2
Moos Late 1	CC	2015/07/29	Groep 91	63-79	125-64	57.5	12.0	0.95	12.6	2.7	T1-3
Moos Late 1	SC	2015/07/29	Groep 91	72-82	88-56	56.3	11.3	1.34	8.4	0.8	T1
Moos Late 2	CC	2015/07/29	Groep 91	67-77	105-72	48.0	12.8	1.40	9.1	0.0	T1-5
Moos Late 2	SC	2015/07/29	Groep 91	70-80	88-64	55.6	11.6	1.27	9.1	0.0	T1-6
Ruby	CC	2015/07/01	Groep 91	65-70	105-88	53.9	11.3	1.27	8.9	1.9	T1
Ruby	SC	2015/07/01	Groep 91	64-74	125-72	56.7	11.0	1.43	7.7	1.9	T1-4

Ruby	CC	2015/07/29	Groep 91	66-77	105-72	52.1	12.9	1.71	7.5	2.5	T1-2
Ruby	SC	2015/07/29	Groep 91	70-75	88-72	58.8	12.0	1.32	9.1	2.1	T1-4
Skilderkrans	CC	2015/07/01	Groep 91	67-76	105-72	54.0	11.4	1.32	8.6	0.3	T1
Skilderkrans	SC	2015/07/01	Groep 91	67-73	105-72	51.9	12.4	1.74	7.1	0.0	T1
Skilderkrans	CC	2015/07/29	Groep 91	73-89	72-48	56.0	12.1	1.70	7.1	0.0	T1
Skilderkrans	SC	2015/07/29	Groep 91	71-82	88-56	56.4	12.3	1.73	7.1	0.0	T1
Turkey	C35	2015/05/06	Bosveld	68-80	88-64	49.3	12.3	1.22	10.1	0.8	T4-6
Turkey	CC	2015/05/06	Bosveld	72-83	88-56	50.7	10.1	0.96	10.5	0.0	T4-6
Turkey	SC	2015/05/06	Bosveld	70-88	88-48	53.8	11.4	1.08	10.6	1.0	T4-6
Turkey	C35	2015/05/27	Bosveld	71-81	88-64	49.8	12.3	1.15	10.7	1.5	T1-5
Turkey	CC	2015/05/27	Bosveld	76-85	72-56	52.9	10.6	0.93	11.4	0.0	T1-4
Turkey	C35	2015/05/06	Groep 91	62-70	125-88	56.3	11.1	1.44	7.7	5.8	T4-6
Turkey	CC	2015/05/06	Groep 91	62-71	125-88	57.8	11.9	1.51	7.9	4.3	T3-5
Turkey	SC	2015/05/06	Groep 91	63-74	125-72	53.5	11.3	1.45	7.8	6.2	T4-5
Turkey	C35	2015/06/11	Groep 91	65-77	105-72	63.2	11.5	1.31	8.8	6.1	T1-3
Turkey	CC	2015/06/11	Groep 91	65-74	105-72	55.1	12.3	1.32	9.3	2.3	T1
Turkey	SC	2015/06/11	Groep 91	65-74	105-72	54.4	12.1	1.39	8.7	3.5	T1-2
Valearly	C35	2015/05/06	Bosveld	68-75	88-72	48.6	12.0	1.08	11.1	0.6	T4-6
Valearly	CC	2015/05/06	Bosveld	72-80	88-64	48.7	11.4	1.05	10.9	0.9	T4-6
Valearly	C35	2015/05/27	Bosveld	68-80	88-64	49.6	13.0	1.02	12.7	0.3	T1-4
Valearly	CC	2015/05/27	Bosveld	62-82	125-56	50.9	11.1	0.95	11.7	0.0	T1-4
Val Late	SC	2015/07/01	Bosveld	70-79	88-64	47.4	11.4	1.70	6.7	0.5	T1-5
Val Late	SC	2015/07/29	Bosveld	77-88	72-64	46.9	10.5	1.67	6.3	0.6	T1-2
Weipe	C35	2015/05/28	Moriah	67-84	105-56	40.3	9.5	0.75	12.7	0.0	T1-3

5.5.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele & Malelane)

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Opsomming

Tango word die vroegste ryp volgens resultate van die 2015 seisoen vir hierdie warm produksie area, met 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 Valley Gold, Winola en Mor 26 met die hoogste Brix vlakke in vergelyking met die ander seleksies (Brix 14). Yosemite Gold en Gold Nugget was volgende om ryp te word en verteenwoordig die mid-laat van die Mandaryn Hibried reeks, ge-evalueer met ŉ 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 63% 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

Tango matures first according to the results of the 2015 season for the warm production areas, with 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 Valley Gold, Winola and Mor 26, which developed the highest Brix levels compared to the other selections (Brix 14). Yosemite Gold and Gold Nugget matured next, towards the mid-late period of the Mandarin Hybrid range evaluated at this trial site, with good internal quality (Brix: acid ratio of 12.5:1) as well as good external colour (T1-2). Tahoe Gold followed, with the highest juice level of 63% 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), Mahela Citrus (Letsitele), Moriah Citrus (Hoedspruit) and Riverside (Malelane) from the Limpopo region. The following varieties were evaluated: African Sunset (B24), Furr (Clemcot), Gold Nugget, Hadass, Mor 26, Orah, Shasta Gold, Tahoe Gold, Tambor, Tango, Valley Gold (B17), Yosemite Gold, Winola.

Table 5.5.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2015 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
Yosemite Gold	CC	2010

Table 5.5.4.2. List of Mandarin Hybrid selections evaluated at Mahela (Letsitele) during the 2015 season.

Selection	Rootstock	Planted
Gold Nugget	CC	2013
Shasta Gold	CC	2013
Tahoe Gold	CC	2013
Tango	CC	2013
Yosemite Gold	CC	2013

Table 5.5.4.3. List of Mandarin Hybrid selections evaluated at Moriah (Hoedspruit) during the 2015 season.

Selection	Rootstock	Topwork
African Sunset (B24)	MxT	2011
Furr (Clemcot)	MxT	2011
Hadas	MxT	2011
Mor 26	MxT	2011
Orah	MxT	2011
Tambor	MxT	2011
Valley Gold (B17)	MxT	2011
Winola	MxT	2011

Table 5.5.4.4. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2015 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 third 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 second season to evaluate African Sunset, Mor 26 and Valley Gold at the Bosveld trial site. The trees at Moriah Citrus were evaluated for the first time this year; the trial site became part of the CRI evaluation criteria in 2015.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over-mature. This process from the start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

African Sunset (B24)
There was enough fruit on the trees to complete one evaluation at the Bosveld and two evaluations at the Moriah trial site, due to a fairly light crop. The large to very large fruit size (count 1XX to 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 improved from average to good with high juice (above 50%), Brix, and acceptable acid levels. External colour remained delayed ranging from T3 to T5, bearing in mind the hot production areas. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be the end of May to middle of June.

Furr (Clemcot)
Furr developed large to extra-large fruit size (count 1X – 1XXX) on the trees at Moriah Estate, one of the characteristics of the cultivar, as well as an excellent crop on the trees (80 kg/tree). The external colour development on the fruit was good for the Hoedspruit area. Internally the fruit quality was very good, developing high juice (above 50%) and Brix (13) levels with acceptable acids. Another quality of the fruit is the high seed count of between 13 and 16 seeds per fruit. Maturity seems to be middle to the end of May for the hot production areas, according to the information in Table 5.5.4.5.

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 (better at Mahela and Riverside than Bosveld) and developed good juice (50%), high Brix (above 12) and lower acid (above 0.7%) levels and an improved external colour (T1-2) compared to last year's T4-5. Keep in mind the fairly young tree age (3/4/5 years old) of this trial. Future evaluations will determine the feasibility of this selection in the hot areas. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be the middle to end of June.

Hadas
Hadas is a very late maturing mandarin selection according to the high acid levels at the end of July (1.85%), but with good external colour (T1). The external colour of the fruit remains a deep yellow intensity. Seed levels were below 1 seed per fruit this season and the fruit size varied from large to extra-large (count 1 to 1XXX). Based on the internal quality results in Table 5.5.4.5, estimated maturity will be end of August to middle of September.

Mor 26

Mor 26 produced an average crop on the trees for the 2015 season. The fruit size was erratic and peaked between count 2 and 1XXX, medium to large/extra-large fruit. The external colour development was yellow and between T1-2. The internal quality was very good with high juice levels of 56%, Brix above 14 and acceptable acid levels. There were on average 2.5 seeds in the fruit at Bosveld and 1.9 at Moriah. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be the middle to end of June.

Orah

Orah was evaluated for the first time this season, producing a good crop on the trees with medium to large (count 2 to 1XX) fruit size. The average seed count in the fruit ranged from 7.8 to 13.8 seeds per fruit, one of the characteristics of the selection. Internal quality was good, the Brix levels were above 13 by t harvest, as well as good juice levels (above 50%) and acceptable acids (0.8%). Early external colour development ranged from T1 to T2. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be end of May to the middle of June.

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 and Riverside trial site was better compared to the Bosveld site. The flavour improved with high juice (above 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. The fruit size peaked from large to very large (count 1X-1XXX). The internal quality was good with juice levels of 50% and higher, Brix above 12 at Mahela and Riverside, and acceptable acid levels (above 1.2%). Based on the internal quality results in Table 5.5.4.5 maturity will be middle of July to end of July at both Mahela and Riverside.

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 the trees compared to the 2014 season. The fruit size peaked from medium to large/very large (count 3-1XXX) and the fruit shape was similar to that of a Minneola tangelo fruit. The external colour improved between T1-3 when the internal quality was optimum. Tahoe produced fruit with soft fibre strength that peels easily, and all the fruit evaluated were completely seedless, except for one evaluation at Riverside with 0.3 seeds per fruit counted. The internal quality was good with juice levels of as high as 63%, Brix averaged 10 and acid levels were acceptable by the time of harvest. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be middle June to middle of July.

Tambor

Tambor is an addition to the late maturing mandarin selections for the hot production areas, producing 1.2 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 50%, Brix above 11 and acids above 1 at the last evaluation. Fruit size peaked between count 1X and 1XXX, large to very large for mandarin varieties. Based on the internal quality results in Table 5.5.4.5, estimated maturity will be end of July to the end 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. 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 and peaked at 13.5 for the Riverside trial, 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 (small to medium). Based on the internal quality results in Table 5.5.4.5, estimated maturity will be end of April to the middle of May.

Valley Gold (B17)

Valley Gold was evaluated for the second time at the Bosveld trial site and for the first time at Moriah. The internal quality was good with acid levels around 0.78% and external colour between T1 and 3 when the second evaluation was completed. Fruit size decreased at Bosveld compared to the 2014 season (count 2 to 1X), and peaked from count 1X to 1XXX at Moriah due to a fairly light crop on the trees. There was limited fruit

split on the trees at Bosveld, but high fruit split was present on the trees at Moriah resulting in up to 30% fruit drop. Maturity is estimated to be middle of June for these hot production areas.

Winola

Winola was completely seedless at Moriah Estate, producing an average crop on the young trees. Fruit size peaked from medium to large (count 2 to 1XX). Internal quality was poor with juice levels below 45%, resulting in severe granulation problems in the fruit. Brix (above 10) and acids were better 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 was very light at the Riverside and Bosveld sites with a good crop on the trees at Mahela. 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 developed up to 0.3 seeds per fruit. Yosemite developed the biggest tree size compared to the other UCR 5 selections. This aggressive growth characteristic may be the reason for the poor crop on the trees (vegetative growth), and must be redirected into fruit set and crop on the trees. Fruit size varied from medium to large/very large (count 2-1XXX), similar to Tahoe Gold, due to the light crop on the trees. The internal quality improved this season with higher juice, Brix and acid levels. External colour developed along with the internal quality towards the end of the evaluations (T1-2). Based on the internal quality results in Table 5.5.4.5, estimated maturity will be mid-June to mid-July.

Conclusion

There was an improvement in the external colour delay in the hot areas that was a problem in the past; future evaluations will clarify the situation. Degreening may be an option for the Gold Nugget and TDEs (fruit colour development was yellow with degreening), but ethylene reacted slowly with Tango (W. Murcott selection) and Nadorcott. Gold Nugget and Yosemite Gold may be a possibility to consider for the hot areas due to stronger fruit with optimal fruit size, and good internal quality when external colour becomes more intense (T1-2). In the hot areas it will be crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit. There was severe sunburn on the Shasta Gold fruit compared to the cooler production areas.

Shasta Gold had the largest fruit size, followed by Orah, Yosemite Gold, Tambor, Gold Nugget, Hadas and Furr, then Winola and Tambor. The smallest fruit size was produced on Tango and Tahoe Gold. Furr and Orah developed the highest number of seeds, followed by Mor 26. There were similar seed numbers this season in Tahoe Gold and Yosemite Gold (0.3), as well as Valley Gold (2.4); all the other selections were completely seedless.

Furr, Hadas, Orah, Tambor and Winola were evaluated for the first time this season with limited information; future evaluations will continue to determine suitability for this production area.

Table 5.5.4.5. Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele), Mahela (Letsitele), Moriah (Hoedspruit) and Riverside (Malelane) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
African Sunset (B24)	C35	2015/05/06	Bosveld	75-97	1XX	51.2	11.8	0.98	13.3	0.6	T5-6
African Sunset (B24)	MxT	2015/05/28	Moriah	80-90	1XXX	52.0	9.4	0.83	11.3	0.0	T1-5
African Sunset (B24)	MxT	2015/07/02	Moriah	85-91	1XXX	45.0	9.7	0.67	14.5	0.0	T1
Furr (Clemcott)	MxT	2015/04/16	Moriah	70-80	1X-1XXX	50.2	11.1	0.95	11.7	16.1	T2-5
Furr (Clemcott)	MxT	2015/05/28	Moriah	70-75	1X-1XX	53.4	13.6	1.00	13.6	13.0	T1-3
Furr (Clemcott)	MxT	2015/06/11	Moriah	77-84	1XX-1XXX	50.4	12.9	0.94	13.7	14.4	T1
Furr (Clemcott)	MxT	2015/07/02	Moriah	75-83	1XX-1XXX	52.8	13.6	0.91	14.9	15.0	T1
Gold Nugget	CC	2015/05/06	Bosveld	63-70	2-1X	47.7	9.5	0.91	10.4	0.0	T5-8
Gold Nugget	CC	2015/05/27	Bosveld	67-75	1-1XX	50.0	10.4	0.80	13.0	0.0	T2-6
Gold Nugget	CC	2015/04/30	Mahela	65-76	1-1XX	53.3	8.9	0.74	12.0	0.0	T5-7
Gold Nugget	CC	2015/05/27	Mahela	65-80	1-1XXX	50.0	10.8	0.64	16.9	0.0	T2-6
Gold Nugget	CC	2015/07/01	Mahela	65-73	1-1XX	49.7	12.5	0.73	17.1	0.0	T1
Gold Nugget	CC	2015/04/22	Riverside	60-70	2-1X	51.4	10.1	1.17	8.6	0.0	T6-8
Gold Nugget	CC	2015/05/11	Riverside	68-76	1-1XX	50.8	10.0	0.97	10.3	0.0	T3-7
Gold Nugget	CC	2015/05/26	Riverside	70-75	1X-1XX	51.5	10.7	0.81	13.2	0.0	T2-5
Gold Nugget	CC	2015/06/23	Riverside	72-80	1XX-1XXX	47.3	11.9	0.75	15.9	0.0	T1
Gold Nugget	CC	2015/07/07	Riverside	74-80	1XX-1XXX	47.7	12.8	0.76	16.8	0.0	T1
Hadas	MxT	2015/07/02	Moriah	66-77	1-1XX	55.5	12.1	2.01	6.0	0.7	T1
Hadas	MxT	2015/07/30	Moriah	67-82	1-1XXX	57.1	12.3	1.85	6.6	0.0	T1
Mor 26	SC	2015/05/06	Bosveld	64-71	1-1XX	40.9	11.7	1.12	10.4	2.9	T2-4
Mor 26	SC	2015/06/10	Bosveld	62-67	2--1	48.8	13.9	1.04	13.4	2.1	T1
Mor 26	MxT	2015/05/28	Moriah	68-79	1X-1XXX	56.0	14.2	1.38	10.3	1.3	T1-2
Mor 26	MxT	2015/06/11	Moriah	63-77	2-1XX	48.4	12.6	0.84	15.0	1.9	T1
Mor 26	MxT	2015/07/02	Moriah	60-66	1--1	50.4	14.6	1.34	10.9	2.5	T1
Orah	MxT	2015/04/16	Moriah	65-76	1-1XX	48.3	10.6	1.08	9.8	13.8	T2-3
Orah	MxT	2015/05/28	Moriah	67-74	1-1XX	53.0	13.5	1.17	11.5	9.2	T1-2
Orah	MxT	2015/06/11	Moriah	70-79	1X-1XXX	52.9	11.5	0.71	16.2	7.8	T1-2
Orah	MxT	2015/07/02	Moriah	62-76	2-1XX	58.6	13.8	0.80	17.3	8.2	T1
Shasta Gold	SC	2015/05/06	Bosveld	73-82	1XX-1XXX	52.0	9.5	1.52	6.3	0.0	T4-6
Shasta Gold	CC	2015/05/27	Bosveld	74-83	1XX-1XXX	52.3	10.2	1.26	8.1	0.0	T2-5
Shasta Gold	CC	2015/04/30	Mahela	69-80	1X-1XXX	57.2	9.0	1.57	5.7	0.0	T6-8
Shasta Gold	CC	2015/05/27	Mahela	73-80	1XX-1XXX	50.9	10.7	1.30	8.2	0.0	T1-5
Shasta Gold	CC	2015/07/01	Mahela	75-81	1XX-1XXX	54.2	12.0	1.19	10.1	0.0	T1
Shasta Gold	CC	2015/04/22	Riverside	70-78	1X-1XXX	51.7	9.1	2.46	3.7	0.0	T3-6
Shasta Gold	SC	2015/05/11	Riverside	68-78	1X-1XXX	55.7	10.0	1.76	5.7	0.0	T2-5
Shasta Gold	CC	2015/05/26	Riverside	72-88	1XX-1XXX	54.8	11.1	1.67	6.6	0.0	T1-4
Shasta Gold	CC	2015/06/23	Riverside	75-85	1XX-1XXX	55.9	11.7	1.51	7.7	0.0	T1-2
Shasta Gold	CC	2015/07/07	Riverside	70-80	1X-1XXX	54.0	12.3	1.54	8.0	0.0	T1
Tahoe Gold	CC	2015/05/06	Bosveld	61-75	2-1XX	57.4	10.1	1.31	7.7	0.0	T3-7
Tahoe Gold	CC	2015/05/27	Bosveld	65-74	1-1XX	48.8	10.3	1.15	9.0	0.0	T4-6
Tahoe Gold	CC	2015/04/30	Mahela	60-70	2-1X	63.3	10.2	1.18	8.6	0.0	T4-5
Tahoe Gold	CC	2015/05/27	Mahela	66-70	1-1X	56.0	9.5	0.97	9.8	0.0	T1-5
Tahoe Gold	CC	2015/07/01	Mahela	63-72	2-1XX	61.2	11.5	0.82	14.0	0.0	T1-3
Tahoe Gold	CC	2015/04/22	Riverside	58-69	3-1X	59.4	10.6	2.21	4.8	0.3	T5-8
Tahoe Gold	CC	2015/05/11	Riverside	65-73	1-1XX	60.5	10.9	1.00	15.1	0.0	T2-4
Tahoe Gold	CC	2015/05/26	Riverside	64-75	1-1XX	59.1	11.9	1.45	8.2	0.0	T2-5

Tahoe Gold	CC	2015/05/07	Riverside	63-74	2-1XX	57.3	12.6	1.25	10.1	0.0	T1
Tahoe Gold	CC	2015/06/23	Riverside	62-80	2-1XXX	58.8	12.3	1.26	9.8	0.0	T1
Tambor	MxT	2015/07/02	Moriah	74-87	1XX-1XXX	52.7	11.4	1.24	9.2	1.5	T1
Tambor	MxT	2015/07/30	Moriah	71-86	1X-1XXX	56.7	11.8	1.25	9.4	1.0	T1
Tango	CC	2015/05/06	Bosveld	65-74	1-1XX	52.0	8.5	0.6	7.7	0.0	T3-6
Tango	CC	2015/05/27	Bosveld	64-73	1-1XX	57.0	9.6	0.68	14.1	0.0	T3-5
Tango	CC	2015/04/30	Mahela	65-73	1-1XX	55.8	8.2	0.52	15.8	0.0	T4-7
Tango	CC	2015/05/27	Mahela	65-75	1-1XX	54.9	9.2	0.45	20.4	0.0	T2-4
Tango	CC	2015/04/14	Riverside	58-65	3--1	57.4	9.4	1.09	8.6	0.0	T1
Tango	CC	2015/04/22	Riverside	65-75	1-1XX	57.1	9.6	0.83	11.6	0.0	T2-4
Tango	CC	2015/05/11	Riverside	65-73	1-1XX	59.5	10.2	1.42	7.2	0.0	T3-6
Tango	CC	2015/06/23	Riverside	60-67	2--1	60.0	11.9	0.77	15.5	0.0	T1
Tango	CC	2015/05/26	Riverside	65-70	1-1XX	58.5	11.9	0.79	15.1	0.0	T1-2
Tango	CC	2015/07/07	Riverside	56-65	3--1	61.5	13.5	0.82	16.5	0.0	T1
Valley Gold (B17)	SC	2015/05/06	Bosveld	63-70	2-1X	58.3	10.9	1.05	10.4	2.1	T4-7
Valley Gold (B17)	MxT	2015/05/28	Moriah	70-77	1X-1XX	49.2	10.0	0.93	10.8	0.0	T1-4
Valley Gold (B17)	MxT	2015/07/02	Moriah	72-82	1XX-1XXX	45.6	10.5	0.77	13.6	2.6	T1
Winola	C35	2015/05/28	Moriah	60-67	2--1	26.2	10.2	1.07	9.5	0.0	T1-2
Winola	MxT	2015/07/02	Moriah	65-72	1-1XX	45.2	10.2	0.99	10.3	0.0	T1
Yosemite Gold	CC	2015/05/06	Bosveld	67-80	1-1XXX	50.2	9.3	1.1	8.5	0.0	T6-8
Yosemite Gold	CC	2015/05/27	Bosveld	80-85	1XXX	48.7	10.0	0.95	10.5	0.0	T4-6
Yosemite Gold	CC	2015/04/30	Mahela	70-75	1X-1XX	55.2	10.7	1.06	10.1	0.2	T3-5
Yosemite Gold	CC	2015/05/27	Mahela	65-80	1-1XXX	53.6	11.6	0.97	12.0	0.0	T1-2
Yosemite Gold	CC	2015/07/01	Mahela	65-75	1-1XX	57.3	11.6	0.81	14.3	0.0	T1
Yosemite Gold	CC	2015/04/22	Riverside	71-83	1X-1XXX	44.3	9.4	1.62	5.8	0.0	T3-8
Yosemite Gold	CC	2015/05/11	Riverside	70-80	1X-1XXX	47.2	10.2	1.35	7.6	0.3	T2-6
Yosemite Gold	CC	2015/05/26	Riverside	72-84	1XX-1XXX	47.3	10.4	1.29	8.1	0.0	T1-4
Yosemite Gold	CC	2015/06/23	Riverside	77-85	1XX-1XXX	48.3	11.2	1.13	9.9	0.0	T1
Yosemite Gold	CC	2015/07/07	Riverside	77-85	1XX-1XXX	50.8	12.5	1.11	11.3	0.0	T1

5.5.5 PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Tom Burke)

Project 941D by J. Joubert and M.P. Cele (CRI)

Opsomming

Hierdie is ŉ nuwe proef en betekenisvolle data kon slegs van Du Roi en McClean SL verkry word. McClean saadloos sal later in die Valencia seisoen ryp word, met totaal saadlose vrugte en medium tot groot vruggrootte, tussen telling 72 tot 48. Du Roi volg as laat rypwordende Valencia, wat lae saadtellings in die vrugte ontwikkel met matige gladde skil tekstuur. Inligting is op hierdie stadium baie beperk weens die derde oes op die bome hierdie seisoen. Hierdie proef sal as ŉ bystand perseel gebruik word vir een van die ander kombinasie proewe om addisionele inligting te genereer. Opvolg evaluasies sal gedoen word wanneer meer seleksies in produksie kom.

Summary

This is a new trial and meaningful data could only be collected from Du Roi and McClean SL. McClean seedless will mature later in the Valencia season, with completely seedless fruit and medium to large fruit size, ranging from counts 72 to 48. Du Roi will follow as a late maturing Valencia, developing low seed counts in the fruit and fairly smooth rind texture. Information at this stage was limited due to this being the third crop on the trees for the season. This trial site will become a back-up site to one of the other combined sites to generate additional information. Future evaluations will be conducted when more selections come into production.

Objective

- To find suitable Valencia selections with superior characteristics for the intermediate inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on McClean SL and Du Roi 2 at Klipbokspruit, Tom Burke.

Table 5.5.5.1. List of Valencia selections evaluated at Klipbokspruit (Tom Burke) during 2015.

Selection	Rootstock	Topwork	No. of trees
Du Roi 2	SC	2011	4
McClean SL	SC	2011	5

Results and discussion

There was a better crop on the Valencia selections available for the 2015 season compared to the previous season. NGB bought Rolemsha (Tom Burke) and the farm name will change back to Klipbokspruit. Du Roi 2 and McClean SL were the only Valencia selections evaluated this season due to having a crop on the trees. The trial site will be used as a back-up site in the future and only valuable information will be captured.

Du Roi 2
Du Roi was evaluated for the first time this season with a good crop on the young trees. The fruit size peaked from medium to large (count 88 to 56), optimum for Valencia production. There were 2.7 seeds per fruit in the Du Roi Valencia and the rind texture was fairly smooth. The internal quality was good with juice levels above 50%, Brix of 9.4 and fairly high acids (1.4%), with delayed external colour (T2-4). Du Roi is one of the late maturing Valencia options and maturity seems to be end of July according to Table 5.5.5.2.

McClean SL
There was a good crop on the young trees. McClean SL remained completely seedless at this trial site. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. Tree condition was good and the internal fruit colour will develop into a deep orange by the time of peak maturity. The external colour of the fruit improved compared to 2014 and ranged from T1 to T3. The fruit size varied from count 64 to 48 on Swingle rootstock combination. The Brix:acid ratio was 8.6, an improvement on the 7 from last season. There were no sunburn, splitting or creasing problems with the fruit, and the fruit peels moderately with high rind oil content. Maturity for this specific production according to Table 5.5.5.2 will be end of June to middle July.

Conclusions

Du Roi and McClean SL will be two of the later maturing selections for this area. Fruit size on both selections ranged from medium to large/extra-large. The external colour development improved on McClean SL in combination with Swingle rootstock, but Du Roi was delayed. McClean SL remained completely seedless compared to Du Roi with low seed levels per fruit.

Hopefully more selections will come into production next season; at this stage information was limited due to young tree age and a limited number of fruit on the trees.

Table 5.5.5.2. Internal fruit quality data for Valencia orange selections at Klipbokspruit (Tom Burke) on 30 June 2015.

Selection	Root-stock	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Du Roi 2	SC	72-83	88-56	51.3	9.4	1.37	6.9	2.7	T2-4
McClean SL	SC	79-87	64-48	49.7	9.4	1.09	8.6	0.0	T1-3

5.5.6 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry production areas (Weipe)

Project 899A by J. Joubert and M.P. Cele (CRI)

Opsomming

Hierdie was die eerste seisoen wat die NGB proef ge-evalueer is as gevolg van voldoende vrugte aan die bome, en betekenisvolle data kon versamel word. Valearly begin die seisoen as die vroegste Valencia by die NGB proef met ‘n vertraagde vrugkleur op oorryp vrugte. McClean SL sal volg met later vrugkleur en totaal saadlose vrugte. Volgende om ryp te word sal die gewone McClean wees met goeie kleur en 1.3 sade per vrug. Novelle la Cotte en Rhode Red sal volg as die mid-rypwordende Valencia seleksies. Jassie is dan volgende met uitstekende interne kwaliteit en optimum kleur ontwikkeling as die mid-laat Valencia gedeelte. Du Toit Late en Moos Late1 sal die seisoen afsluit vir hierdie proef met goeie kleur en die beste vrugkwaliteit.

Summary

This was the first season to evaluate the NGB trial site due to fruit numbers on the trees, and meaningful data could be collected. Valearly will start the season as the earliest maturing Valencia at the NGB site with a colour delay on the overmature fruit. McClean SL will follow with delayed colour and completely seedless fruit. Next to mature will be McClean with advanced colour and 1.3 seeds per fruit. Novelle la Cotte and Rhode Red will follow as the mid-maturing Valencia selections. Jassie will be next, towards the mid-late Valencia section, with excellent internal quality and optimum colour development. Du Toit Late and Moos Late 1 will end f the season for this trial site with good fruit colour and the best quality fruit.

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 Du Toit Late, Jassie, McClean, McClean SL, Mooslate 1, Novelle la Cotte, Rhode Red and Valearly at NGB, Weipe.

Table 5.5.6.1. List of Valencia selections evaluated at NGB (Weipe) during 2015.

Selection	Rootstock	Topwork
Du Toit Late	X639	2012
Jassie	X639	2012
McClean	X639	2012
McClean SL	X639	2012
Mooslate 1	X639	2012
Novelle la Cotte	X639	2012
Rhode Red	X639	2012
Valearly	X639	2012

Results and discussion

The Alicedale trial site at Tshipise had a very poor crop on the trees and no evaluations were done this season. There was a good fruit set on the trees for 2015 and evaluations will be possible in the next season.

Fruit size on all the selections at NGB peaked from medium to large (count 88 to count 56). Du Toit late and Moos Late 1 developed the best juice levels for this season, above 60%. Jassie outperformed the other Valencia selections with the highest seed count per fruit (5.5), a Brix of 11.6 and Brix:acid ratio of 11.5, followed by Rhode Red with a Brix of 10.9. Acid levels in the fruit were medium to high for all the combinations, except on Valearly with fairly low acids (0.6%) due to the earliness of the cultivar.

Conclusions

Valearly's colour was delayed atpeak maturity (T3-T5) and resulted in low acids for the cultivar later in the season. All the selections evaluated developed seeds in their fruit, except for McClean seedless and Novelle la Cotte. McClean and Novelle la Cotte were the only two selections that did not comply with minimum export standards due to juice levels below 48%. The ideal fruit size distribution for Valencia exports was achieved and peaked from count 88 to count 56 (excellent).

Hopefully more selections will come into production next season; at this stage information was limited due to young tree age and a limited number of fruit on the trees.

Table 5.5.6.2. Internal fruit quality data for Valencia orange selections at NGB (Weipe) during the 2015 season.

Selection	Root-stock	Date harvested	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Du Toit Late	X639	2015/06/10	70-82	88-56	49.1	9.8	1.05	9.3	1.9	T3-6
Du Toit Late	X639	2015/08/03	72-80	88-64	61.3	10.1	0.99	10.2	3.4	T1-2
Jassie	X639	2015/08/03	72-84	88-56	59.3	11.6	1.01	11.5	5.5	T1-2
McClean	X639	2015/06/10	71-83	88-56	47.8	10.3	1.15	9.0	1.3	T1-4
McClean SL	X639	2015/06/10	73-83	72-56	48.4	9.0	0.89	10.1	0.0	T4-6
Mooslate 1	X639	2015/08/03	71-82	88-56	62.7	10.4	1.18	8.8	1.8	T1-2
Novelle la Cotte	X639	2015/06/10	71-84	88-56	47.4	9.9	1.21	8.2	0.0	T2-4
Rhode Red	X639	2015/06/10	70-81	88-64	49.1	9.6	1.10	8.7	0.0	T3-7
Rhode Red	X639	2015/08/03	74-81	72-64	58.6	10.9	1.09	10.0	3.3	T1
Valearly	X639	2015/05/19	71-77	88-72	48.0	10.1	0.60	16.8	0.3	T3-5
Valearly	X639	2015/06/10	74-84	72-56	44.9	10.0	0.66	15.2	0.0	T1-4

5.5.7 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall & Tom Burke)**
Project 941C by J. Joubert and M.P. Cele (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die twee produksie areas, a.g.v. die klimaatsone en boom ouderdom (2009, 2011 teenoor 2012). Die resultate vir die Tom Burke produksie area (effens warmer) het aangedui dat Tango die vroegste ryp geword het, met die kleinste vruggrootte en gemiddelde tot goeie interne kwaliteit, gevolg deur Tahoe Gold met groot tot baie groot vrugte vir hierdie seisoen. Tango en Shaste Gold het lae saad tellings ontwikkel hierdie seisoen. Yosemite Gold het geen oes op die bome gehad en alternatiewe drag patrone moet ondersoek word (Gibb bespuitings krities). Gold Nugget was volgende gereed vir oes, gevolg deur Valley Gold. Shasta Gold was die laatste seleksie gereed vir oes, teen einde Junie tot middel Julie, 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 two different production areas was similar, due to the climatic region (intermediate areas) and tree age (2009, 2011 versus 2012). The results indicated that in the Tom Burke (slightly warmer) production area, Tango matures first with the smallest fruit size and fair to good internal quality, followed by Tahoe Gold with large to extra-large fruit size for this season. Tango and Shasta Gold developed low seed numbers this season. The Yosemite Gold trees remained fruitless for another season and alternate bearing patterns must be investigated (Gibb applications crucial). Gold Nugget was next to mature, followed by Valley Gold. Shasta Gold was the last selection to mature, at the end of June to middle of July, 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), Schoonbee Estate (Marble Hall) and Klipbokspruit (Tom Burke) from the Limpopo region. The following varieties were evaluated: African Sunset, Gold Nugget, IRM 1&2, Monica, Nadorcott ARC & LS, Phoenix (2PH SL), Reina, Shasta Gold, Tahoe Gold, Tango, Yosemite Gold and Valley Gold.

Table 5.5.7.1. List of Mandarin Hybrid selections evaluated at Engelbrecht Trust (Marble Hall) during the 2015 season.

Selection	Rootstock	Planted
Nadorcott ARC	CC	2011
Nadorcott LS	CC	2011

Table 5.5.7.2. List of Mandarin Hybrid selections evaluated at Moosrivier Estate (Marble Hall) during the 2015 season.

Selection	Rootstock	Planted
African Sunset (B24)	C35	2009
IRM 1	SC	2009
IRM 2	SC	2009
Monica	SC	2009
Phoenix (2 PH SL)	SC	2009
Reina	SC	2009
Valley Gold (B17)	C35	2009

Table 5.5.7.3. List of Mandarin Hybrid selections evaluated at Schoonbee Estate (Marble Hall) during the 2015 season.

Selection	Rootstock	Topwork
Gold Nugget	CC & Lina/CC	2012
Shasta Gold	CC & Lina/CC	2012
Tahoe Gold	CC & Lina/CC	2012
Tango	CC & Lina/CC	2012

Table 5.5.7.4. List of Mandarin Hybrid selections evaluated at Klipbokspruit (Tom Burke) during the 2015 season.

Selection	Rootstock	Topwork
Gold Nugget	CC/RL/SC	2011
Shasta Gold	SC	2011
Tahoe Gold	CC/SC	2011
Tango	CC/RL/SC	2011

Results and discussion

The trees at Schoonbee Estate were topworked 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. Limited information was available at Moosrivier and Engelbrecht Trust due to fruit numbers (hail damage) and fruit was harvested before final evaluation.

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 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.

There was no crop on the Yosemite Gold trees at Schoonbee Estate or Klipbokspruit to evaluate this season. Crop manipulation measures will be essential to ensure future evaluations and results.

Lina navel on Carrizo was used as interstock in combination with all the UCR 5 selections during the top-working process, to compare the impact between topworking directly on to the rootstock versus onto Lina navel (interstock) and then Carrizo rootstock. Information improved due to a better crop on the trees and evaluations will continue next season.

African Sunset (B24)
There was enough fruit on the trees to complete one evaluation at the Moosrivier due to a fairly light crop. The very large fruit size (count 1XXX) is a selection quality and the light crop contributes to this scenario. African Sunset developed a protruding navel-end on most of the fruit; the bigger the fruit size the more visible the navel-end. The internal quality was average with low juice (above 40%), Brix above 10 and acceptable acid levels. External colour peaked at T1 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 Klipbokspruit and Schoonbee were completely seedless, and fruit size was large to extra-large on all rootstock combinations (1-1XXX) at Klipbokspruit compared to medium-large (count 2-1XX) at Schoonbee (due to better crop). The internal quality of the fruit at both sites was average, low juice (37-50%) was captured throughout the season; Brix and acid levels were better at Schoonbee (above 11.0 and 1.0%) compared to Klipbokspruit (average 10 and below 1.0%). The external colour improved this season and peaked between T1 and T2. Based on the internal quality results in Table 5.5.7.5, estimated maturity will be the middle to end of June.

IRM1&2
IRM1 produced 60 to 80 kg/tree compared to IRM2 with 30 to 40 kg/tree. IRM2 developed better external colour (orange) earlier in the season and IRM1 a more yellowish colour, with less ribbing on the fruit. IRM1 produced better internal quality with juice 53%, Brix above 12 and high acids (late maturing selection).

Monica
Yield production on the trees varied from 60 to 80 kg/tree and the tree size was large compared to the other Mandarin selections (shong an aggressive growth pattern). The internal quality was average with low juice levels (45%), but high Brix (above 11) and acid (1.1%) when external colour peaked at T1. There were 2.4 seeds per fruit for this trial site. Monica developed a very course rind and deep external colour.

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 60 kg/tree compared to ARC with 40 kg/tree and the fruit size varied from 2-1XX to 1-1XX due to the crop load. Both selections evaluated were completely seedless. Maturity seems to be two weeks earlier on the LS selection, according to Table 5.5.7.5. but information was limited due to only one evaluation being done (middle to end of June).

Phoenix
Rind texture on this selection was smooth and developed a deep yellow colour by the time of maturity. Two evaluations were possible at Moosrivier and crop production peaked at 60 kg per tree. Seed count per fruit peaked from 2.9 to 3.8. The juice levels were below 50%, Brix up to 12 and acceptable acids (0.8%), when the external colour varied from T1 to 2.

Reina
Reina developed a medium coarse rind compared to Monica. The juice content was very low (32%) on this selection, Brix average (9.6) and fairly high acids were present when the external colour peaked from T1 to 2. Reina developed the highest seed count for this trial site with 21.2 seeds per fruit.

Shasta Gold
The crop on the Shasta Gold trees improved (20 kg/tree) at Klipbokspruit and Schoonbee (light crop due to smaller and younger trees). Shasta Gold developed fairly round fruit (Minneola tangelo type) on the trees at both trial sites. There was less ribbing and sunburn on the fruit, and better tree canopy development for protection. There were a lot of thorns on the bearing branches of the trees. Rind texture was smoother this season. Shasta Gold produced fruit with soft fibre strength that peels easily, and the fruit evaluated was completely seedless, except for the final evaluation at Schoonbee (0.3 seeds per fruit). The fruit size peaked at large to very large (count 1-1XXX). The flavour was fair with average juice (up to 48%) and high rind oil content. The internal quality improved this season with a higher Brix of up to 11, and high acid levels (above 1.3% with final evaluation). Based on the internal quality results in Table 5.5.7.5 maturity will be end of June to the middle of July depending on rootstock choice.

Tahoe Gold
Tahoe Gold produced 30 to 40 kg/tree at Klipbokspruit compared to 40 to 60 kg/tree at Schoonbee. This selection 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 medium to large/very large (count 2-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was no delay this season in the external colour development at the Klipbokspruit trial site compared to the Schoonbee site (T1). Tahoe produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated was completely seedless. The internal quality improved to average/good; juice up to 50%, Brix up to 11 and fair acids (Table 5.5.7.5). Estimated maturity is end of May to middle June.

Tango
Tango remained completely seedless at the Klipbokspruit site, and had 0.6 seeds per fruit at the Schoonbee trial site. There was a fair crop on the trees 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 50%), Brix was average (up to 10) and the acid levels stabilizing below 1.0 towards the end of the season. Fruit size peaked at count 3 to 1XXX (small to medium/large). Based on the internal quality results in Table 5.5.7.5, estimated maturity wil be end of May to middle of June.

Valley Gold (B17)
The internal quality was average to good with a juice content average below 50%, Brix 12 and acid levels around 1.0 %; external colour between T1 and 3 when the third evaluation was completed. Fruit size peaked from count 2 to 1XX due to a good crop on the trees. There was limited fruit split on the trees at Moosrivier this season. Maturity is estimated to be middle of June based on the internal quality results in Table 5.5.7.5 for this intermediate production area.

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 (T1-2) due to temperature drop in winter. The appearance of the Shasta Gold's fruit improved this season (older trees), 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 withsmoother fruit, medium to large fruit size and fair to good internal quality. Shasta Gold had the largest fruit size, followed by Tahoe Gold, and then Gold Nugget. The smaller fruit size was produced on Tango, reaching up to 1XX/1XXX, with a lighter crop on the trees at both sites. There were no incidences of seed in the UCR 5 fruit at the two trial sites, except for Shasta (0.3) and Tango (0.6) at Schoonbee Estate.

This was the first evaluation of African Sunset, IRM1&2, Monica, Nadorcott ARC & LS, Phoenix, Reina and Valley Gold at the Moosrivier and Engelbrecht Trust site, so information is limited and future evaluations will improve recommendations on these varieties. Nadorcott ARC, Nadorcott LS and African Sunset were

completely seedless for this trial. All the other selections developed seed in the fruit with Reina having the highest numbers (21 seeds/fruit).

Table 5.5.7.5. Internal fruit quality data for Mandarin hybrid selections at Schoonbee Estate (Marble Hall), Moosrivier (Marble Hall), Engelbrecht Trust (Marble Hall) and Klipbokspruit (Tom Burke) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
African Sunset (B24)	C35	2015/07/15	Moosrivier	82-97	1XXX	42.9	10.1	0.90	11.2	0.0	T1
Gold Nugget	CC	2015/05/26	Klipbokspruit	77-88	1XX-1XXX	40.5	9.6	0.79	12.2	0.0	T2-4
Gold Nugget	SC	2015/05/26	Klipbokspruit	70-85	1X-1XXX	44.8	10.6	0.87	12.2	0.0	T2-4
Gold Nugget	RL	2015/05/26	Klipbokspruit	65-76	1-1XX	50.5	8.6	0.75	11.5	0.0	T2-5
Gold Nugget	RL	2015/06/30	Klipbokspruit	67-77	1-1XX	38.4	9.5	0.88	10.8	0.0	T1-2
Gold Nugget	CC	2015/06/30	Klipbokspruit	67-88	1-1XXX	42.7	11.1	0.87	12.8	0.0	T1
Gold Nugget	CC	2015/05/14	Schoonbee	60-66	2-1X	45.5	10.4	1.06	9.8	0.0	T5-7
Gold Nugget	CC	2015/06/03	Schoonbee	62-70	2-1X	45.2	11.7	1.14	10.3	0.0	T1-4
Gold Nugget	CC	2015/07/09	Schoonbee	63-76	2-1XX	46.2	12.2	1.10	11.1	0.0	T1
Gold Nugget/Lina	CC	2015/05/14	Schoonbee	60-69	2-1X	47.6	9.4	1.04	9.0	0.0	T4-6
Gold Nugget/Lina	CC	2015/06/03	Schoonbee	62-70	2-1X	45.5	11.5	1.13	10.2	0.0	T3-5
Gold Nugget/Lina	CC	2015/07/09	Schoonbee	64-75	1-1XX	37.6	11.7	1.19	9.8	0.0	T1
IRM1	SC	2015/07/15	Moosrivier	68-70	1X	53.1	12.7	1.95	6.5	4.2	T1-2
IRM2	SC	2015/07/15	Moosrivier	67-73	1-1XX	47.4	10.6	0.74	14.4	7.1	T1-2
Monica	SC	2015/07/15	Moosrivier	75-77	1XX	45.3	11.4	1.11	10.3	2.4	T1
Nadorcott ARC	CC	2015/06/04	Engelbrecht	65-72	1-1XX	44.6	9.6	0.85	11.3	0.0	T1-4
Nadorcott LS	CC	2015/06/04	Engelbrecht	65-78	1-1XXX	45.6	9.2	0.65	14.2	0.0	T1
Phoenix (2PH SL)	SC	2015/06/03	Moosrivier	64-72	1-1XX	43.6	10.5	0.77	13.6	2.9	T2-3
Phoenix (2PH SL)	SC	2015/07/15	Moosrivier	64-75	1-1XX	45.8	11.9	0.82	14.5	3.8	T1-2
Reina	SC	2015/07/15	Moosrivier	72-82	1XX-1XXX	32.1	9.6	1.35	7.1	21.2	T1-2
Shasta Gold	SC	2015/05/26	Klipbokspruit	85-96	1XXX	48.0	7.8	1.45	5.4	0.0	T1-3
Shasta Gold	CC	2015/05/14	Schoonbee	63-75	2-1XX	48.0	9.3	1.55	6.0	0.0	T3-6
Shasta Gold	CC	2015/06/03	Schoonbee	75-85	1XX-1XXX	46.2	10.1	1.57	6.4	0.0	T1
Shasta Gold	CC	2015/07/09	Schoonbee	78-90	1XXX	40.7	11.0	1.38	8.0	0.0	T1
Shasta Gold/Lina	CC	2015/05/14	Schoonbee	74-88	1XX-1XXX	48.0	9.4	1.47	6.4	0.0	T2-4
Shasta Gold/Lina	CC	2015/06/03	Schoonbee	73-93	1XX-1XXX	42.4	9.9	1.43	6.9	0.0	T1-2
Shasta Gold/Lina	CC	2015/07/09	Schoonbee	80-92	1XXX	38.0	10.5	1.29	8.1	0.3	T1
Tacle	SC	2015/06/03	Moosrivier	72-88	1XX-1XXX	47.0	10.9	1.33	8.2	0.0	T1
Tahoe Gold	CC	2015/05/26	Klipbokspruit	78-90	1XXX	48.9	8.8	0.90	9.8	0.0	T3-5
Tahoe Gold	SC	2015/05/26	Klipbokspruit	80-87	1XXX	48.3	8.10	0.90	9.0	0.0	T2-5
Tahoe Gold	CC	2015/06/30	Klipbokspruit	77-90	1XX-1XXX	36.7	9.5	0.76	12.5	0.0	T1
Tahoe Gold	CC	2015/05/14	Schoonbee	63-68	2-1X	53.6	9.5	1.13	8.5	0.0	T3-7
Tahoe Gold	CC	2015/06/03	Schoonbee	70-82	1X-1XXX	48.2	10.0	0.95	10.5	0.0	T1-3
Tahoe Gold	CC	2015/07/09	Schoonbee	67-80	1-1XXX	47.8	11.1	1.08	10.3	0.0	T1
Tahoe Gold/Lina	CC	2015/05/14	Schoonbee	61-74	2-1XX	54.7	9.2	1.08	8.4	0.0	T3-6
Tahoe Gold/Lina	CC	2015/06/03	Schoonbee	70-81	1X-1XXX	52.3	10.1	1.08	9.4	0.0	T1-5
Tahoe Gold Lina	CC	2015/07/09	Schoonbee	65-82	1-1XXX	46.3	11.0	0.98	11.2	0.0	T1
Tango	CC	2015/06/30	Klipbokspruit	65-75	1-1XX	42.8	9.6	0.79	12.2	0.0	T1-2
Tango	SC	2015/06/30	Klipbokspruit	70-75	1X-1XX	42.6	10.8	0.84	12.9	0.0	T1
Tango	CC	2015/05/26	Klipbokspruit	67-84	1-1XXX	49.8	9.0	0.71	12.7	0.0	T3-5
Tango	RL	2015/05/26	Klipbokspruit	65-74	1-1XX	51.5	8.2	0.60	13.7	0.0	T2-4

Tango	SC	2015/05/26	Klipbokspruit	65-75	1-1XX	53.0	9.5	0.84	11.3	0.0	T3-4
Tango	CC	2015/05/14	Schoonbee	58-68	3-1X	50.0	9.5	0.78	12.2	0.0	T3-5
Tango	CC	2015/06/03	Schoonbee	66-82	1-1XXX	46.7	10.3	0.85	12.1	0.6	T1-5
Tango	CC	2015/07/09	Schoonbee	62-72	2-1XX	43.2	10.6	0.87	12.2	0.0	T1
Tango/Lina	CC	2015/05/14	Schoonbee	62-70	2-1X	51.5	8.9	0.84	10.6	0.0	T3-6
Tango/Lina	CC	2015/06/03	Schoonbee	65-75	1-1XX	47.9	9.4	0.84	11.2	0.0	T2-4
Tango/Lina	CC	2015/07/09	Schoonbee	62-75	2-1XX	41.7	10.4	0.95	10.9	0.0	T1
Valley Gold (B17)	C35	2015/06/03	Moosrivier	60-70	2-1X	53.2	10.7	1.14	9.4	1.2	T1-3
Valley Gold (B17)	C35	2015/06/26	Moosrivier	67-77	1-1XX	49.5	10.7	0.95	11.3	0.0	T1-3
Valley Gold (B17)	C35	2015/07/15	Moosrivier	66-75	1-1XX	46.7	12.1	1.28	9.5	1.5	T1

5.5.8 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise)

Project 899B by J. Joubert and M.P. Cele (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat ŉ baie belangrike punt uitlig wanneer dit by die keuse van kultivars vir aanplantings kom, sowel as die onderstam wat gebruik word. Die resultate van die 2015 seisoen vir hierdie warm produksie areas het aangedui dat Tango die vroegste ryp geword het met die kleinste vruggrootte en goeie interne kwaliteit (suurvlakke daal vining in begin van seisoen). Daarna het Tahoe Gold gevolg, met beter eksterne vrug kleur. Al die seleksie was totaal saadloos hierdie seisoen. Gold Nugget en Yosemite Gold was volgende om ryp te word, nader aan die einde van die Mandaryn Hibried reeks, met ŉ gemiddelde tot goeie interne kwaliteit, asook goeie eksterne kleur ontwikkeling (T1). Shasta Gold was die laatste seleskie 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. 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 variety to plant where, as well as the suitable rootstock for that area. The results of the 2015 season indicate 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. All the selections evaluated 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 (T1). 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. 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) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.5.8.1. List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2015 season.

Selection	Rootstock	Topworked
Shasta Gold	RL/X639	2010
Tahoe Gold	RL/X639	2010
Yosemite Gold	RL/X639	2010
Gold Nugget	RL/X639	2010
Tango	RL/X639	2010

Table 5.5.8.2. List of Mandarin Hybrid selections evaluated at NGB (Weipe) during the 2015 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

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 was medium to large/extra large (count 2-1XXX) and the fruit on all the trees was completely seedless. The internal quality of the fruit deteriorated from good to fair and developed juice (avg 45%), Brix (10) and acid levels below 1.0% avg and an external colour from T1-2. Future evaluations will determine the feasibility of Gold Nugget in the hot areas. Based on the internal quality results in Table 5.5.8.3, 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 light crop on the Shasta Gold trees (20-40 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 average to low juice (41-55%) and 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 fair with low juice (avg 49%) and Brix (9) levels, but high acid levels (above 1.3% final evaluation). Based on the internal quality results in Table 5.5.8.3, maturity will be end of June.

Tahoe Gold

Tahoe Gold produced a good crop on the trees at Alicedale (rough lemon). 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 increased this season and peaked from large to very large (count 1X-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was an improvement in the external colour when the internal quality was optimal. Rough lemon developed better acid levels this season, improving fruit quality, flavour and shelf life. Tahoe Gold produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated were completely seedless at Alicedale and NGB. The internal quality was good with juice levels reaching 60%, Brix averaging 9 and acid levels were acceptable (0.8% and higher). Based on the internal quality results in Table 5.5.8.3, estimated maturity was the end of May to beginning of June.

Tango

Tango remained completely seedless at Alicedale and NGB where there was a better crop (60 kg/tree) 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 50%), 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 peaked at count 3 to 1XXX (medium to large). Based on the internal quality results in Table 5.5.8.3, estimated maturity will be end of April to middle of May.

Yosemite Gold

Yosemite Gold cropped a light yield on Rough lemon and X639, 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 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. Fruit size varied from large to very large (count 1-1XXX), 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 (average 10). External colour developed along with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.5.8.3, estimated maturity will be the middle to end of June.

Conclusion

The external colour delay (internal quality improved with more mature trees) in the hotter areas remained a problem; future evaluations will confirm this. Degreening may be an option for the Gold Nugget and TDEs, but ethylene reacted slowly or not at all for Tango (W. Murcott selection) and Nadorcott. Shasta Gold may be a possibility to consider for the hot areas due to higher acid levels late in the season, when external colour becomes more intense (T1-2) due to temperature drop (winter time). The appearance of Shasta Gold's fruit in the Tshipise and Weipe area (hot) may be a problem. In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack-out percentage (Shasta had severe sunburn at Alicedale and NGB). Gold Nugget improved considerably with good internal quality, better production and medium to large fruit size. 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. All the selections were completely seedless this season.

Table 5.5.8.3. Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) and NGB (Weipe) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	RL	2015/04/28	Alicedale	61-73	2-1XX	54.2	8.8	0.78	11.3	0.0	T5-7
Gold Nugget	RL	2015/05/19	Alicedale	62-86	2-1XXX	32.5	8.5	0.67	12.7	0.0	T4-6
Gold Nugget	RL	2015/06/09	Alicedale	71-89	1X-1XXX	42.7	8.9	0.75	11.9	0.0	T1
Gold Nugget	X639	2015/04/28	Alicedale	65-70	1-1X	50.8	9.7	0.98	9.9	0.0	T4-7
Gold Nugget	X639	2015/05/19	Alicedale	65-75	1-1XX	49.4	9.7	0.83	11.7	0.0	T3-5
Gold Nugget	X639	2015/06/09	Alicedale	65-78	1-1XXX	41.7	11.1	0.88	12.6	0.0	T1
Gold Nugget	X639	2015/04/29	NGB	60-70	2-1X	49.7	9.6	0.78	12.3	0.0	T6-8
Gold Nugget	X639	2015/05/19	NGB	60-72	2-1XX	42.9	10.4	0.70	14.9	0.0	T4-7
Gold Nugget	X639	2015/06/10	NGB	67-79	1-1XXX	42.8	10.7	0.78	13.7	0.0	T1-6
Shasta Gold	RL	2015/04/28	Alicedale	71-77	1X-1XX	51.7	8.8	1.34	6.6	0.0	T5-7
Shasta Gold	RL	2015/05/19	Alicedale	70-87	1X-1XXX	50.9	9.2	1.32	7.0	0.0	T3-6
Shasta Gold	RL	2015/06/09	Alicedale	75-85	1XX-1XXX	46.0	9.6	1.27	7.6	0.0	T1-3
Shasta Gold	X639	2015/05/19	Alicedale	83-101	1XXX	41.5	8.5	1.67	5.1	0.0	T3-4
Shasta Gold	X639	2015/04/29	NGB	71-89	1X	55.6	8.9	1.20	7.4	0.0	T5-7
Shasta Gold	X639	2015/05/19	NGB	70-86	1X-1XXX	50.7	10.6	1.24	8.5	0.0	T4-6
Shasta Gold	X639	2015/06/10	NGB	80-92	1XXX	48.8	9.7	1.03	9.4	0.0	T2-5
Tahoe Gold	RL	2015/04/28	Alicedale	70-77	1X-1XX	60.2	8.0	1.35	5.9	0.0	T4-6
Tahoe Gold	RL	2015/06/09	Alicedale	80-90	1XXX	33.5	8.1	0.82	9.9	0.0	T3-6
Tahoe Gold	X639	2015/05/19	Alicedale	70-87	1X-1XXX	48.5	7.8	1.12	7.0	0.0	T3-6
Tahoe Gold	X639	2015/06/09	Alicedale	73-85	1XX-1XXX	50.6	9.0	1.00	9.0	0.0	T1
Tahoe Gold	X639	2015/04/29	NGB	71-80	1X-1XXX	58.0	10.1	1.28	7.9	0.0	T6-8
Tahoe Gold	X639	2015/05/19	NGB	70-80	1X-1XXX	57.4	10.0	1.09	9.2	0.0	T3-6
Tahoe Gold	X639	2015/06/10	NGB	74-90	1XX-1XXX	54.0	9.7	0.91	10.7	0.0	T1-3
Tango	RL	2015/04/28	Alicedale	62-65	2--1	57.7	8.4	0.69	12.2	0.0	T5-7
Tango	RL	2015/05/19	Alicedale	55-68	3-1X	43.3	8.5	0.68	12.5	0.0	T4-6
Tango	X639	2015/04/28	Alicedale	60-70	2-1X	57.2	7.9	0.84	9.4	0.0	T4-6
Tango	X639	2015/05/19	Alicedale	65-75	1-1XX	50.0	8.3	0.79	10.5	0.0	T3-5
Tango	X639	2015/06/09	Alicedale	60-72	2-1XX	50.0	9.4	0.87	10.8	0.0	T1-3
Tango	X639	2015/04/29	NGB	62-70	2-1X	55.4	9.5	0.68	14.0	0.0	T4-6
Tango	X639	2015/05/19	NGB	55-65	1--3	56.1	10.8	0.81	13.3	0.0	T3-5
Tango	X639	2015/06/10	NGB	63-75	2-1XX	54.7	10.0	0.65	15.4	0.0	T1-3
Yosemite Gold	RL	2015/04/28	Alicedale	67-75	1-1XX	52.9	10.1	1.06	9.5	0.0	T4-7
Yosemite Gold	RL	2015/05/19	Alicedale	67-75	1-1XX	48.0	11.3	1.22	9.3	0.0	T3-6
Yosemite Gold	RL	2015/06/09	Alicedale	70-77	1X-1XX	45.2	11.5	1.34	8.6	0.0	T1
Yosemite Gold	X639	2015/05/19	Alicedale	74-90	1XX-1XXX	25.0	8.4	1.21	6.9	0.0	T4-6
Yosemite Gold	X639	2015/04/29	NGB	74-80	1XX-1XXX	52.2	9.2	1.07	8.6	0.0	T6-8
Yosemite Gold	X639	2015/05/19	NGB	75-77	1XX	46.3	10.5	0.97	10.8	0.0	T3-5

5.5.9 **PROGRESS REPORT: Evaluation of Mandarin Hybrid selections in the cool inland areas (Burgersfort)**

Project 990 by J. Joubert and M.P. Cele (CRI)

Opsomming

Goeie eksterne kleur ontwikkeling tesame met goeie interne vrug kwaliteit was een van die sterkpunte van die koeler produksie areas. Die sap inhoud moet noukeurig met opvolg evaluasies gekontroleer word, want dit kan effens aan die laer kant wees. Die resultate van die 2015 seisoen vir hierdie koel binnelandse produksie area het aangedui dat Tango die vroegste ryp geword het met medium tot groot vrugte, asook baie goeie interne kwaliteit en smaak. Yosemite Gold wat vir die eerste keer hierdie seisoen vrugte gedra het, sal volgende wees met sterk ferm vrugte. Daarna volg Gold Nugget met die beste interne kwaliteit vir die seisoen, hoë Brix en goeie suur vlakke wat belowende rakleeftyd sal verseker. Daar was geen saad in enige van die Mandaryn Hibried vrugte hierdie seisoen nie, met die uitsondering van Tahoe Gold. Tahoe Gold was volgende om ryp te word, nader aan die einde van die laat Mandaryn Hibried seisoen met goeie interne kwaliteit vrugte wat van die hoogste sap vlakke opgelewer het. Shasta Gold was die laatste seleskie gereed vir oes, 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

Good external colour development was one of the strong points in the cooler production areas together with good internal fruit quality. Monitor the juice levels closely on this selection, because they tend to be on the low side. The results of the 2015 season indicated that Tango matures first for the cool inland production area with medium to large fruit size, and very good internal quality and flavour. Yosemite, bearing a crop for the first time this season, will be next to mature with strong firm fruit. Gold Nugget followed with the best internal quality for the season with high Brix and good acid levels extending the shelf life of the fruit. There were no seeds present in any of the Mandarin Hybrid fruit this season with the exception of Tahoe Gold. Tahoe Gold matured next towards the end of the Mandarin Hybrid range evaluated at this trial site, with good internal quality resulting in highest juice levels in the fruit. Shasta Gold was the last selection to mature, 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 hot production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Viljoen Farm (Burgersfort) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.5.9.1. List of Mandarin Hybrid selections evaluated at Viljoen (Burgersfort) during the 2015 season.

Selection	Rootstock	Topwork
Shasta Gold	CC	2011
Tahoe Gold	CC	2011
Yosemite Gold	CC	2011
Gold Nugget	CC	2011
Tango	CC	2011

Results and discussion

All the selections were bearing fruit for the second time this season. 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 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, and to set more smooth textured fruit on the lower branches. This unique selection is familiar for its rough textured fruit with coarse rinds. After completing the evaluations, it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. Gold Nugget developed good internal quality (Brix: acid ratio above 11), Brix content of 12.9 and high acid (1.7%) levels for longer shelf life at optimal external colour (T1). Juice levels throughout all the evaluations at the Burgersfort site remained in the 40s (range from 43 to 45.2%), which is low to compete in the export market. Bearing in mind the young tree age, future evaluations will determine the optimal internal quality of this selection in the cooler areas. The fruit was completely seedless during all the evaluations. Based on the internal quality results in Table 5.5.9.2, estimated maturity will be the end of July to middle of August.

Shasta Gold

There was less ribbing compared to the hot production trial sites (Tshipise, Letsitele and Malelane) on most of the fruit, and barely any sunburn. Shasta Gold developed fairly flat fruit on the trees at the trial sites. Rind texture was smoother this season. The fruit size was slightly smaller and peaked from large to very large (avg. count 1-1XX), similar to hot production areas. The internal quality was good with juice levels of up to 50%, Brix as high as 12 and good acid levels of 1.7% when the colour peaked at T1. The higher acid may delay harvest time (Brix: acid ratio of 7.1), but improve shelf life of the fruit. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless (0.1 seeds 2013). Based on the internal quality results in Table 5.5.9.2, maturity will be middle to end of August (ultra late).

Tahoe Gold

Tahoe Gold developed the smallest tree size (compact round tree shape) when compared to the other UC5 varieties. The fruit size peaked from medium to large (count 3-1XX) and the fruit shape was similar to that of a Minneola tree. The external colour was optimum (T1) when the internal quality reached peaked maturity with acid level above 1.6%. Tahoe produced fruit with soft fibre strength that peels easily, and the fruit evaluated contained seed levels ranging from 0.2 to 0.4 seeds per fruit. The internal quality on Tahoe was not outstanding compared to 2013 and 2014 (Brix: acid ratio of 7.0). With the second last evaluation the juice levels were above 50% with high acid levels of 1.8%. Based on the internal quality results in Table 5.5.9.2, estimated maturity will be the middle to end of July.

Tango

The fruit shape was similar to the Nadorcott selection. Tango was completely seedless at the Viljoen trial site in Burgersfort and the trees developed a good crop (60 to 70 kg/tree). Rind texture was very smooth with a natural shine (similar to packhouse waxing). The Tango trees were thornless and V-shaped with medium growth rate on Carrizo rootstock. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was one of the top two selections evaluated in this trial, high in juice content (50%), Brix remained very good for this selection (11.5) and excellent acid level (1.2%) at external colour ranging from T1-4. Brix:acid ratio peaked at 9.3 and the fruit displayed a deep orange fibre colour. Fruit size improved and peaked from count 2 to 1X (medium to large). Based on the internal quality results in Table 5.5.9.2, estimated maturity will be the middle of June to the beginning of July.

Yosemite Gold

There was limited fruit on the trees to evaluate due to poor fruit set. Yosemite tends to have severe alternate cropping patterns. Future evaluations will determine the magnitude of the problem. The fruit was firm and completely seedless through the three evaluations completed, with deep orange rind colour. Fruit size peaked from count 2 to 1XX (medium to large). The internal quality developed good Brix (12) and acid (1.5%) levels, unfortunately the juice content was the lowest for this trial (40%) when the external colour peaked at T1. Based on the internal quality results in Table 5.5.9.2, estimated maturity will be the middle to the end of July.

Conclusion

There was no external colour delay in the cooler area and the optimal colour development correlated with a good to very good internal quality (acid levels on the lower side); future evaluation will support this statement.

In the cooler areas it may be necessary to cover the mandarin orchards with shade net to minimise possible hail damage and sunburn, to improve percentage packout of the fruit.

The best internal quality fruit was between Tahoe (juice levels above 50%) and Gold Nugget, developing Brix content of up to 12.9. Shasta Gold and Gold Nugget will mature late in the Mandarin hybrid season due to high acid levels (1.7% with last evaluation). Shasta Gold had the largest fruit size, followed by Yosemite Gold, then Gold Nugget. Tahoe developed smaller fruit size and peaked from count 3 to count 2, on the smaller side. All the selections were completely seedless this season, except for Tahoe with a limited number of seeds.

Table 5.5.9.2. Internal fruit quality data for Mandarin hybrid selections on Carrizo citrange at Viljoen Farm (Burgersfort) during the 2015 season.

Selection	Root-stock	Date harvested	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	CC	2015/05/04	54-76	4-1XX	45.2	11.2	1.92	5.8	0.0	T6-8
Gold Nugget	CC	2015/06/05	68-75	1X-1XX	44.4	11.2	1.70	6.6	0.0	T3-5
Gold Nugget	CC	2015/07/14	65-70	1-1X	43.4	12.9	1.77	7.3	0.0	T1
Shasta Gold	CC	2015/05/04	64-72	1-1XX	50.0	9.5	2.10	4.5	0.0	T4-6
Shasta Gold	CC	2015/06/05	70-79	1X-1XXX	45.3	10.3	1.81	5.7	0.0	T1-4
Shasta Gold	CC	2015/07/14	66-76	1-1XX	47.6	12.3	1.74	7.1	0.0	T1
Tahoe Gold	CC	2015/04/16	58-65	3--1	54.9	9.6	2.47	3.9	0.4	T6-8
Tahoe Gold	CC	2015/05/04	61-69	2-1X	54.9	10.5	1.96	5.4	0.2	T6-8
Tahoe Gold	CC	2015/06/05	60-73	2-1XX	52.6	10.9	1.76	6.2	0.3	T3-5
Tahoe Gold	CC	2015/07/14	62-73	2-1XX	47.9	11.4	1.68	6.8	0.0	T1
Tango	CC	2015/04/16	60-68	2-1X	47.8	9.2	1.25	7.4	0.0	T5-7
Tango	CC	2015/06/05	60-68	2-1X	49.6	11.5	1.23	9.3	0.0	T1-4
Yosemite Gold	CC	2015/05/04	60-75	2-1XX	49.1	9.5	1.43	6.6	0.0	T4-8
Yosemite Gold	CC	2015/06/05	66-77	1-1XX	45.8	10.5	1.40	7.5	0.0	T3-5
Yosemite Gold	CC	2015/07/14	64-75	1-1XX	40.6	12.2	1.54	7.9	0.0	T1

5.5.10 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino)

Project 963C by J. Joubert and M.P. Cele (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 (intermediate areas) en boom ouderdom (2011). Die resultate vir die Nelspruit produksie area het aangedui dat Sonet die vroegste ryp geword het, met die kleinste vruggrootte en gemiddelde interne kwaliteit (lae sure), gevolg deur Nadorcott LS en Nadorcott ARC met medium tot groot vrugte vir hierdie seisoen en baie goeie kleur ontwikkeling. IRM 2 was vlogende gereed vir oes gewees; met minder ribbing op die vrugte in vergelyking met IRM 1 by die ander proef persele. Al die vrugte was total saadloos gewees met die evaluasies.

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, Sonet matures first with the smallest fruit size and fair internal quality (low acids), followed by Nadorcott LS and Nadorcott ARC with medium to large fruit size for this season and excellent colour development. IRM 2 matures next with less ribbing on the fruit compared to IRM 1 at the other trial sites. All the fruit evaluated this season was completely seedless.

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) from the Mpumalanga region. The following varieties were evaluated: IRM 2, Nadorcott ARC & LS and Sonet.

Table 5.5.10.1. List of Mandarin Hybrid selections evaluated at Karino-koöp (Nelspruit) during the 2015 season.

Selection	Rootstock	Planted
IRM 2	CC	2011
Nadorcott ARC	CC	2011
Nadorcott LS	CC	2011
Sonet	CC	2011

Results and discussion

The trees at Karino-koöp were evaluated for the first time this season; this having an impact on the quality and quantity of the fruit. Limited information was available and future evaluations will be conducted.

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.

<u>IRM 2</u>
IRM 2 produced a good crop on the trees and fruit size peaked from medium to large (count 2 to 1XX). The fruit was completely seedless and there was some ribbing on the fruit (limited). Juice levels averaged below 50%, Brix was good (up to 11) and acids were below 1.0% compared to the first evaluation (30 April 2015). Based on the internal quality results in Table 5.5.10.2, estimated maturity will be the middle to the end of May.

<u>Nadorcott ARC & LS</u>
The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections developed good internal quality with high juice levels (up to 60%), Brix averaging 10 and acceptable acids (0.8%). Nadorcott LS produced a better crop on the trees compared to the ARC selection and the fruit size due to the crop load varied from 2-1XX. Both selections evaluated were completely seedless. Maturity seems to be two weeks earlier on the LS selection, but information was limited due to the first year of evaluation (middle to end of May), according to Table 5.5.10.2.

<u>Sonet 2</u>
Fruit split on the Sonet 2 seems to be a problem and 30 to 40% of the crop was dropped during the season. Fruit size varied from count 3 to 1X (medium to large); internal quality was fair to good, juice levels were on the higher side and peaked above 54%, Brix 10 and acids low early in the season. The fruit was completely seedless during all the evaluations. External colour was delayed, T2 to T7. Based on the internal quality results in Table 5.5.10.2, estimated maturity will be the middle to the end of March.

Conclusion

This was the first evaluation of IRM 2, Nadorcott ARC, Nadorcott LS and Sonet 2 at the Karino site, so information is limited and future evaluations will improve recommendations on these varieties. IRM 2, Nadorcott ARC, Nadorcott LS and Sonet 2 were completely seedless for this trial. Sonet 2 developed problems

with fruit splitting and low acids. There was a good external colour development on the Nadorcott selections (deep orange).

Table 5.5.10.2. Internal fruit quality data for Mandarin hybrid selections at Karino- koöp (Nelspruit) during the 2015 season.

Selection	Root-stock	Date harvested	Size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
IRM2	CC	2015/04/30	60-72	2-1XX	51.7	9.6	1.01	9.5	0.0	T4-6
IRM2	CC	2015/05/12	62-69	2-1X	50.8	9.9	0.87	11.4	0.0	T4-6
IRM2	CC	2015/05/26	62-79	2-1XXX	46.3	10.3	0.75	13.7	0.0	T1-4
IRM2	CC	2015/06/09	60-74	2-1XX	49.4	11.2	0.83	13.5	0.0	T1-2
IRM2	CC	2015/06/23	62-73	2-1XX	49.7	11.4	0.71	16.1	0.0	T1
Nadorcott ARC	CC	2015/04/30	63-70	2-1X	58.6	9.2	0.87	10.6	0.0	T3-6
Nadorcott ARC	CC	2015/05/12	60-70	2-1X	56.5	10.1	0.74	13.6	0.0	T3-6
Nadorcott ARC	CC	2015/05/26	65-73	1-1XX	56.3	10.3	0.70	14.7	0.0	T1-4
Nadorcott ARC	CC	2015/06/09	62-75	2-1XX	54.0	10.4	0.82	12.7	0.0	T1-2
Nadorcott ARC	CC	2015/06/23	60-67	2--1	60.3	11.8	0.88	13.4	0.0	T1
Nadorcott LS	CC	2015/04/30	62-71	2-1X	56.0	9.3	0.66	14.1	0.0	T3-6
Nadorcott LS	CC	2015/05/12	62-70	2-1X	55.9	10.3	0.76	13.6	0.0	T4-6
Nadorcott LS	CC	2015/05/26	65-72	1-1XX	55.1	10.5	0.73	14.4	0.0	T1-4
Nadorcott LS	CC	2015/06/09	62-72	2-1XX	52.8	10.6	0.73	14.5	0.0	T1
Nadorcott LS	CC	2015/06/23	53-70	4-1X	58.4	10.3	0.84	12.3	0.0	T1
Sonet 2	CC	2015/02/20	56-64	3--1	55.2	8.2	1.25	6.6	0.0	T7-8
Sonet 2	CC	2015/03/17	66-70	3-1X	58.3	9.0	0.67	13.4	0.0	T2-7
Sonet 2	CC	2015/04/10	62-70	2-1X	54.4	9.0	0.51	17.6	0.0	T2-5
Sonet 2	CC	2015/04/30	58-65	3--1	54.5	10.8	0.47	23.0	0.0	T1-3

5.5.11 PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Karino)

Project 963B by J. Joubert and M.P. Cele (CRI)

Opsomming

In die Nelspruit produksie area word M7 eerste ryp met die beste interne kwaliteit (lae sure) vir hierdie proef, gevolg deur Fukumoto 2 met klein tot medium vruggrootte vir hierdie seisoen en goeie kleur ontwikkeling. Newhall word volgende ryp met redelike sap vlakke en vertraagde eksterne kleur op die vrugte. Fischer en Dream volg, om die middel van die nawel soetlemoen reeks te vul, met medium tot groot vruggrootte en hoë Brix vlakke. Clarke en 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 (low acids) for the trial, followed by Fukumoto 2 with small to medium fruit size for this season and good colour development. Newhall matures next with fair juice levels and delayed external colour on the fruit. Fischer and Dream matured next, towards the middle of the navel orange range, with medium to large fruit size and high Brix levels. Due to limited evaluation data and poor internal quality (low juice and acids) this season, maturity estimates were not possible for Clarke and 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) from 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 5.5.11.1. List of navel selections evaluated at Karino-koöp (Nelspruit) during the 2015 season.

Selection	Rootstock	Planted
Clarke	CC	2011
Dream	SC	2011
Fischer	SC	2011
Fukumoto 2	SC	2011
Hutton	CC	2011
Newhall	SC	2011
M7	CC/SC	2011
Newhall	SC	2011

Results and discussion

The trees at Karino-koöp were evaluated for the first time this season; this having an impact on the quality and quantity of the fruit. Limited information was available and future evaluations will be conducted.

Clarke
Clarke developed medium to large (count 88 to count 40) fruit size on the trees at Karino and the external colour peaked at T1-2. The navel-end was fairly open and the fruit shape oblong; rind texture was coarse. Clarke produced fruit with poor juice levels (up to 43%), Brix 10.4 and acids 0.8% from the first evaluation. Based on the internal quality results in Table 5.5.11.2, 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 47%, Brix of 10 and similar acids (0.8%). Dream fruit developed a fairly smooth rind, small navel-end and fairly round fruit shape. There was a delay on the external colour of the fruit, peaking between T2 and T6 with the second last evaluation. Maturity seems to be end of April to middle May according to Table 5.5.11.2.

Fischer (control)
The acid levels on Fischer navel were even lower, averaging 0.7% for the season. Juice and Brix improved up to 47% and 11 respectively. Externally the fruit colour development peaked from T1 to T3. Fruit size was optimum for navel production, medium to large fruit size (count 88 to 56). To estimate maturity was difficult due to the low acid levels early in the season, future evaluations will provide more information.

Fukumoto 2
Fukumoto 2 was selected 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 varied from small to medium (count 105 to count 64). Juice levels were low (43%), Brix of 10 and low acids (0.7%).

Hutton
There was a range of different fruit sizes on the Hutton trees for the season, from small to medium to large

fruit (count 105 to count 48). Juice levels in the beginning of the season were very low (average 30%) and increased up to 40% in the final evaluation. Rind texture remained fairly coarse due to the young tree age and navel-ends were open on 60% of the fruit.

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 T1-2, Swingle T2-4). There was chimeras and mutations on a number of the fruit in the trial block. The juice levels (up to 49%) were the best for the season compared to the other navel selections at the trial site. Fruit size on CC was bigger (count 88 to count 56) compared to SC (count 105 to count 56) due to the crop load on the trees. Brix and acid content on both rootstocks was good to very good, up to 11.6 and 0.8% respectively. Based on the internal quality results in Table 5.5.11.2, estimated maturity will be the end of March to the middle of April.

Newhall

Newhall produced medium to large fruit (count 105 to 56) on the trees with low acids (0.73%) 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 was 9 and juice levels peaked at a low 45%. The Newhall fruit remained fairly green (T4-6) when the third evaluation was completed. Maturity seems to be middle of April to end of April.

Conclusion

This was the first 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 (even on SC) was low to very low; below the minimum export requirement of 48%. 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 navel selections for the Karino area.

Table 5.5.11.2. Internal fruit quality data for Navel selections at Karino- koöp (Nelspruit) during the 2015 season.

Selection	Root-stock	Date harvested	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Clarke	CC	2015/05/12	75-83	72-56	39.9	9.6	0.80	12.0	0.0	T4-6
Clarke	CC	2015/05/26	77-80	72-64	39.9	10.5	0.79	13.3	0.0	T2-5
Clarke	CC	2015/06/09	76-91	72-40	41.4	10.3	0.83	12.4	0.0	T1-2
Clarke	CC	2015/06/23	70-80	88-64	43.1	11.0	0.72	15.3	0.0	T1
Dream	SC	2015/03/17	70-80	88-64	45.3	7.9	0.99	8.0	0.0	T5-8
Dream	SC	2015/04/30	70-82	88-56	49.6	10.2	0.82	12.4	0.0	T3-5
Dream	SC	2015/05/12	73-83	72-56	46.3	10.6	0.76	13.9	0.0	T2-6
Dream	SC	2015/05/26	76-83	72-56	47.0	11.1	0.63	17.6	0.0	T1-4
Fischer	SC	2015/03/17	70-76	88-72	41.6	8.7	0.90	9.7	0.0	T6-8
Fischer	SC	2015/04/30	75-80	72-64	50.2	10.2	0.66	15.5	0.0	T3-6
Fischer	SC	2015/05/12	73-84	72-56	47.1	11.1	0.64	17.3	0.0	T1-2
Fischer	SC	2015/05/26	75-82	72-56	47.9	11.0	0.58	19.0	0.0	T1-3
Fukumoto 2	SC	2015/03/17	67-79	105-64	43.2	8.7	0.85	10.2	0.0	T5-7
Fukumoto 2	SC	2015/04/10	70-75	88-72	43.5	10.3	0.67	15.4	0.0	T3-4
Fukumoto 2	SC	2015/04/30	75-80	72-64	44.6	10.9	0.82	13.3	0.0	T1-4
Fukumoto 2	SC	2015/05/12	73-80	72-64	41.0	10.7	0.51	21.0	0.0	T1
Fukumoto 2	SC	2015/05/26	70-80	88-64	43.3	10.1	0.52	19.4	0.0	T1
Hutton	CC	2015/03/17	66-79	105-64	24.7	7.6	1.09	7.0	0.0	T6-8
Hutton	CC	2015/05/12	67-79	105-64	35.1	10.3	0.80	12.9	0.0	T4-6
Hutton	CC	2015/05/26	73-82	72-56	42.4	10.6	0.72	14.7	0.0	T3-5
Hutton	CC	2015/06/09	72-88	88-48	40.3	10.8	0.68	15.9	0.0	T1-4
Hutton	CC	2015/06/23	74-82	72-56	41.9	10.9	0.66	16.5	0.0	T1
M7	CC	2015/03/17	70-78	88-64	43.7	9.3	1.02	9.1	0.0	T5-6
M7	CC	2015/04/10	77-83	72-56	49.1	10.7	0.85	12.6	0.0	T2-4
M7	CC	2015/04/30	72-83	88-56	49.0	11.6	0.75	15.5	0.0	T1
M7	CC	2015/05/12	77-83	72-56	46.5	10.4	0.72	14.4	0.0	T2-4
M7	SC	2015/03/17	70-75	88-72	42.6	9.6	0.95	10.1	0.0	T4-6
M7	SC	2015/04/10	67-78	105-64	44.1	10.7	0.75	14.3	0.0	T2-4
M7	SC	2015/04/30	72-80	88-64	48.0	11.6	0.77	15.1	0.0	T1
M7	SC	2015/05/12	68-79	88-64	42.9	11.9	0.73	16.3	0.0	T1
M7	SC	2015/05/26	72-82	88-56	44.1	11.6	0.60	19.3	0.0	T1
Newhall	SC	2015/03/17	70-82	88-56	40.6	7.4	1.10	6.7	0.0	T6-7
Newhall	SC	2015/04/10	67-70	105-88	45.4	9.4	0.80	11.8	0.0	T4-6
Newhall	SC	2015/04/30	71-80	88-64	48.6	9.6	0.63	15.2	0.0	T4-6
Newhall	SC	2015/05/12	70-79	88-64	45.7	10.0	0.61	16.4	0.0	T3-5
Newhall	SC	2015/05/26	73-85	72-56	43.2	9.9	0.51	19.4	0.0	T1-3

5.5.12 **PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Marble Hall)**

Project 941A by J. Joubert and M.P. Cele (CRI)

Opsomming

Die Nawel proef by Moosrivier en Schoonbee was vir die eerste keer hierdie seisoen ge-evalueer. Hierdie produksie area ontwikkel lae suur vlakke in die nawel vrugte vroeg in die seisoen en kompensasie vir laer uitvoer suur standaarde moet versoek word. Painter Early was die eerste seleksie om ryp te word met goeie kleur ontwikkeling. Santa Catherina sal volg met medium vruggrootte, gevolg deur Kirkwood Red wat rooi interne pigment ontwikkel, soortgelyk aan Cara Cara nawel. De Wet 2 met vertraagde kleur en Robyn 2 (optimum kleur) was volgende gereed om te oes, meer in die middle van die nawel seisoen. Gloudi sal die seisoen vir hierdie proef afsluit, met slegs Lane Late en Carninka Late wat later rypword in die laatnawel reeks. Evaluasies sal voortgaan as gevolg van die beperkte inligting beskikbaar.

Summary

The navel trial at Moosrivier and Schoonbee was evaluated for the first time this season. 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. Painter Early will be the first selection to mature with good colour development. Santa Catherina will follow with medium fruit size, followed by Kirkwood Red developing red internal pigmentation similar to the Cara Cara navel. De Wet 2 with delayed colour and Robyn 2 (optimum colour) were next to mature towards the middle of the navel season. Gloudi will end of the season at this trial site, with only Lane Late and Carninka Late to mature later in the latenavel range. 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 Moosrivier Estate (Marble Hall) and Schoonbee Estate (Marble Hall) from the Limpopo region. The following early, mid and late maturing selections were evaluated: Clarke, De Wet 2, Gloudi, Golden Buch Eye, Kirkwood Red, Painter Early, Robyn 2 and Santa Catherina.

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 5.5.12.1. List of navel selections evaluated at Moosrivier Estate (Marble Hall) during the 2015 season.

Selection	Rootstock	Planted
Clarke	SC	2009
Gloudi	SC	2009
Kirkwood Red	SC	2009

Table 5.4.12.2. List of navel selections evaluated at Schoonbee Estate (Marble Hall) during the 2015 season.

Selection	Rootstock	Topwork
De Wet 2	CC	2012
Golden Buch Eye	CC	2012
Painter Early	CC	2012
Robyn 2	CC	2012
Santa Catherina	CC	2012

Results and discussion

The trees at Karino-koöp were evaluated for the first time this season; this having an impact on the quality and limiting the quantity of the fruit available to evaluate. Limited information was available and future evaluations will be conducted.

Clarke developed the lowest juice levels (35%) in the fruit with delayed external colour (T1-5) whilst Gloudi developed the best juice of 48%, as well as Brix above 10 and acids (0.82%) (Table 5.5.12.3). Santa Catherina developed poor acids in the fruit (0.5%), the lowest for the trial site. The lowest Brix was on De Wet 2 in combination with Carrizo rootstock (high IQ rootstock). Brix:acid ratios peaked from 12.1 to 17 (well overmatured) due to the fairly low acids. External colour was delayed on 5 of the 8 selections, except for Gloudi, Painter Early and Robyn 2. Evaluations will continue before any recommendations are made.

Conclusion

The internal quality of the fruit was poor to average and all the juice levels were below the minimum standards (48.5%). All the Brix levels for this trial were below 10, except for Gloudi. The acid levels were low (0.8%) from the first evaluation and the shelf life of the fruit will be questionable (short shelf life). Painter early and Santa Catherina were below the minimum acid standard of 0.6%. Colour development was delayed on the overmatured fruit early in the season.

Table 5.5.12.3. Internal fruit quality data for Navel selections at Moosrivier Estate and Schoonbee Estate (Marble Hall) during the 2015 season.

Selection	Root-stock	Date harvested	Site	Size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Clarke	SC	2015/06/03	Moosriver	82-95	56-36	34.8	9.7	0.80	12.1	0.0	T1-5
De Wet 2	CC	2015/05/14	Schoonbee	84-98	56-36	43.9	8.3	0.68	12.2	0.0	T4-6
Gloudi	SC	2015/06/03	Moosriver	78-85	64-56	40.4	9.4	0.77	12.2	0.0	T3-6
Gloudi	SC	2015/07/15	Moosriver	75-84	72-56	48.2	10.6	0.82	12.9	0.0	T1-2
Golden Buck Eye	CC	2015/05/14	Schoonbee	83-92	56-40	40.6	8.5	0.68	12.5	0.0	T4-6
Kirkwood Red	SC	2015/06/03	Moosriver	84-95	56-36	41.1	9.3	0.74	12.6	0.0	T2-5
Painter Early	CC	2015/03/19	Schoonbee	72-90	88-40	43.9	8.5	0.65	13.1	0.0	T6-8
Painter Early	CC	2015/05/14	Schoonbee	75-88	72-48	46.9	9.7	0.57	17.0	0.0	T1-4
Painter Early	CC	2015/06/03	Schoonbee	80-85	64-56	43.1	9.5	0.57	16.7	0.0	T1-2
Robyn 2	CC	2015/07/09	Schoonbee	79-97	64-36	42.8	9.1	0.62	14.7	0.0	T1
Santa Catherina	CC	2015/05/14	Schoonbee	70-76	88-72	40.5	8.8	0.55	16.0	0.0	T4-6

5.5.13 **PROGRESS REPORT: Evaluation of Lemon selections in the intermediate production areas (Tom Burke)**

Project 941B by J. Joubert and M.P. Cele (CRI)

Opsomming

Die 2015 seisoen was die eerste drag op die bome gewees vir die suurlemoen proef by Tom Burke. 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 ŉ beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseël. 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. Hoë temperature gedurende blom periodes het swak vrugset tot gevolg gehad vir sekere van die seleksies. Al die seleksies was getopwerk aan die einde van die 2012 seisoen op X639, vir die aanpasbaarheid by die hoër pH gronde en verenigbaarheid met Eureka-tipe suurlemoene.

Summary

The 2015 season produced the first crop on the trees for the Lemon trial at Tom Burke. Willow Tree 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. 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. High temperatures during the flowering periods induced poor fruit set on some of the selections. All the selections were topworked at the end of the 2012 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 Klipbokspruit (Tom Burke) on Eureka, Genoa, Limoneira 8A, Lisbon, Lisbon Yen Ben and Willow Tree Long.

Table 5.5.13.1. List of lemon selections evaluated at Klipbokspruit (Tom Burke) during the 2015 season.

Selection	Rootstock	Topworked
Eureka	X639	2012
Genoa	X639	2012
Limoneira 8A	X639	2012
Lisbon	X639	2012
Lisbon Yen Ben	X639	2012
Willow Tree Long	X639	2012

Results and discussion

Limoneira 8A (38%) and Lisbon (39.5%) developed the lowest juice percentages for the season, but Willow Tree Long had the highest juice percentage of 44%. Genoa produced the biggest fruit size and peaked from count 4 to count 1 through the season. The highest seed content per fruit was on Lisbon (11.7 seeds per fruit), followed by Willow Tree Long (10.6 seeds per fruit). The external colour ranged from T3 to T5 at the second 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, Lisbon, Limoneira 8A and Genoa performed well and were more suitable for the intermediate production areas compared to the experimental selections.

Table 5.5.13.2. Internal fruit quality data for Lemon selections at Klipbokspruit (Tom Burke) during the 2015 season.

Selection	Root-stock	Date harvested	Size (mm)	Count	Juice (%)	Avg. seed	Colour
Eureka	X639	2015/05/26	57-66	5--3	42.7	8.2	T4-6
Eureka	X639	2015/06/30	52-58	6--5	41.7	9.0	T4-5
Genoa	X639	2015/05/26	60-75	4--1	38.4	7.7	T5-7
Genoa	X639	2015/06/30	60-71	4--2	42.7	10.1	T4-6
Limoneira 8A	X639	2015/05/26	57-65	5--3	36.7	6.3	T4-7
Limoneira 8A	X639	2015/06/30	54-63	5--3	38.4	7.0	T4-5
Lisbon	X639	2015/05/26	57-66	5--3	42.8	10.5	T4-6
Lisbon	X639	2015/06/30	65-69	3--2	36.1	12.8	T4-5
Lisbon Yen Ben	X639	2015/05/26	51-57	6--5	42.6	0.3	T3-6
Lisbon Yen Ben	X639	2015/06/30	43-53	6	35.8	0.6	T4-5
Willow Tree Long	X639	2015/05/26	55-67	5--3	43.3	10.1	T3-5
Willow Tree Long	X639	2015/06/30	60-66	4--3	43.7	11.0	T4-5

5.5.14 PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area

Project 416A by J. Joubert and M.P. Cele (CRI)

Opsomming

Midknight, met ʼn gesonde entlas verbinding, het bewys dit is verenigbaar met Sunki 812, ʼn 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 ʼn groot boom oplewer. Die produksie hierdie seisoen was gemiddeld en het met 54.4 kg per boom afgeneem (50% laer), met ʼn ooreenstemmende toename in vruggrootte, met pieke by telling 56.

Delta toon vereenigbaarheid met Sunki 812, HRS 802 en FF-6 onderstamme vir hierdie proef perseel. Die entlas tussen die onderstam en bostam was glad met geen tekens van onverenigbaarheid (geen groeipunte by entlas) nie. Daar was ʼn geringe afname in oes produksie op die bome hierdie seisoen. Vruggrootte het effens toegeneem by al drie onderstam kombinasies en het by telling 105/125 en 72 gepiek.

Evaluasies tot op datum toon aan dat hierdie onderstamme waardevol kan wees vir die sitrus produsente; meer spesifiek Sunki 812, waar hoë pH vlakke en kalkagtige gronde voorkom. Sunki 812 was vir sy hoë verdraagsaamheid teen Phytophthora, sitrus aalwurms en tristeza, asook beter weerstand vir hoër pH en kalkagtige gronde, geselekteer.

Summary

Visual evaluations of the Midknight: Sunki 812 bud-union, indicated that the union was in good condition and the combination compatible. Sunki 812 is a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliate (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. Yield production this season was average and decreased to 54.4 kg per tree (50% lower), with a corresponding increase in fruit size, peaking at count 56.

Delta seems to be compatible with Sunki 812, HRS 802 and FF-6 rootstocks at this trial site. The bud-union between the rootstock and scion was fairly smooth without any signs of incompatibility (no growth tips at bud-

union). There was a slight decrease on crop production this season on all three rootstocks. Fruit size on all three rootstock combinations increased slightly and peaked at count 105/125 and 72.

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 Midknight and Delta Valencias on new, imported rootstocks on replant soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

Seeds of Sunki 812 (Sunki x Beneke), HRS 809 and FF-6 were imported and propagated in 1996 by Esselen Nursery, a CIS accredited nursery in the Malelane region of Mpumalanga.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: HRS 802 (Siamese pummelo x Gotha Road trifoliate), Sunki 812 (Sunki mandarin x Beneke trifoliate) and FF-6 (Sunki x MTO trifoliate orange). Midknight Valencia was budded onto US 812 (Sunki x Beneke). The trees were planted at Esselen Nursery in March 1999.

Table 5.5.14.1. Number of trees per rootstock in the Delta and Midknight Valencia trial at Malelane.

Selection	Rootstock	No. of trees
Midknight	Sunki 812	4
Delta	Sunki 812	4
Delta	HRS 802	4
Delta	FF-6	5

Results and discussion

Midknight Valencia

This is the first trial of Midknights planted on US 812 (Sunki x Beneke) in South Africa. There was a significant decrease in crop production for the 2015 season from 108.9 to 54.5 kg/tree in comparison with the previous season (Table 5.5.14.4), and the 9-year mean was 62.0 kg/tree (Table 5.5.14.4). Internally fruit quality was good with Brix (11.9) and juice levels slightly lower compared to 2014 (57.2%) with 54.2% (Table 5.5.14.2).

The acid content increased this season from 1.0 to 1.4%, similar to the 2013 season, improving the Brix:acid ratio to 10.4. The crop production between Midknight and Delta on US 812 (Sunki x Beneke) was very different compared to 2014 (107 kg/tree), Midknight decreased yield production with 50% from 107 kg/tree to 54 kg/tree. The Midknight trees were slightly smaller compared to the Delta trees, but the difference was not significant enough in this case and the difference in yields cannot be attributed to vigour and tree size. Fruit size peaked at count 56 (36%), followed by count 72 (28%) and count 48 (17%), producing a bigger fruit size on the trees for this season, due to the lighter crop (good fruit size distribution).

Delta Valencia

Delta on HRS 802 produced the best juice content (58.2%), as well as the highest Brix:acid ratio of 13:6, followed by Sunki 812 with the highest Brix level (12.8) and acid of 1.5% (Table 5.5.14.2). There was an improvement on the external colour development on all three rootstock combinations peaking at T1 to T2 compared to T2/3-4/5 last season. Fruit size increased on HRS 802 and peaked at count 72, followed Sunki 812 and FF-6 at count 105/125. There was a slight drop in crop production this season on Sunki 812 (from 105.5 to 94.0 kg/tree), HRS 803 (from 107.3 to 106.0 kg/tree) and FF-6 (134.8 to 100.7 kg/tree).

Conclusions

The crop production remained impressive on all the Delta combinations this season due to excellent fruitset, less theft due to better security; as well as trees in peak production at this trial site, with the exception of Midknight on Sunki 812 with 50% less fruit on the trees.

Midknight on Sunki 812 (Sunki x Beneke) performed well, producing an average crop compared to the previous season (109 to 54.4 kg per tree), with slightly bigger fruit size due to the lighter yield, and good internal qualities. The acid levels (1.5%) increased slightly this season compared to 2014 (1.0%). The Brix:acid ratio was lower (8:1) due to the higher acid level, slightly delaying the maturity of the selection, with external colour at T1-2.

Delta was evaluated on three hybrid rootstocks: Sunki 812 (Sunki x Beneke), HRS 802 and FF-6. The most important combination of the above mentioned was Sunki 812 (Sunki x Beneke). Sunki 812 (Sunki x Beneke) was selected for replant conditions, very specific high pH and calcareous soils. Delta performed well on all three combinations this season, producing fruit with good internal quality and slightly bigger fruit size. There was a decrease in crop production on all three rootstock combinations (least on HRS 802). HRS 802 had the best internal quality (Brix: acid ratio 13.6). The fruit size peaked at counts 105/125 for Sunki 812 and FF-6, and count 72 for HRS 802 for this trial site.

The FF-6 rootstock was also selected for high pH soils, and future trials in the Musina and Kakamas areas must be conducted to confirm these characteristics.

Table 5.5.14.2. Internal fruit quality of Midknight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 26 August 2015.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Delta	FF-6	54.2	11.9	1.14	10.44	0.0	T1-2
Delta	HRS 802	58.2	12.5	0.92	13.59	0.0	T1-2
Delta	Sunki 812	53.8	12.8	1.53	8.37	0.0	T1-2
Midknight	Sunki 812	53.7	12.7	1.49	8.52	0.0	T1-2

Table 5.5.14.3. Fruit size distribution at Esselen nursery during the 2015 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	17.46	Delta	HRS 802	48	0.84
Midknight	Sunki 812	56	35.15	Delta	HRS 802	56	14.60
Midknight	Sunki 812	72	28.23	Delta	HRS 802	72	30.46
Midknight	Sunki 812	88	13.46	Delta	HRS 802	88	26.07
Midknight	Sunki 812	105/125	5.33	Delta	HRS 802	105/125	25.14
Midknight	Sunki 812	144	0.34	Delta	HRS 802	144	2.89
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	Sunki 812	48	1.79	Delta	FF-6	48	0.95
Delta	Sunki 812	56	12.81	Delta	FF-6	56	9.36
Delta	Sunki 812	72	24.80	Delta	FF-6	72	22.31
Delta	Sunki 812	88	26.17	Delta	FF-6	88	26.96
Delta	Sunki 812	105/125	28.11	Delta	FF-6	105/125	33.17
Delta	Sunki 812	144	6.33	Delta	FF-6	144	7.24

Table 5.5.14.4. Production per tree of Midknight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2015 season.

Cultivar	Root-stock	Kg/tree 2007	Kg/tree 2008	Kg/tree 2009	Kg/tree* 2010	Kg/tree* 2011	Kg/tree* 2012	Kg/tree 2013	Kg/tree 2014	Kg/tree 2015	9 Year Total	9 Year Mean
Midknight	Sunki 812	98.7	50.4	51.7	40.2	34.1	47.6	72.0	108.9	54.4	558.0	62.0
Delta	Sunki 812	120.5	66.4	78.6	58.7	42.7	56.5	114.0	105.4	94.0	736.8	81.9
Delta	HRS 802	120.6	102.3	102.4	81.4	74.7	51.1	112.0	107.3	106.0	857.8	95.3
Delta	FF-6	134.1	97.2	80.7	79.8	61.4	33.3	106.1	134.8	100.7	828.1	92.0

*Note: Heat wave experienced during fruit set period (fruit also stolen from this specific section)

5.5.15 **PROGRESS REPORT: Evaluation of various Navel selections on different rootstocks in the Marble Hall area**

Project 590A by J. Joubert and M.P. Cele (CRI)

Opsomming

Albei die vroeë nawel seleksies (Fukumoto en Newhall) het nie aan die uitvoer standaarde voldoen nie, a.g.v. lae sap en Brix vlakke. The eksterne kleur op albei seleksies was vertraag in vergelyking met die vorige seisoen. Vruggroote op albei seleksies het by telling 56 gepiek (Newhall op CC piek by telling 48), ideale vruggrootte vir nawel produksie. Fukumoto en Newhall het ‘n beter oes opgelewer in vergelyking met die 2013 seisoen met die uitsondering van Carrizo citrange (Fukumoto en Newhall) en X639 (Newhall).

Summary

The two early navel varieties (Fukumoto and Newhall) did not comply with the export requirements, due to low juice and Brix levels. The external colour development on both selections was delayed compared to the previous season. Fruit size peaked at count 56 on both selections (Newhall on CC peaked at count 48), optimum fruit size for navel production. Fukumoto and Newhall cropped a better yield on the trees compared to 2013 with the exception of Carrizo citrange (Fukumoto and Newhall) and X639 (Newhall).

Objectives

- Evaluate and assess the horticultural performance and capability of various new Navel selections on different rootstocks.
- Determine the superior rootstock combinations for these new selections.
- Be able to make credible commercial recommendations.

Materials and methods

Trees were planted at Schoeman Boerdery in 2005, and the additional trees established at BBE Boerdery late 2004. A visual evaluation was done to determine production per tree, trueness to type and compatibility with scion, and each tree was harvested with the sizer to determine production per tree as well as fruit size distribution per tree. Samples were taken and internal quality tested and analysed. Fruit colour was evaluated and analysed.

Table 5.5.15.1. List of cultivar and rootstock combinations in the Navel trial at Schoeman Boerdery in the Marble Hall area.

Selection	Rootstock	Qty Trees
Fukumoto	C35	4
Fukumoto	MxT	4
Fukumoto	CC	5
Fukumoto	SC	5
Fukumoto	X639	5
Fukumoto	Terra Bella	3
Newhall	C35	5
Newhall	MxT	2
Newhall	CC	3
Newhall	SC	5
Newhall	X639	5
Newhall	Terra Bella	1

Results and discussion

This was the second evaluation for the trial site, due to severe cold and hail damage in the past, and poor crop set on the trees.

<u>Fukumoto Navel</u>

Fukumoto on Carrizo produced the best juice (46.2%); best Brix, in combination with C35 (9.5); and highest acid levels (0.85%) for this trial; as well as the highest Brix:acid ratio of 11.45. All the scion:rootstock

combinations were below the minimum juice level for export of 48%, not complying with the standards. The external colour on all the combinations was delayed and peaked between T3 and T6 (Table 5.5.15.2). Fruit size peaked at count 56 on all rootstock selections (Table 5.5.15.3). MxT produced the best crop on the trees with 97.8 kg per tree, followed by C35 with 74.3 kg per tree and CC with 65.1 kg per tree. Swingle remained the lowest with 47.4 kg per tree (Table 5.5.15.4).

Newhall Navel

Newhall on Carrizo developed the highest juice content of 47.3%, followed by Terra Bella (47.2%) and X639 (46.4%). The juice levels increased this season but did not comply with the standard. The Brix levels on all the rootstock combinations was too low for export, except on Terra Bella (9.2). The acid level ranged from 0.79 to 0,90%, complying with the minimum standards. The external colour on all the combinations was delayed between T4 and T7 (Table 5.5.15.2). Carrizo peaked at count 48 and the rest of the rootstocks at count 56 (Table 5.5.15.3). MxT was the best option for good yield production with 56.8 kg per tree, followed by C35 with 56.4 kg per tree and SC with 44.8 kg per tree. The lowest crop was on Terra Bella. (Table 5.5.15.4).

Conclusions

Fukumoto on MxT performed well, bearing in mind that the trial was harvested for the first time in 2013 and for the second time in 2015. The internal quality was average with low juice and Brix levels; only acids complied with export standards. Fruit size was large and the yield production peaked at 97.8 kg per tree. The internal quality of Newhall was similar to Fukumoto and the acids were acceptable, but not the low juice and Brix levels. The external colour was delayed (T4 to T7) and the fruit size peaked at count 56; Carrizo peaked at count 48. MxT and C35 produced the best crop on the trees, averaging 57 kg per tree.

Table 5.5.15.2. Internal fruit quality data for Navels on different rootstocks at Schoeman Boerdery, Marble Hall on 1 April 2015.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Fukumoto	C35	41.1	9.5	0.83	11.45	0.0	T3-5
Fukumoto	MxT	42.6	8.5	0.82	10.37	0.0	T3-5
Fukumoto	CC	46.2	7.6	0.76	10.00	0.0	T4-6
Fukumoto	SC	41.8	7.3	0.70	10.43	0.0	T4-6
Fukumoto	X639	44.5	7.9	0.74	10.68	0.0	T4-6
Fukumoto	TB	41.0	6.9	0.75	9.20	0.0	T4-6
Newhall	C35	46.2	8.0	0.87	9.20	0.0	T5-6
Newhall	MxT	44.7	7.9	0.78	10.13	0.0	T5-7
Newhall	CC	47.3	7.7	0.79	9.75	0.0	T4-6
Newhall	SC	44.0	7.9	0.92	8.59	0.0	T4-6
Newhall	X639	46.4	8.4	0.85	9.88	0.0	T4-6
Newhall	TB	47.2	9.2	0.90	10.22	0.0	T4-6

Table 5.5.15.3. Fruit size distribution per rootstock at Schoeman Boerdery, Marble Hall on 1 April 2015.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fukumoto	C35	48	11.23	Newhall	C35	48	18.83
Fukumoto	C35	56	34.49	Newhall	C35	56	30.17
Fukumoto	C35	72	25.92	Newhall	C35	72	20.83
Fukumoto	C35	88	14.77	Newhall	C35	88	12.67
Fukumoto	C35	105/125	11.86	Newhall	C35	105/125	11.50
Fukumoto	C35	144	1.73	Newhall	C35	144	6.00
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	MxT	48	12.53	Newhall	MxT	48	17.13
Fukumoto	MxT	56	31.06	Newhall	MxT	56	33.71
Fukumoto	MxT	72	28.03	Newhall	MxT	72	22.19
Fukumoto	MxT	88	15.08	Newhall	MxT	88	14.04
Fukumoto	MxT	105/125	9.26	Newhall	MxT	105/125	11.52
Fukumoto	MxT	144	4.04	Newhall	MxT	144	1.40
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	CC	48	22.24	Newhall	CC	48	52.65
Fukumoto	CC	56	32.31	Newhall	CC	56	31.45
Fukumoto	CC	72	20.66	Newhall	CC	72	7.77
Fukumoto	CC	88	13.30	Newhall	CC	88	4.24
Fukumoto	CC	105/125	9.32	Newhall	CC	105/125	2.12
Fukumoto	CC	144	2.18	Newhall	CC	144	1.77
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	SC	48	31.67	Newhall	SC	48	20.73
Fukumoto	SC	56	36.99	Newhall	SC	56	34.84
Fukumoto	SC	72	17.17	Newhall	SC	72	23.70
Fukumoto	SC	88	9.19	Newhall	SC	88	11.91
Fukumoto	SC	105/125	3.10	Newhall	SC	105/125	6.28
Fukumoto	SC	144	1.88	Newhall	SC	144	2.54
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	X639	48	11.17	Newhall	X639	48	11.32
Fukumoto	X639	56	28.66	Newhall	X639	56	26.48
Fukumoto	X639	72	25.89	Newhall	X639	72	23.19
Fukumoto	X639	88	17.64	Newhall	X639	88	15.82
Fukumoto	X639	105/125	13.51	Newhall	X639	105/125	17.69
Fukumoto	X639	144	3.13	Newhall	X639	144	5.49
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	TB	48	26.71	Newhall	TB	48	41.21
Fukumoto	TB	56	39.02	Newhall	TB	56	34.55
Fukumoto	TB	72	18.10	Newhall	TB	72	12.12
Fukumoto	TB	88	3.05	Newhall	TB	88	6.06
Fukumoto	TB	105/125	5.49	Newhall	TB	105/125	4.24
Fukumoto	TB	144	1.63	Newhall	TB	144	1.82

Table 5.5.15.4. Production per tree of Navel selections on different rootstocks at Schoeman Boerdery, Marble Hall on 1 April 2015.

Cultivar	Rootstock	Kg/tree 2013	Kg/tree 2015
Fukumoto	C35	41.8	74.3
Fukumoto	CC	67.6	65.1
Fukumoto	MxT	65.3	97.8
Fukumoto	SC	21.6	47.4
Fukumoto	TB	37.2	57.8
Fukumoto	X639	50.1	63.7
Newhall	C35	17.2	56.4
Newhall	CC	53.4	32.0
Newhall	MxT	20.5	56.8
Newhall	SC	14.2	44.8
Newhall	TB	0.0	44.7
Newhall	X639	98.0	40.1

5.5.16 **PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area**

Project 590B by J. Joubert and M.P. Cele (CRI)

Opsomming

Delta op Koethen citrange het ʼn Brix:suur verhouding bo 12.5 opgelewer. Delta se vruggrootte op al die onderstam kombinasies het by telling 72 gepiek, behalwe vir Swingle (105/125) a.g.v. ʼn swaar oeslading. Die oes produksie op al die onderstam kombinasies het toegeneem behalwe by MxT (82 na 64 kg/boom). Geen onverenigbaarheids tekens was by enige van die kombinasies sigbaar nie.

McClean SL op al die onderstam kombinasies het aan die uitvoer standaarde voldoen met die interne kwaliteit wat heelwat verbeter het op die vrugte. Terra Bella het die hoogste sap inhoud opgelewer met Carrizo die hoogste Brix:suur verhouding. Vruggrootte vir Valencias was uitstekend gewees; vruggrootte tellings het gepiek tussen telling 56 en 72. Daar was ‘n beter oes op die McClean SL bome met die uitsondering van MxT.

Midknight het baie goeie interne kwaliteit ontwikkel en aan die uitvoer staandaarde voldoen, behalwe vir X639. Vruggrootte het by telling 56 gepiek; Koethen Citrange by telling 105/125. Oor die algemeen was daar ʼn ligte oes op die Midknight bome gewees; beste oes produksie was op Swingle met 44 kg per boom.

Portsgate het hierdie seisoen goed gevaar met die uitsondering van hoe suurvlakke op C35 en Terra Bella. Die vruggrootte het tussen telling 88 en telling 56 gepiek. Die beste produksie kombinasie was Portsgate op Swingle gewees wat 105 kg per boom gedra het.

Summary

Delta on Koethen citrange developed a Brix:acid ratio above 12.5. Delta peaked on all the rootstock combinations with fruit size at count 72, except for Swingle (105/125) due to heavy crop load. Yield production increased on all rootstock selections except for MxT (82 to 64 kg/tree). Noo incompatibility problems were visible on the rootstock combinations.

McClean SL on all the rootstock combinations complied with the minimum export standards due to a considerable improvement in the internal quality of the fruit. Terra Bella developed the best juice levels with Carrizo the highest Brix:acid ratio. Fruit size for Valencias was excellent; fruit counts peaked between count 56 and 72. There was a better yield on the McClean SL trees with the exception of MxT.

Midknight developed very good internal quality and complied with export standards, except for X639. Fruit size peaked at count 56; Koethen Citrange peaked at count 105/125. There was a very light crop on the Midknight trees in general; the best yield was on Swingle with 44 kg per tree.

Portsgate performed well this season with the exception of high acids on C35 and Terra Bella. Fruit size peaked from count 88 to count 56. The best production combination was Portsgate on Swingle, cropping 105 kg per tree.

Objectives

- Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks.
- Determine the superior rootstock combinations for these new selections.
- Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar rootstock combination were planted in 2002. These were evaluated visually to determine production per tree, trueness to type and compatibility with scion. Each tree was harvested and the fruit sized to determine production per tree as well as fruit size distribution per tree. Samples were taken for internal quality testing and analysis. Fruit colour was also evaluated.

Table 5.5.16.1. List of cultivar and rootstock combinations in the Valencia trial at Golden Frontiers Citrus, Hectorspruit in the Komatipoort area.

Selection	Rootstock	Qty Trees
Delta (Control)	C35	5
Delta (Control)	CC	5
Delta (Control)	KC	5
Delta (Control)	MxT	5
Delta (Control)	SC	5
Delta (Control)	Terrabella	5
Delta (Control)	X639	5
McClean SL	C35	5
McClean SL	CC	5
McClean SL	KC	5
McClean SL	MxT	5
McClean SL	SC	5
McClean SL	Terrabella	5
McClean SL	X639	5
Midknight	C35	5
Midknight	CC	5
Midknight	KC	4
Midknight	MxT	5
Midknight	SC	5
Midknight	Terrabella	5
Midknight	X639	4
Portsgate	C35	5
Portsgate	CC	5
Portsgate	KC	5
Portsgate	MxT	5
Portsgate	SC	5
Portsgate	Terrabella	5
Portsgate	X639	5

Results and discussion

Delta Valencia

Delta on KC produced the best Brix (12.6) and C35 the highest acid level (1.43%). The optimum Brix:acid ratio of 11.9 was developed by Terra Bella, followed by SC with the highest juice level (57.6%) and acids (1.55). The external colour on all the selections was T1. All the rootstock combinations complied with the export standards (Table 5.5.16.2). Fruit size on all the rootstock combinations peaked at count 72, except for Swingle at count 105/125 (Table 5.5.16.3). Six of the seven scion: rootstock combinations increased crop production this season except for MxT which decreased from 83 to 64 kg per tree. The lowest production was on Koethen with 34.6 kg per tree. The highest production was in combination with Swingle cropping 105 kg of fruit per tree (Table 5.5.16.4).

McClean SL Valencia

Terra Bella in combination with McClean SL developed the highest juice content of 59.7%, in combination with 1.4% acid, resulting in a low Brix:acid ratio of 8. The lowest acid levels for this trial were produced on Koethen and CC (0.96% & 0.97%). C35, as in the 2013 & 2014 trials, outperformed the other scion:rootstock combinations with the best Brix (12.8); and CC with the Brix:acid ratio (12.8). The external colour on all the selections was T1 and McClean SL on all rootstocks was completely seedless (Table 5.5.16.2). All the rootstocks peaked at count 72 except for C35 and Terra Bella with count 56 (bigger fruit this season) (Table 5.5.16.3). There was an increase on six of the scion:rootstock combinations in crop production this season. Highest yield production this season was on X639 (142 kg/tree), followed by Swingle with 108 kg per tree. The lowest crop was on C35 with 39 kg per tree (Table 5.5.16.4).

Midknight Valencia

CC in combination with Midknight peaked with juice levels of 59.9% and C35 produced Brix of 13.1. Terra Bella developing the best Brix:acid ratio of 11.4 and the lowest acid level of 1.1%. The external colour on all the selections was T1 (Table 5.5.16.2). C35, All the rootstock combinations peaked at count 56, followed by Koethen at count 105/125 (Table 5.5.16.3). Six of the seven scion:rootstock combinations decreased crop production this season, except for X639 (39 kg/tree). The highest 8-year total was on C35 with 450 kg and 56 kg per tree 8-year mean (Table 5.5.16.4).

Portsgate Valencia

Portsgate on Carrizo produced the best juice (57%) levels, followed by C35 with the best Brix level (13.1) and X639 the highest Brix:acid ratio above 12, as well as lowest acid level of 0.91% (earlier maturing combination). The external colour on all the selections was T1 (Table 5.5.16.2). Four of the seven rootstocks peaked at count 72, followed by two combinations with count 56 (); except for Swingle at count 88, due to the excellent yield production (Table 5.5.16.3). There was a yield production increase on all the scion:rootstock combinations this season. Swingle outperformed the other combinations with 105 kg per tree. The second best was X639 with 91 kg per tree, 38 kg/tree more compared to 2014. The lowest crop was on Koethen with 31 kg per tree (Table 5.5.16.4).

Conclusions

Delta on all the rootstocks produced seedless fruit with very good internal quality. The fruit size production for Delta peaked at count 72 with the exception of Swingle. Production increased compared to the 2014 season; MxT was the only rootstock setting a lighter yield on the trees. The fruit was completely seedless at this trial site.

McClean seedless performed well and Terra Bella produced the highest juice levels (60%) for this trial. C35 outperformed the other rootstocks with the highest Brix level (13). Five of the rootstocks peaked at count 72, except for C35 and Terra Bella at count 56. McClean seedless produces on average, a good fruit size for a Valencia selection. X639 and Swingle peaked from 108 kg to 142 kg per tree, excellent yield production for a Valencia selection.

Midknight's internal quality performed better this season and improved from average to very good. There were 0.3 seeds per fruit on X639. Midknight always tends to have larger fruit and this season was the norm, with six of the rootstocks peaking at count 56 (C35, CC, SC, TB), except for Koethen peaking at count 105/125. Crop set on the trees remained low and six of the seven combinations dropped in yield production (20 kg to 40 kg per tree).

Portsgate performed well internally with juice levels on all seven of the combinations, above 53%. The highest juice was on CC (58%); Brix on C35 (13.1); and Brix:acid ratio on X639 (12.2%), with X639 also developing the

lowest acid (0.91%). Portsgate produced fruit size ranging from count 72 to 56 on the trees this season. There was a better yield production at the GFC trial site; the biggest crop was in combination with Single.

Table 5.5.16.2. Internal fruit quality data for Valencias on different rootstocks at Golden Frontiers Citrus, Hectorspruit on 28 July 2015.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Delta	C35	52.8	12.6	1.43	8.8	0.0	T1
Delta	CC	53.1	12.0	0.90	13.3	0.0	T1
Delta	KC	56.3	12.7	1.01	12.6	0.0	T1
Delta	MxT	57.3	11.8	1.35	8.7	0.0	T1
Delta	SC	57.6	10.7	1.55	6.9	0.0	T1
Delta	TB	52.7	11.5	0.97	11.9	0.0	T1
Delta	X639	53.2	11.8	1.36	8.7	0.0	T1
McClean SL	C35	53.0	12.8	1.46	8.8	0.0	T1
McClean SL	CC	56.8	11.8	0.97	12.2	0.0	T1
McClean SL	KC	55.7	11.3	0.96	11.8	0.0	T1
McClean SL	MxT	56.0	11.6	1.35	8.6	0.0	T1
McClean SL	SC	58.4	11.5	1.14	10.1	0.0	T1
McClean SL	TB	59.7	11.5	1.33	8.6	0.0	T1
McClean SL	X639	56.0	11.2	1.40	8.0	0.0	T1
Midknight	C35	54.4	13.1	1.41	9.3	0.0	T1
Midknight	CC	59.9	11.6	1.43	8.1	0.0	T1
Midknight	KC	55.1	10.3	1.24	8.3	0.0	T1
Midknight	MxT	54.9	11.0	1.34	8.2	0.0	T1
Midknight	SC	53.9	12.5	2.05	6.1	0.0	T1
Midknight	TB	53.5	12.5	1.10	11.4	0.0	T1
Midknight	X639	53.5	12.0	1.67	7.2	3.0	T1
Portsgate	C35	56.7	13.1	2.02	6.5	0.0	T1
Portsgate	CC	57.8	11.2	1.10	10.2	0.0	T1
Portsgate	KC	55.7	12.2	1.56	7.8	0.0	T1
Portsgate	MxT	55.1	10.9	1.01	10.8	0.0	T1
Portsgate	SC	53.5	10.5	1.76	6.0	0.0	T1
Portsgate	TB	52.4	10.2	2.24	4.6	0.0	T1
Portsgate	X639	56.6	11.1	0.91	12.2	0.0	T1

Table 5.5.16.3. Fruit size distribution per rootstock at Golden Frontiers Citrus, Hectorspruit on 28 July 2015.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	2.80	Midknight	C35	48	21.36
Delta	C35	56	24.18	Midknight	C35	56	27.92
Delta	C35	72	29.88	Midknight	C35	72	25.58
Delta	C35	88	18.96	Midknight	C35	88	13.79
Delta	C35	105/125	19.73	Midknight	C35	105/125	8.79
Delta	C35	144	4.45	Midknight	C35	144	2.56
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	CC	48	2.53	Midknight	CC	48	21.42
Delta	CC	56	15.18	Midknight	CC	56	33.94
Delta	CC	72	25.19	Midknight	CC	72	24.05
Delta	CC	88	23.05	Midknight	CC	88	12.36
Delta	CC	105/125	28.79	Midknight	CC	105/125	7.41
Delta	CC	144	5.25	Midknight	CC	144	0.82
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	KC	48	1.92	Midknight	KC	48	4.13
Delta	KC	56	23.56	Midknight	KC	56	11.91
Delta	KC	72	29.33	Midknight	KC	72	12.70
Delta	KC	88	20.79	Midknight	KC	88	18.80
Delta	KC	105/125	20.55	Midknight	KC	105/125	39.67
Delta	KC	144	3.85	Midknight	KC	144	12.80
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	MxT	48	5.06	Midknight	MxT	48	12.24
Delta	MxT	56	22.79	Midknight	MxT	56	26.53
Delta	MxT	72	24.55	Midknight	MxT	72	22.68
Delta	MxT	88	20.32	Midknight	MxT	88	16.55
Delta	MxT	105/125	23.12	Midknight	MxT	105/125	19.95
Delta	MxT	144	4.16	Midknight	MxT	144	2.04
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	SC	48	0.65	Midknight	SC	48	19.08
Delta	SC	56	9.93	Midknight	SC	56	35.02
Delta	SC	72	25.02	Midknight	SC	72	27.38
Delta	SC	88	29.10	Midknight	SC	88	12.57
Delta	SC	105/125	32.15	Midknight	SC	105/125	4.71
Delta	SC	144	3.15	Midknight	SC	144	1.23
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	TB	48	2.51	Midknight	TB	48	40.45
Delta	TB	56	23.14	Midknight	TB	56	32.93
Delta	TB	72	30.38	Midknight	TB	72	10.98
Delta	TB	88	22.66	Midknight	TB	88	9.55
Delta	TB	105/125	18.03	Midknight	TB	105/125	4.07
Delta	TB	144	3.28	Midknight	TB	144	2.03

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	1.92	Midknight	X639	48	12.19
Delta	X639	56	14.62	Midknight	X639	56	30.10
Delta	X639	72	28.13	Midknight	X639	72	24.96
Delta	X639	88	26.85	Midknight	X639	88	15.71
Delta	X639	105/125	24.58	Midknight	X639	105/125	13.80
Delta	X639	144	3.90	Midknight	X639	144	3.23
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	C35	48	12.86	Portsgate	C35	48	5.63
McClean SL	C35	56	35.22	Portsgate	C35	56	26.31
McClean SL	C35	72	24.64	Portsgate	C35	72	25.39
McClean SL	C35	88	15.99	Portsgate	C35	88	20.52
McClean SL	C35	105/125	9.13	Portsgate	C35	105/125	19.68
McClean SL	C35	144	2.16	Portsgate	C35	144	2.47
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	CC	48	6.70	Portsgate	CC	48	2.84
McClean SL	CC	56	26.72	Portsgate	CC	56	21.97
McClean SL	CC	72	30.41	Portsgate	CC	72	30.27
McClean SL	CC	88	19.85	Portsgate	CC	88	22.84
McClean SL	CC	105/125	15.03	Portsgate	CC	105/125	17.81
McClean SL	CC	144	1.29	Portsgate	CC	144	4.26
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	KC	48	5.05	Portsgate	KC	48	3.23
McClean SL	KC	56	23.95	Portsgate	KC	56	21.24
McClean SL	KC	72	30.20	Portsgate	KC	72	28.09
McClean SL	KC	88	22.93	Portsgate	KC	88	23.66
McClean SL	KC	105/125	15.91	Portsgate	KC	105/125	20.16
McClean SL	KC	144	1.97	Portsgate	KC	144	3.63
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	MxT	48	6.35	Portsgate	MxT	48	7.73
McClean SL	MxT	56	26.01	Portsgate	MxT	56	24.93
McClean SL	MxT	72	35.80	Portsgate	MxT	72	30.37
McClean SL	MxT	88	20.19	Portsgate	MxT	88	21.04
McClean SL	MxT	105/125	10.72	Portsgate	MxT	105/125	13.91
McClean SL	MxT	144	0.94	Portsgate	MxT	144	2.02
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	SC	48	1.91	Portsgate	SC	48	0.65
McClean SL	SC	56	15.44	Portsgate	SC	56	9.93
McClean SL	SC	72	31.48	Portsgate	SC	72	25.02
McClean SL	SC	88	28.00	Portsgate	SC	88	29.10
McClean SL	SC	105/125	21.53	Portsgate	SC	105/125	32.15
McClean SL	SC	144	1.64	Portsgate	SC	144	3.15
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	TB	48	13.25	Portsgate	TB	48	10.40

McClean SL	TB	56	39.34	Portsgate	TB	56	38.20
McClean SL	TB	72	28.99	Portsgate	TB	72	30.00
McClean SL	TB	88	11.90	Portsgate	TB	88	14.70
McClean SL	TB	105/125	4.35	Portsgate	TB	105/125	5.80
McClean SL	TB	144	2.17	Portsgate	TB	144	0.90
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	X639	48	2.11	Portsgate	X639	48	2.06
McClean SL	X639	56	15.56	Portsgate	X639	56	20.09
McClean SL	X639	72	28.72	Portsgate	X639	72	27.83
McClean SL	X639	88	26.43	Portsgate	X639	88	23.27
McClean SL	X639	105/125	23.06	Portsgate	X639	105/125	23.53
McClean SL	X639	144	4.12	Portsgate	X639	144	3.22

Table 5.5.16.4. Production per tree of Valencia selections on different rootstocks at Golden Frontiers Citrus, Hectorspruit on 28 July 2015.

Cultivar	Rootstock	Kg/tree								8 Year	8 Year
		2008	2009	2010	2011	2012	2013	2014	2015	Total	Mean
Delta	C35	60.1	45.6	51.7	37.6	50.5	53.8	40.2	43.4	382.9	47.9
Delta	CC	33.4	25.6	21.5	38.4	58.3	60.2	33.0	40.2	310.6	38.8
Delta	KC	38.5	26.2	10.4	32.6	49.0	30.6	25.7	34.6	247.6	31.0
Delta	MxT	39.4	40.0	6.4	13.8	48.4	73.8	82.8	64.1	368.7	46.1
Delta	SC	73.9	76.8	65.7	41.7	70.1	140.4	60.8	105.1	634.5	79.3
Delta	TB	34.9	30.5	32.3	31.0	44.4	87.8	40.6	43.6	345.1	43.1
Delta	X639	35.1	51.8	44.7	51.1	69.1	107.7	48.7	68.0	476.2	59.5
McClean SL	C35	81.0	64.3	32.5	46.3	46.1	69.0	26.0	39.3	404.5	50.6
McClean SL	CC	29.2	54.6	22.6	27.5	32.4	83.7	21.6	51.5	323.1	40.4
McClean SL	KC	24.7	58.3	21.6	38.8	46.7	90.3	19.4	50.4	350.2	43.8
McClean SL	MxT	19.4	26.6	10.1	4.5	16.1	71.8	49.0	43.1	240.6	30.1
McClean SL	SC	35.7	98.1	49.7	47.5	74.0	170.8	61.1	108.4	645.3	80.7
McClean SL	TB	46.9	39.4	49.6	37.2	55.5	57.6	36.0	47.2	369.4	46.2
McClean SL	X639	29.3	96.2	49.9	80.6	49.2	182.2	71.4	141.8	700.6	87.6
Midknight	C35	33.9	83.4	54.8	39.0	49.7	76.4	69.4	43.4	450.0	56.3
Midknight	CC	20.9	32.1	3.6	12.3	41.8	28.6	42.6	30.1	212.0	26.5
Midknight	KC	11.3	29.7	15.2	16.9	44.0	81.4	41.7	45.9	286.1	35.8
Midknight	MxT	8.2	27.0	1.2	8.6	30.3	20.3	46.7	19.7	162.0	20.3
Midknight	SC	13.8	57.7	8.1	30.7	53.0	95.9	53.3	44.2	356.7	44.6
Midknight	TB	19.6	53.7	14.3	15.5	36.6	56.0	52.7	26.3	274.7	34.3
Midknight	X639	5.2	17.9	3.3	13.0	36.3	68.7	34.5	31.1	210.0	26.3
Portsgate	C35	33.6	53.5	22.3	26.2	68.4	60.4	39.3	55.7	359.4	44.9
Portsgate	CC	26.6	31.3	9.6	4.2	50.9	35.0	32.7	38.2	228.5	28.6
Portsgate	KC	19.0	31.3	4.9	11.8	58.2	47.4	26.1	30.9	229.6	28.7
Portsgate	MxT	30.5	19.4	3.3	7.5	25.1	76.0	65.7	83.0	310.5	38.8
Portsgate	SC	55.2	44.0	19.9	15.3	106.0	89.2	62.7	105.1	497.4	62.2
Portsgate	TB	48.0	40.8	30.4	31.8	55.8	77.7	40.0	48.0	372.5	46.6
Portsgate	X639	35.6	73.0	37.1	73.1	91.7	96.9	53.4	91.0	551.8	69.0

5.5.17 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)**
Project 57D by S. Meeding (CRI)

Opsomming

Hierdie spesifieke Satsuma proef word bestempel as een van die beste Satsuma proewe in die land. Die proef se ligging is goed geskik vir Satsuma produksie. Die bome is ongeveer 10 jaar oud (geplant in 2006). Die 2015 seisoen was die laaste jaar van evaluasie. Die bome is afgesaag vir nuwe Satsuma seleksies. Die bome is volwasse met groot boom volume. Imamura het ontwikkel in die grootste boom in vergelyking met die ander seleksies. Owari en Dobashi Beni was meer kompakte bome wat kleiner was in boomgrootte. Dobashi Beni en Imamura het ‘n alternatiewe drag patroon, en Imamura set geen vrugte alternatief nie. In die 2014 seisoen het Imamura ‘n baie goeie drag gehad en Dobashi Beni het geen vrugte op die boom gehad nie. In die 2015 seisoen het Imamura geen vrugte op die boom gehad nie en Dobashi Beni het ‘n baie goeie drag gehad. Die orde van rypwording was as volg: Ueno, Aoshima op Carizzo citrange, Dobashi Beni, Ohtsu, Owari en Aoshima op Swingle citrange wat die seisoen eindig.

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 regarded as one of the best Satsuma trials in the country. The trial location is in an area well suited for Satsuma production. The trees are 10 years old (planted 2006). The 2015 season was the last year for evaluations. The trees were cut down to add new Satsuma selections to the trial. The trees are mature with large tree canopies. Imamura developed into the biggest tree compared to the other selections. Owari and Dobashi Beni were more compact trees with smaller tree size. Two of the selections, Dobashi Beni and Imamura had alternative bearing patterns with Imamura having the more serious bearing problem. In the 2014 season Imamura had an excellent crop with Dobashi Beni having no crop on the trees. In the 2015 season Imamura had no fruit on the trees and Dobashi Beni had a very good yield. The order of ripening was as follows; Ueno, Aoshima on Carizzo citrange, Dobashi Beni, Ohtsu, Owari and Aoshima on Swingle citrange ended 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 Late Satsuma selections from the Paarl region of the Western Cape. The following selections were evaluated: Aoshima, Dobashi Beni, Imamura, Ohtsu, Owari and Ueno.

Table 5.5.17.1. List of Satsuma selections evaluated at Lustigaan (Paarl) during 2015.

Selection	Rootstock	Planted
Aoshima	Carrizo/Swingle	2006
Dobashi Beni	Carrizo	2006
Imamura	Carrizo	2006
Ohtsu	Carrizo	2006
Owari	Carrizo	2006
Ueno	Carrizo	2006

Results and discussion

Aoshima

Aoshima was planted on two rootstocks at this trial site, Carrizo citrange and Swingle citrumello. The difference between the two rootstocks was clearly visible from a distance (tree size and vigour). Both rootstocks developed a good fruit size (count 1x) on the trees. Aoshima in combination with Swingle matured 3-4 weeks later compared to Carrizo and the rind texture was rough. Aoshima in combination with Carrizo was second to mature, and Aoshima on Swingle ended the Satsuma season. Aoshima on Swingle had an excellent juice percentage before peak maturity (61%), but the juice percentage came down to 52% at peak maturity. On both rootstocks there was a very good yield on the trees.

Dobashi Beni

Dobashi Beni had a very good yield after having no fruit on the trees in the 2014 season due to overcropping in 2013. The fruit had a very good internal juice percentage 57.4% at peak maturity. The selection also had good external colour development for a satsuma; colour plate (T3). Dobashi Beni is one of the selections with the best external rind colour after degreening. The rind has a deep orange colour.

Imamura

There was no crop on the trees for the 2015 season. The selection had a serious alternate bearing problem.

Ohtsu

Ohtsu was the fourth Satsuma selection to mature at the Paarl trial site. Ohtsu had a good fruit size this season and peaked from count 1-1xx (medium to large). The internal quality was good with one of the highest juice percentages (over 57%). Ohtsu is one of the selections with the best yields and medium to large fruit size year after year. The fruit has a smooth rind texture.

Owari

Owari is one of the older Satsuma selections at the trial site and was used as control. Owari, overall, had the smallest fruit, peaking at count 1 on average. Owari's fruit tends to get puffy before peak maturity. The fruit has a pronounced neck. Owari is one of the selections with the best external colour development and peaked at colour plate T3.

Ueno

Ueno was the first selection to mature this season. The tree canopy size was large with a heavy crop on the trees. Fruit size development remained above average ranging from count 1x-1xx, despite the heavy crop on the trees. Colour development was slightly delayed. Ueno had a low seed count with only 0.3 seeds per fruit. The selection has a very good yield year after year as well as very good average fruit size (1x-1xx).

For Satsuma mandarins, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which the fruit is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Conclusion

Dobashi Beni, Ohtsu and Ueno had the best fruit size (count 1xx) with Aoshima on Swingle and Owari having the best juice percentages: 61% and 58.2% respectively. Imamura was the only selection that had no yield on the trees. Aoshima on Swingle had the roughest rind texture, possibly due to the rootstock choice. Ueno had the highest Brix° of all the Satsuma selections (12.6°). Aoshima on Carizzo had the highest seed count with 0.7 seeds per fruit.

Table 5.5.17.2. Internal fruit quality data for Satsuma selections in the Paarl region (Lustigaan) of the Western Cape during the 2015 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/04/17	Aoshima	CC	1x	52.1	10.5	0.82	12.8	0.0	T6
2015/04/28	Aoshima	CC	1x	52.2	12.4	0.72	17.1	0.7	T4
2015/05/08	Aoshima	CC	1	53.4	11.6	0.91	12.8	0.1	T4
2015/04/17	Aoshima	SC	1x	61.0	9.4	0.95	9.9	0.2	T6
2015/04/28	Aoshima	SC	1x	52.7	11.6	1.01	11.5	0.2	T6
2015/05/08	Aoshima	SC	1x	52.0	11.1	0.96	11.5	0.3	T5
2015/04/17	Dobashi Beni	CC	1xx	57.4	10.4	0.88	11.9	0.2	T5
2015/04/28	Dobashi Beni	CC	1x	55.1	11.4	0.86	13.2	0.3	T5
2015/05/08	Dobashi Beni	CC	2	52.9	11.2	1.02	11.0	0.1	T3
2015/03/31	Ohtsu	CC	1	55.5	10.7	0.95	11.3	0.0	T6
2015/04/17	Ohtsu	CC	1x	54.5	10.7	0.86	12.4	0.1	T6
2015/04/28	Ohtsu	CC	1xx	55.1	11.8	0.80	14.7	0.1	T4
2015/05/08	Ohtsu	CC	1x	57.3	11.4	0.90	12.7	0.3	T4
2015/04/17	Owari	CC	1	58.2	10.3	1.01	10.2	0.0	T6
2015/04/28	Owari	CC	1	54.2	11.5	0.97	11.8	0.0	T5
2015/05/08	Owari	CC	1	55.1	11.4	0.92	12.5	0.1	T3
2015/04/17	Ueno	CC	1x	57.5	11.9	0.80	14.9	0.0	T5
2015/04/28	Ueno	CC	1xx	54.2	12.6	0.79	15.9	0.3	T5
2015/05/08	Ueno	CC	1x	54.5	11.6	0.90	12.9	0.1	T5

5.5.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)

Project 1000D by S. Meeding (CRI)

Opsomming

Die seisoen het begin met Clemenpons, gevolg deur Marisol, Nules en ge-eindig met Bonanules. In die 2015 seisoen was Marisol se rypwordingstyd voor Nules waar dit in die 2013 en 2014 seisoen andersom was. Daar was ‘n definitiewe vertraging in eksterne vrugkleur met ‘n kleurplaat T6 vir Clemenpons, Nules en Marisol. Bonanules het ‘n beter eksterne kleurontwikkeling met ‘n kleurplaat T5 en was die laaste seleksie om ryp te word.

Summary

The season started with Clemenpons, followed by Marisol, Nules and ended with Bonanules. In the 2015 season Marisol matured before Nules whereas in the 2013 and 2014 season, Nules matured before Marisol. There was a clear indication of a delay in external fruit colour with a colour plate T6 for Clemenpons, Nules and Marisol. Bonanules, being the last selection to mature, had a more promising colour development and peaked at colour plate T5.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Wellington region of the Western Cape; the planting age is unknown. The following varieties were evaluated: Bonanules, Clemenpons, Marisol and Nules.

Table 5.5.18.1. List of Clementine selections evaluated at Bonathaba (Wellington) during 2015.

Selection	Rootstock	Planted
Bonanules	Troyer	Unknown
Clemenpons	Carrizo	Unknown
Marisol	Troyer	Unknown
Nules	Troyer	Unknown

Results and discussion

Bonanules

Bonanules was last of the four Clementine selections to mature, but showed the best external colour development of all the selections (colour plate T5) (Table 5.5.18.2). The fruit has a thin rind and peels easily. The internal colour of the fruit was light in colour (dry) and some of the fruit was granulated, however, the Bonanules had the best juice percentage of all four selections (60.5%). The fruit was completely seedless, as expected when planted in a solid Clementine orchard.

Clemenpons

Clemenpons was the first selection to mature with the lowest acid percentage (0.81%) and also the lowest Brix (10.5°). The selection had the second highest juice percentage of 58.6%. Some of the fruit showed signs of granulation.

Marisol and Nules

Marisol and Nules are two older selections used as controls for Clementine trials. Both selections remained seedless. Marisol was second to mature with a good fruit size (count 1) and Nules had the smallest fruit of all 4 selections at this trial site (count 3). The colour development on both selections was bad (colour plate T6).

Conclusion

All the selections evaluated were seedless at this trial, with a serious delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Marisol had the highest Brix (11.4°). Nules had the smallest fruit size and peaked at count 3. All four selections showed signs of granulation that can be a result of the windbreak directly next to the trial trees. All four selections had a very good juice percentage: all over 50%, with Bonanules the highest (60.5%) and Clemenpons second highest (58.6%).

Table 5.5.18.2. Internal fruit quality data for Clementine selections in the Wellington region (Bonathaba) of the Western Cape during the 2015 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/03/31	Bonanules	TC	1	60.5	11.0	0.96	11.4	0	T5
2015/03/31	Clemenpons	TC	1	58.6	10.5	0.81	12.9	0	T6
2015/03/31	Marisol	TC	1	55.3	11.4	0.91	12.5	0	T6
2015/03/31	Nules	TC	3	53.2	11.1	0.94	11.8	0	T6

5.5.19 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)**
Project 997A by S. Meeding and Z. Zondi (CRI)

Opsomming

Die 2015 seisoen was die tweede oes jaar vir hierdie mandaryn proef in die Oos-Kaap Middelande. Weens die swaar drag van die 2014 seisoen het al vyf van die UCR seleksies tekens van alternatiewe drag getoon. Die drie seleksies wat die swakste drag gehad het was Tahoe Gold, Shasta Gold, en Yosemite Gold. Op hierdie drie seleksies was daar min tot geen vrugte. Tango en Gold Nugget was ook geaffekteer deur 2014 se swaar drag, maar die bome het nog steeds ‘n aanvaarbare tot goeie drag gehad in vergelyking met die boomgrootte.

Summary

The 2015 season was the second harvest for this Mandarin trial in the East Cape Midlands area. Due to a very heavy crop in the 2014 season, all five of the UCR selections showed a tendancy towards alternate bearing. The three selections that were effected most were Tahoe Gold, Shasta Gold and Yosemite Gold. On these three selections there was little to no fruit on the trees. Tango and Gold Nugget were also affected but both selections still had a fair to good yield on the trees compared to the tree size.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Cookhouse and Fort Beaufort region of the East Cape Midlands. A range of new mandarin hybrids have been added to this area and should be bearing fruit in the 2015 season. 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 5.5.19.1. List of mandarin hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2015 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Yosemite Gold	CC	2010

Results and discussion

Gold Nugget
Gold Nugget was the second selection to mature with a Brix of 14.8° and the Acid still being at 1.07%. This indicates that the fruit will likely have an extended shelf life. The selection had a low seed count with four of the five samples being seedless and only one sample having an average of 0.3 seeds per fruit. Gold Nugget has a very upright growth habbit. The fruit higher up in the tree are much coarser compared to the rest, but the fruit texture tends to become smoother as the tree ages. The external colour of the fruit was yellow to dark

yellow. In the 2015 season the trees had a fair crop due to over cropping in the 2014 season. Some of the fruit has stylar-end split of about 3-5 mm that turns black as the scar callus forms. In the warmer areas the scar will heal and will not become black. The selection had good fruit size with counts from 1-1xx.

Nadorcott

Nadorcott is used as a control for the mandarin varieties, and more specifically Tango. Nadorcott developed the second smallest fruit size (count 1) for this trial site. The selection was not seedless with a seed count of 0.6 seeds per fruit. There was a good colour development (T1) at peak maturity. The selection also had a problem with acids remaining high.

Shasta Gold

Shasta Gold was the fourth selection to mature. The selection had a high Brix of 13.3° when the acid was still at 1.24% (late maturing selection). There was little to no fruit on the trees due to the over cropping in the 2014 season. Fruit was coarse and also develop ribbing. Shasta Gold had the best colour development of all the selections at the trial site and peaked at colour plate T1 well before peak maturity was reached. The selection had the lowest internal juice content of 46.9%. This could be due to the fact that there was a light crop on the trees and with fruit counts being 1xx-1xxx, fruit can granulate.

Tahoe Gold

Tahoe Gold was the first selection to mature with a Brix:acid ratio of 14:1. The selection matured a month later than normal. This could be because the East Cape Midlands is one of South Africa's coldest citrus production areas. The selection had a very light crop on the trees due to over cropping in the 2014 season. The fruit was already puffy at full maturity The selection had the second highest seed count with an average of 0.6 seeds per fruit. Tahoe Gold had a very high juice content (above 58%) and excellent fruit size count of 1x-1xxx. The flesh of the fruit has a very fine texture that improves the eating quality of the fruit. Tahoe Gold developed the smallest tree size of all five selections with a round shape and very dense canopy.

Tango

Tango was the last selection to mature due to a high acid percentage. In the colder production areas, acids tend to drop more slowly compared to the hotter production areas. The selection had a better yield than the rest of the selections but had the smallest fruit size and peaked at count 2 to count 1. External colour development was very good with a deep orange rind colour. Tango had the highest juice levels above 60%.

Yosemite Gold

Yosemite Gold was the third selection to mature with a very good external colour development. The fruit was smooth with a deep red rind colour. This selection is prone to alternate bearing with little to no fruit in the off year. The 2015 season was the off season for Yosemite Gold with just a few fruit on the trees and large fruit size, peaking from count 1xx to 1xxx. The selection developed a very large tree with thorns. The thorns on the bearing branches were small to none with bigger thorns on the stronger growing shoots. The selection had a low seed count with 0.7 seeds per fruit. Juice percentages were good and tested above 54%.

Conclusion

In the previous season (2014), Gold Nugget and Tango showed a better resistance against freezing cold temperatures with minimum damage on the trees and the fruit. In this season (2015), both these selections also showed that it is not that prone to alternate bearing. Yosemite- and Shasta Gold is the two selections with the largest fruit size (1xxx) and also the best colour development. The fruit had a deep red rind colour. Gold Nugget is likely to have a better shelf life because of the selections high Brix: Acid ratio. Tahoe Gold, Nadorcott and Tango had the best juice percentages (58.7%, 58.9% and 60.8%). Yosemite Gold had the highest number of seeds per fruit (0.7).

Table 5.5.19.2. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2015 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/05/18	Golden Nugget	CC	1x	50.3	9.8	1.51	6.5	0	T7
2015/05/18	Golden Nugget	CC	1	50.9	9.8	1.86	5.3	0	T7
2015/06/23	Golden Nugget	CC	1xx	52.6	11.9	1.22	9.8	0	T4-5
2015/07/06	Golden Nugget	CC	1xx	53.2	11.7	1.33	8.8	0.3	T2-3
2015/08/14	Golden Nugget	CC	1x	53.5	14.8	1.07	13.8	0	T1
2015/05/18	Nadorcott	CC	1x	54.5	9.5	1.49	6.4	0	T6
2015/06/23	Nadorcott	CC	1	56.9	11.6	1.21	9.6	0	T3
2015/07/06	Nadorcott	CC	1	58.9	11.0	1.28	8.6	0.6	T1
2015/05/18	Shasta Gold	CC	1xxx	53.6	8.8	1.76	5.0	0.4	T5-6
2015/05/18	Shasta Gold	CC	1xx	52.9	9.2	2.01	4.6	0	T6
2015/06/23	Shasta Gold	CC	1xxx	46.9	9.5	1.10	8.6	0	T1
2015/07/06	Shasta Gold	CC	1xxx	49.7	11.1	1.35	8.2	0.5	T1
2015/08/14	Shasta Gold	CC	1xx	51.7	13.3	1.24	10.7	0.6	T1
2015/05/18	Tahoe Gold	CC	1xx	57.0	8.6	1.27	6.8	0	T6-7
2015/05/18	Tahoe Gold	CC	1xx	55.8	9.4	1.58	5.9	0.6	T6-7
2015/06/23	Tahoe Gold	CC	1x	52.6	10.7	1.06	10.1	0	T2-3
2015/07/06	Tahoe Gold	CC	1xxx	58.7	10.5	1.10	9.5	0.4	T1-2
2015/08/14	Tahoe Gold	CC	1xx	50.1	11.7	0.83	14.1	0.3	T1
2015/05/18	Tango	CC	1	55.3	9.3	1.46	6.4	0	T6
2015/05/18	Tango	CC	1	54.6%	9.4	1.81	5.2	0	T6-7
2015/06/23	Tango	CC	1	56.8%	11.3	1.25	9.0	0	T3
2015/07/06	Tango	CC	2	60.8%	11.2	1.27	8.8	0	T1
2015/05/18	Yosemite Gold	CC	1xxx	54.9%	8.7	1.51	5.8	0	T7
2015/05/18	Yosemite Gold	CC	1xx	53.1%	9.1	1.87	4.9	0	T7
2015/06/23	Yosemite Gold	CC	1xxx	54.3%	10.4	1.12	9.3	0	T4-5
2015/07/06	Yosemite Gold	CC	1xxx	55.4%	10.9	1.30	8.4	0.5	T3-4
2015/08/14	Yosemite Gold	CC	1xxx	53.1%	12.0	1.01	11.9	0.7	T1

5.5.20 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)**
Project 997B by S. Meeding and Z. Zondi (CRI)

Opsomming

Die mandaryn proef is opgedeel in twee verskillende proef persele. African Sunset (B24), Nova en Nova ARC is in Addo geplant en is ongeveer 20 km vanaf Kirkwood, maar vorm steeds deel van die Sondagsrivier Vallei. Nova word gebruik as ʼn kontrole vir die vroeë-mid mandaryn seleksies. Beide Nova ARC en African Sunset (B24) was deur die LNR in Addo ontwikkel. Nova ARC is steeds in die eksperimentele fase waar African Sunset (B24) op ʼn groter skaal aangeplant word.

Die 2015 seisoen was die UCR 5 seleksies se derde oes op die bome by hierdie proefperseël. Die Yosemite Gold en Tahoe Gold bome moes herplant word weens die pakhuis wat verleng was, en nie al die bome het die herplant oorleef nie. Furr word as kontrole gebruik in hierdie mandaryn proef. Hierdie proefperseël is ook geleë in die Kirkwood area.

Summary

The mandarin trial is divided into two different trial sites. The African Sunset (B24), Nova and Nova ARC were planted in Addo about 20 km from Kirkwood, also part of the Sundays River Valley. Nova was used as a control for the early-mid mandarin selections. Both the Nova ARC and the African Sunset (B24) were developed by the ARC in Addo. Nova ARC is still in the experimental phase whereas African Sunset (B24) has been planted on a larger scale.

In the 2015 season, the UCR 5 selections produced their third crop at this trial site. The Yosemite Gold and Tahoe Gold trees were relocated due to the packhouse being extended. Some of the trees did not survive the transplant. Furr was used as a control for this mandarin trial. This trial is also situated in the Kirkwood area.

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 had been added to this area and were bearing fruit in the 2015 season. The following varieties were evaluated: Furr, Gold Nugget, Shasta Gold, Tahoe Gold, Tango, Yosemite Gold, African Sunset (B24), Nova and Nova ARC.

Table 5.5.20.1. List of Mandarin hybrid selections evaluated in the Sundays River Valley (H. Ehlers) region during the 2015 season.

Selection	Rootstock	Planted
Furr (Clemcott)	Carrizo	2004

Selection	Rootstock	Topwork
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Yosemite Gold	Carrizo	2011
Tango	Carrizo	2011

Table 5.5.20.2. List of Mandarin hybrid selections evaluated in the Sundays River Valley (Penhill) region during the 2015 season.

Selection	Rootstock	Planted
African Sunset (B24)	Carrizo	2011
Nova	Carrizo	2011
Nova ARC	Carrizo	2011

Results and discussion

<u>African Sunset (B24)</u>
The selection had a poor yield with very large fruit size (1xx-1xxx) on the trees. There were a number of out of season fruit on the trees that was typical in the area for the 2015 season. Fluctuating temperatures during the flowering period can cause trees to set out-of-season fruit. The fruit was completely seedless this season. The fruit had a low Brix and did not even reach 10° at peak maturity.

<u>Nova</u>
Nova is a well known commercial selection used as a control in this mandarin trial. The selection developed a

deep intense red rind colour. Nova is known to peel with a bit more difficulty compared to the other easy peelers. The selection had a low seed count of 0.3 seeds per fruit on average.

Nova ARC

Nova ARC is an experimental selection. The selection was evaluated from a semi-commercial orchard. The trees had a very good yield with fruit size ranging from count 1-1x. Tree size was very small compared to the normal Nova selection. The trees also suffer from “die-back” and gumming on the tree stem. Internally the fruit developed a good juice percentage (over 55%).

Furr (Clemcott)

Furr is a Clementine x Murcott cross and was used as a control for the mandarin trial site. The tree size is large (aggressive growth pattern) and has consistently cropped a good yield every production year. In the 2015 season, Citrus Scab was identified as the cause of some external problems on the fruit rind. Furr has a very distinct flavor and produces a very good eating quality fruit. The selection had a high seed count of 8.1 seeds per fruit. Fruit size was extra large (1xxx) with a high juice percentage (above 55%). Furr had a very good colour development with a colour plate T1 at peak maturity.

Gold Nugget

Gold Nugget cropped a fair to good yield this year for the tree age and size, after a very good yield in the 2014 season. The fruit rind was coarse, but will get smoother as the tree matures. There was light ribbing and sunburn present on some of the fruit. The Gold Nugget trees have a very upright growth habit. Some of the fruit (less than 5%) started to split on the stylar-end. In the colder areas, the area around the split at the fruit end turns black. Due to these lengthy upright shoots many of the trees will break when the shoots bear a heavy crop on the trees. The selection had good fruit size with counts ranging from1x-1xx.

Shasta Gold

Shasta Gold produced a good yield and fruit size for the young tree age. The fruit developed some ribbing but will become smoother as the trees mature. The Shasta fruit shape was fairly flat and fruit size peaked at count 1xxx. Shasta Gold has a very good external colour development (deep orange-red) and colour development of the fruit was at colour plate T1 long before peak maturity. The selection had very good juice percentages with over 57%. Shasta Gold was completely seedless this season.

Tahoe Gold

Tahoe Gold developed a smaller tree volume compared to the other five UCR selections evaluated. Trees were transplanted at 3 years old and some did not survive. The surviving trees are still catching up with the rest of the selections. Tahoe Gold tends to have larger fruit in the first 2 years of production. Thereafter the fruit size stabilizes to a smaller, but still very good size, due to the heavier crop. The selection had a low seed count of just 0.3 seeds per fruit on average. The fruit tends to get puffy before peak maturity is reached. The juice percentage is high with over 60%.

Tango

Tango had a very good crop on the trees. Fruit shape was flat and had a very smooth rind with good external colour development. The selection was completely eedless with high juice percentages (over 60%) at peak maturity. The fruit size peaked at count 1 to 1x. Tango has a very upright growth habit.

Yosemite Gold

Yosemite Gold has good external colour development and fruit was fully coloured before peak maturity was reached. The fruit peels easily. The selection had a better yield compared to the 2014 season which could be due to the fact that the replanted trees are maturing in age. The selection had a good juice percentage with over 55% close to peak maturity. Yosemite Gold was seedless until the last evaluation when the selection had 0.3 seeds per fruit on average. Yosemite Gold normally has the largest tree of all the five UCR selections but due to replanting, these trees became stunted.

Conclusion

Nova ARC had a very good yield and the tree size was small compared to the standard Nova selection (control). The selection peels like a Nova (a bit difficult and messy). Nova ARC is prone to gumming and also “die back”. The selection had the highest seed count of 0.7 seeds per fruit for this trial. African Sunset (B24) had a few out-of-season fruit, which was a problem in the area and could be due to untimely cold weather during flower and fruit set. The selection had a poor yield with very large fruit (count 1xxx). A characteristic of the African Sunset is the small “navel like” stylar-end (protruding navel-end).

Yosemite Gold, Shasta Gold and Furr had the largest fruit size (count 1xxx). All the selections showed very good external colour development and were fully coloured at peak maturity. Furr developed the highest seed numbers with 8.1 seeds per fruit. Furr, Tango and Gold Nugget had the highest Brix of 13.1, 13.2 and 13.4 respectively. Tahoe Gold tends to get puffy before peak maturity. Shasta Gold has ribbing on the fruit which decreases as the tree matures.

Table 5.5.20.3. Internal fruit quality data for Mandarin hybrid selections from various regions of the Sundays River Valley (Penhill) during the 2015 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/05/07	African Sunset (B24)	CC	1xx	53.4	8.3	1.08	7.7	0	T7
2015/06/08	African Sunset (B24)	CC	1xxx	53.1	9.0	0.90	10.0	0	T4
2015/04/22	Nova	CC	1x	58.8	10.7	0.87	12.3	0.3	T4
2015/05/07	Nova	CC	1x	56.0	10.5	0.84	12.5	0	T1-2
2015/06/08	Nova	CC	1xx	52.3	11.5	0.69	16.7	0	T1
2015/04/22	Nova ARC	CC	1x	55.7	9.1	0.79	11.5	0.7	T4
2015/05/07	Nova ARC	CC	1	56.7	11.0	0.82	13.4	0.3	T1-2

Table 5.5.20.4. Internal fruit quality data for Mandarin hybrid selections from various regions of the Sundays River Valley (H. Ehlers) during the 2015 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/06/16	Furr	CC	1xxx	56.8	12.6	0.93	13.5	5.8	T2-3
2015/07/07	Furr	CC	1xxx	57.9	13.1	1.09	12.0	8.1	T1
2015/06/02	Golden Nugget	CC	1xx	50.4	10.3	1.40	7.4	0	T7
2015/06/16	Golden Nugget	CC	1xx	57.5	10.7	1.18	9.1	0	T5
2015/07/16	Golden Nugget	CC	1x	54.7	11.2	0.98	11.4	0	T2
2015/07/28	Golden Nugget	CC	1xx	48.6	11.8	1.07	11.0	0	T1
2015/08/12	Golden Nugget	CC	1xx	54.8	13.2	0.90	14.7	0	T1
2015/05/20	Shasta Gold	CC	1xxx	54.9	9.7	1.58	6.1	0	T6
2015/06/02	Shasta Gold	CC	1xxx	58.5	10.5	1.33	7.9	0	T1
2015/06/16	Shasta Gold	CC	1xxx	57.6	10.7	1.36	7.9	0	T1
2015/07/16	Shasta Gold	CC	1xxx	57.1	10.6	1.16	9.1	0	T1
2015/07/28	Shasta Gold	CC	1xxx	58.7	11.0	1.06	10.3	0	T1
2015/08/12	Shasta Gold	CC	1xxx	57.8	12.2	1.04	11.7	0	T1
2015/08/20	Shasta Gold	CC	1xxx	59.8	12.2	1.12	10.9	0	T1
2015/05/20	Tahoe Gold	CC	1xx	60.9	9.9	1.43	8.8	0	T7
2015/06/02	Tahoe Gold	CC	1xxx	63.1	9.8	1.10	8.9	0	T5-6
2015/06/16	Tahoe Gold	CC	1xx	59.1	10.0	1.65	6.1	0	T4-5
2015/07/16	Tahoe Gold	CC	1	58.8	10.4	0.90	11.6	0.3	T1
2015/07/28	Tahoe Gold	CC	1xxx	54.9	11.8	0.75	15.7	0	T1
2015/08/12	Tahoe Gold	CC	1x	61.8	12.9	0.80	16.1	0	T1
2015/05/20	Tango	CC	1x	55.8	9.7	1.06	9.2	0	T6
2015/06/02	Tango	CC	1	55.2	10.7	0.91	11.8	0	T3
2015/06/16	Tango	CC	1	63.1	12.0	1.23	9.8	0	T2-3
2015/07/16	Tango	CC	1	65.0	12.8	1.17	10.9	0	T1
2015/07/28	Tango	CC	1x	61.2	12.3	1.02	12.1	0	T1
2015/08/12	Tango	CC	1	58.6	13.3	0.80	16.6	0	T1
2015/08/20	Tango	CC	1	61.2	13.4	0.83	16.0	0	T1
2015/05/20	Yosemite Gold	CC	1xx	49.7	8.1	1.76	4.6	0	T8
2015/06/02	Yosemite Gold	CC	1xx	53.1	8.6	1.59	5.4	0	T8
2015/06/16	Yosemite Gold	CC	1xxx	56.2	8.9	1.43	6.2	0	T7
2015/07/16	Yosemite Gold	CC	1xxx	56.6	9.4	1.20	7.8	0	T3-4
2015/07/28	Yosemite Gold	CC	1xxx	55.5	10.1	1.13	8.9	0	T1
2015/08/12	Yosemite Gold	CC	1xxx	56.6	11.7	1.07	10.9	0.3	T1

5.5.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)

Project 997C by S. Meeding and Z. Zondi (CRI)

Opsomming

Die hoof proefperseël is in die Patensie area en dan is daar ook ‘n bystand perseël in die Hankey area. Beide die proef persele maak deel uit van die Gamtoosrivier Vallei. In beide persele het al die URC 5 seleksies dieselfde interne en eksterne eienskappe. In Patensie het die seisoen begin met Tango gevolg deur Tahoe

Gold, Nadorcott, Gold Nugget, Yosemite Gold en die seisoen word afgesluit met Shasta Gold. Yosemite Gold, Shasta Gold en Tahoe Gold het in 2014 ŉ baie swaar oes op die bome gehad by die Hankey perseel, wat ŉ ligte set vir 2015 tot gevolg gehad het. Gold Nugget was die minste geaffekteer deur die alternatiewe drag patroon met ‘n goeie set vir die 2015 seisoen.

Summary

There is a main trial site in the Patensie area, as well as a back-up site in the Hankey area. Both trial sites form part of the Gamtoos River Valley. In both sites all the UCR 5 selections have the same internal and external characteristics. In Patensie the season started with Tango followed by Tahoe Gold, Nadorcott, Gold Nugget, Yosemite Gold, and the season ended off with Shasta Gold. Due to a very heavy crop in 2014 at theHankey site, and this being the first crop on the trees at the other site, Yosemite Gold, Shasta Gold and Tahoe Gold had a very poor crop to no fruit on the trees. Gold Nugget was least affected by alternate bearing pattern.

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 and were bearing fruit in the 2015 season. The following varieties were evaluated: Shasta Gold, Tahoe Gold and Yosemite Gold, as well as Gold Nugget and Tango.

Table 5.5.21.1. List of experimental mandarin hybrid selections evaluated in the Patensie (L. Ferreira) region of the Gamtoos River Valley during the 2015 season.

Selection	Rootstock	Topwork
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Tango	Carrizo	2011
Yosemite Gold	Carrizo	2011

Results and discussion

Gold Nugget
Gold Nugget performed similarly to the other production areas. The trees have a very upright growth habit; the fruit higher up in the trees tends to be coarser than fruit lower down. Some fruit shows signs of ribbing. Trees produced a fair to good yield although tree manipulation (pruning) is necessary to prevent aggressive growth patterns. Gold Nugget was fourth to mature (end of July to end of August) after Tango, Tahoe Gold and Nadorcott. The selection had 0.3 seeds per fruit in just one sample. The remaining five samples were seedless. The selection has high Brix° and Acid (%) ratios with good colour development. The external colour is a deep yellow colour and internal juice percentages were over 51%.

Shasta Gold
Shasta Gold ended off the mandarin season. The fruit was completely seedless with excellent colour development (colour plate T1) 4-6 weeks before peak maturity. Trees with a heavier crop had smaller fruit with a smoother rind texture compared to the trees with a lighter crop. There was ribbing on most of the fruit. Shasta Gold developed a good juice percentage, well over 50% this season. The selection had extra large fruit size and peaked at count 1xxx.

Tahoe Gold
Tahoe Gold developed into a smaller, more compact tree. The trees bore a heavy crop and the grower had to

support the branches to prevent any damage to bearing branches. Despite the heavy crop on the trees, fruit size remained at count 1xxx. External colour development was very good this season (deep orange). The rind is puffy before peak maturity is reached. Tahoe Gold had the best juice percentages with over 60% and the selection was completely seedless.

Tango

Tango, similar to Gold Nugget, developed a very upright growth habit (V-shape tree). Tango sets a heavy crop on the trees with good fruit size ranging from count 1 -1xx. The rind texture was smooth with a deep orange external colour (natural wax shine). Out of the six samples that were evaluated, only one sampled had 0.5 seeds per fruit on average. The selection had very good external colour development and the fruit was fully coloured before peak maturity.

Yosemite Gold

Yosemite Gold grows vigorously and developed the largest tree volume of the UCR 5 varieties. There was a problem with fruit set on the trees this season. Some trees had very good yields while other trees had a poor yield. This selection is affected the most by alternate bearing patterns. The fruit is very firm with a good fruit size peaking at count 1xxx. The fruit rind is thin and peels easily and cleanly. The seed count on this selection was lower than in the 2014 season with only 0.4 seeds per fruit. Yosemite Gold developed an exceptional rind colour (deep red).

Conclusion

All the selections had a very good external colour development and were at colour plate T1 at peak maturity. Tango, Shasta Gold and Yosemite Gold had the best rind colour. Tango developed a deep orange rind colour with a natural shine where Yosemite Gold and Shasta Gold have the deepest red rind colour. Gold Nugget had the highest Brix (13.6°) as well as very high Brix:acid ratio, as did Tango, which should improve shelf life. Gold Nugget, Shasta Gold, Yosemite- Gold and Tahoe Gold had the largest fruit size (count 1xxx). Tahoe Gold and Nadorcott had the highest juice percentages (over 60%). Tango and Nadorcott had the smallest fruit size (count 1).

Table 5.5.21.2. Internal fruit quality data for experimental mandarin hybrid selections from the Patensie (L. Ferreira) region of the Gamtoos River Valley region during the 2015 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/05/14	Golden Nugget	CC	1x	51.4	9.6	1.49	6.4	0.3	T7
2015/06/03	Golden Nugget	CC	1xx	51.3	9.9	0.99	10.0	0	T6
2015/08/04	Golden Nugget	CC	1x	52.1	12.6	0.90	14.0	0	T1
2015/08/19	Golden Nugget	CC	1xx	54.7	13.6	0.79	17.2	0	T1
2015/09/22	Golden Nugget	CC	1x	57.0	12.1	0.63	19.2	0	T1
2015/09/22	Golden Nugget	CC	1xxx	47.9	12.5	0.68	18.4	0	T1
2015/05/14	Nadorcott	CC	1	52.9	9.7	1.16	8.4	0	T6
2015/05/28	Nadorcott	CC	1	57.0	10.1	1.46	6.9	0	T3-4
2015/06/03	Nadorcott	CC	1x	57.0	10.1	1.09	9.3	0	T4
2015/06/25	Nadorcott	CC	1x	56.4	10.1	0.96	10.5	0.8	T1
2015/07/09	Nadorcott	CC	1	64.0	11.3	1.16	9.7	0.3	T2
2015/08/04	Nadorcott	CC	1	58.9	12.3	0.94	13.1	0.3	T1
2015/05/14	Shasta Gold	CC	1xxx	57.2	9.9	1.44	6.9	0	T6
2015/06/03	Shasta Gold	CC	1xxx	57.2	10.3	1.45	7.1	0	T3-4
2015/06/25	Shasta Gold	CC	1xxx	59.1	9.7	1.14	8.5	0	T1
2015/08/04	Shasta Gold	CC	1xxx	50.9	11.8	0.96	12.3	0	T1
2015/05/14	Tahoe Gold	CC	1xxx	59.9	9.0	1.20	7.5	0	T7-8
2015/06/03	Tahoe Gold	CC	1xxx	60.8	9.6	0.86	11.2	0	T4
2015/06/25	Tahoe Gold	CC	1xxx	61.7	9.3	0.81	11.5	0	T1
2015/05/14	Tango	CC	1xx	55.7	9.4	1.01	9.3	0.5	T6
2015/05/28	Tango	CC	1x	53.0	9.5	0.94	10.1	0	T2-3
2015/06/03	Tango	CC	1x	57.1	11.1	1.02	10.9	0	T3-4
2015/06/25	Tango	CC	1	52.0	10.2	0.87	11.7	0	T1
2015/07/09	Tango	CC	1	56.9	11.8	0.70	16.9	0	T1
2015/08/04	Tango	CC	1	52.6	10.8	0.68	15.9	0	T1
2015/05/14	Yosemite Gold	CC	1xxx	56.3	8.7	1.49	5.8	0	T7
2015/06/03	Yosemite Gold	CC	1xxx	59.1	9.4	1.22	7.7	0	T4-5
2015/06/25	Yosemite Gold	CC	1xxx	54.4	9.2	1.13	8.1	0	T1
2015/08/04	Yosemite Gold	CC	1xxx	56.6	11.7	0.91	12.9	0.4	T1
2015/08/18	Yosemite Gold	CC	1xxx	57.1	11.9	0.84	14.2	0	T1

5.5.22 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (Western Cape)**
Project 997D by S. Meeding (CRI)

Opsomming

Die proef perseël in die Paarl wat hier bespreek word, is een van die oudste persele in die land wat Tahoe Gold, Gold Nugget, Shasta Gold en Yosemite Gold ingesluit het. Die bome is volwasse en groeikragtig, produseer goeie kwaliteit vrugte asook goeie vruggrootte. In die Wes-Kaap se proef persele het Tahoe Gold die kleinste boom met ŉ baie swaar drag gelewer. Yosemite Gold het die grootste boom van die vier seleksies, maar het ŉ baie ernstige alternatiewe drag probleem. Soos die Shasta Gold bome volwasse raak verminder die persentasie ribbing op die vrugte. Gold Nugget set ŉ groot aantal vrugte en die lang raamtakke breek sonder ondersteuning. Tahoe Gold se skil raak powwerig kort na optimal rypheid wat dan vrugval veroorsaak.

Die proef perseël in Citrusdal bevat meeste van die nuwe eksperimentele seleksies van vroeg tot laat rypwordend. Die kruisbestuiwing in hierdie proef perseël is baie hoog weens al die verskillende seleksies teenwoordig.

Summary

The Paarl trial site discussed here is one of the oldest sites in the country with plantings of Gold Nugget, Tahoe Gold, Shasta Gold and Yosemite Gold. The trees are mature and vigorous, producing very good fruit quality and fruit size. In the Western Cape trial sites, Tahoe Gold developed the smallest tree with a very heavy crop load. Yosemite Gold developed the largest tree of the four selections with a serious alternate bearing problem. On mature Shasta Gold trees, the ribbing on the fruit tends to decrease. Gold Nugget sets a heavy crop which may cause the long bearing branchesto break. Tahoe Gold becomes puffy soon after peak maturity and fruit starts to drop from the trees.

The tial site in Citrusdal is 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.

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, Porterville and Paarl region of the Western Cape.

Table 5.5.22.1. List of experimental mandarin hybrid selections evaluated in the Paarl region of the Western Cape during the 2015 season.

Selection	Rootstock	Planted
Tahoe Gold	CC	2008
Shasta Gold	CC	2008
Yosemite Gold	CC	2008
Gold Nugget	CC	2008

Table 5.5.22.2. List of experimental mandarin hybrid selections evaluated in the Porterville region of the Western Cape during the 2015 season.

Selection	Rootstock	Topwork
Tahoe Gold	CC	2010
Shasta Gold	CC	2010
Yosemite Gold	CC	2010
Gold Nugget	CC	2010
Tango	CC	2010

Table 5.5.22.3. List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2015 season.

Selection	Rootstock	Topwork	Planted
African Sunset (B24)	CC		2009
Furr (Clemcott)	CC	2011	
Golden Nugget	CC	2010	
Hadas	CC	2011	
I22	CC		2009
IRM 2	CC	2010	
Mor 26	CC		Unsure
Nadorcott	CC		2009
Nadorcott ARC	CC	2010	
Nadorcott SL	CC	2010	
Nova ARC	CC		Unsure
Or 4	CC		Unsure
Shasta Gold	CC	2010	
Sonet	CC		2009
Sonet 2	CC	2011	
Tahoe Gold	CC	2010	
Tango	CC	2010	
Tanor late	CC	2012	
Winola	CC		2009

Results and discussion

African Sunset (B24)
African Sunset had a very poor crop on the trees and the fruit size was extra large (1xxx) as result of this. Colour development was very good (T1) before peak maturity. The larger fruit had a coarser rind compared to the smaller fruit. Internal juice percentages were low (44.9%) close to peak maturity and the selection was completely seedless.

Furr (Clemcott)
Furr is used as a control for the mid-maturing mandarin selections. The selection had a very good rind colour development (deep orange) as well as very good juice percentages (above 55%). Furr peels easily and has very good eating quality. The selection had the third highest Brix (13.0) for this trial. Due to the high cross pollination Furr produced the most seed: 16.3 seeds per fruit on average. The trees had a very good yield.

Gold Nugget
In the first two years Gold Nugget tends to have a coarse rind. Fruit higher up in the trees tend to develop a coarser rind texture compared to fruit lower down. After Year 2 the fruit rind becomes smoother, but will never be as smooth as Tango or Nadorcott. 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 13.7 in Porterville. 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 sets a heavy crop on the trees every year. The fruit develops a deep yellow external colour.

Hadas
Hadas is a late maturing mandarin with very high acid percentages. The acid was still 1.60 in the second week of August and the fruit was already fully coloured. The external rind colour is a light yellow colour with a smooth rind texture. The selection developed 2.0 seeds per fruit on average. Hadas had a very good yield for the medium tree size.

IRM 2
The IRM 2 is one of two irradiated Murcott selections from Australia. The selection is low seeded with good internal and external colour. IRM 2 developed some ribbing on the fruit, but less compared to IRM 1. The fruit is firm and peels easily and cleanly. The fruit has a very good eating quality with a good Brix:acid ratio (14.0). In the 2015 season there was a light crop on the trees, and the trees have a very upright growth habit.

Mor 26
Mor 26 is a late mandarin selection that is currently available for commercial plantings, and has ribbing on the fruit. The fruit was very firm with a thin rind and colour development was very good (T1) at peak maturity. The selection is prone to alternate bearing patterns so crop manipulation is advisable. There was a poor crop on the trees in the 2015 season. The internal juice percentage was above 50%, Brix was very high (16.0°) with good acid levels above 1.10%. The fruit was of high eating quality and fruit size peaked at count 1. Despite heavy cross pollination, the selection only had 1.5 seeds per fruit on average.

Nadorcott ARC & Nadorcott SL
Nadorcott ARC and Nadorcott SL are induced Nadorcott selections to minimise the average seed per fruit. Both selections have the same growth habit and charactaristics as the Nadorcott. Fruit size on both selections peaked at count 1. The internal juice percentages were over 55% and developed good Brix (12.3°) with acids of 1.13%, ensuring a good balance for good eating quality. There were only 0.3 seeds per fruit on average, despite heavy cross pollination and the fruit was fully coloured (T1) before peak maturity.

Nova ARC
Nova ARC is a selection from the ARC that was irradiated to improve the selection as seedless. The trees are very small and in some trees gumming is observed on the branches as well as “die back” on the trees. The fruit had a very good external colour development (T1) before peak maturity was reached, and fruit size for Nova ARC is small to medium (count 2). The selection was completely seedless and the internal juice percentages were over 50%. Nova ARC had a very high Brix level of 15.

Or 4
Or is a late maturing mandarin that is currently planted commercially. The fruit has a smooth rind with less ribbing compared to Mor. Or developed a very firm fruit and peels easily; fruit size peaked from count 2 to count 1. The juice percentages were high (58%) at peak maturity and the selection had high Brix (14.9°) with an acid of 1.09%, ensuring very good eating quality fruit. In this variety block the average seed development per fruit was 2.8.

Shasta Gold
Shasta Gold had no fruit on the tree in the 2015 season due to over cropping in the 2014 season. The fruit has severe ribbing problems when the trees are still in their youth phase. The fruit shape was flat with large to very large fruit size (1xx-1xxx). Shasta Gold developed a very good external fruit colour (deep orange at peak maturity). The fruit rind was thin and peeled cleanly and easily.

Sonet
Sonet is an early mandarin. The selection developed into a small tree with very good yields. The selection had severe creasing problems which indicates that the fruit has to be picked early in the season. Sonet had the highest juice percentages with over 60%, but very bad colour development (T5-6) at peak maturity.

Tahoe Gold
Tahoe Gold struggled with high acids in the 2014 season, and similar levels in the 2015 season. The acid levels did not drop low enough for the fruit to reach the peak maturity ratio of 12:1. The fruit had a very good colour development and peaked at colour plate T1 to 2 before fruit was fully matured. Due to the high acids, the fruit becomes puffy before it reaches peak maturity. Tahoe Gold developed seeds at all the trial sites: up to 2.8 seeds per fruit. The selection has the smallest tree size (compact) of the UCR 5 selections with a rounder shape and dense canopy. At the Paarl trial site, the selection over cropped in the 2014 season and had a poor yield in the 2015 season.

Tango
Tango developed a very smooth rind texture (similar to Nadorcott) with a natural shine. The fruit had a very good colour development in the cooler areas (colour plate T1) at peak maturity. Tango was completely seedless at the Citrusdal site. The selection had a good yield for the medium tree size and the fruit size peaked at count 1.

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. Crop manipulation will be necessary to control the vegetative growth and fruit size. The external colour development is very good and can be compared to the Furr selection. This was the Tanor late selection’s first

crop at this trial site and the internal juice percentage was on the low side (45.9%). Tanor late peels easily and cleanly, and has extra large fruit (count 1XXX).

Winola
Winola is a late maturing mandarin selection with a very high acid percentage. The fruit had a very good colour development (T1) before peak maturity. Winola had good Brix (13.0-14.2°) with high acids (ranging from 1.5 to 1.9%). The selection had 2.2 seeds per fruit on average as well as a problem with alternate bearing.

Yosemite Gold
Yosemite Gold has a serious alternate bearing problem. At the Citrusdal site, Yosemite Gold did not bear a crop for the third year in a row. At the Paarl site, Yosemite Gold outperformed the other two sites, with large fruit size (count 1xxx) and good crop on the trees. The fruit had an excellent colour development with a deep orange rind colour. Yosemite Gold is a very firm (strong) fruit with thin rind that peels easily and cleanly.

Conclusion

African Sunset, Shasta Gold, Yosemite Gold and Tanor late had the largest fruit size (1xxx). Shasta Gold, Yosemite Gold and Tahoe Gold showed signs of severe alternate bearing patterns due to over cropping in the 2014 season. Very poor yields were seen on the trees with extra large fruit size. Furr had the most seeds per fruit on average (16.3). Sonet had the highest juice percentages with over 60%.

Table 5.5.22.4. Internal fruit quality data for experimental mandarin hybrid selections from the Paarl region of the Western Cape during the 2015 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/06/19	Golden Nugget	2	58.6	12.4	1.20	10.3	0.0	T3
2015/06/19	Tahoe Gold	1	64.7	11.4	1.57	7.3	0.5	T1
2015/07/06	Tahoe Gold	1xx	54.3	11.5	1.13	10.1	2.8	T1
2015/08/14	Yosemite Gold	1xxx	45.3	12.2	1.13	10.8	0.3	T1

Table 5.5.22.5. Internal fruit quality data for experimental mandarin hybrid selections from the Porterville region of the Western Cape during the 2015 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/06/18	Golden Nugget	1x	52.4	11.9	1.05	11.3	0.2	T4
2015/08/14	Golden Nugget	1	51.8	13.7	0.71	19.4	0.0	T1
2015/07/08	Shasta Gold	1xxx	49.9	11.1	1.34	8.3	1.2	T1
2015/06/18	Tahoe Gold	1x	53.8	11.8	1.57	7.5	0.7	T2
2015/07/08	Yosemite Gold	1xxx	51.9	11.3	1.19	9.5	1.0	T1

Table 5.5.22.6. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2015 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/06/18	African Sunset (B24)	1xxx	44.9	10.5	1.03	10.2	0.0	T1
2015/06/18	Furr	1x	55.1	13.0	1.09	11.9	16.3	T1
2015/06/18	Golden Nugget	1x	48.1	12.5	1.11	11.3	0.0	T3
2015/07/08	Golden Nugget	1xx	45.3	13.0	0.96	13.5	0.2	T1
2015/07/08	Hadas	1x	50.2	11.1	1.73	6.4	2.0	T1
2015/08/14	Hadas	1x	48.6	12.3	1.60	7.7	1.6	T1
2015/07/08	IRM 2	1	45.7	12.5	1.22	10.2	2.7	T1
2015/08/14	IRM 2	1	45.7	13.8	0.98	14.0	1.8	T1
2015/08/14	Mor 26	1	51.9	16.0	1.10	14.5	1.5	T1
2015/06/18	Nadorcott SL	1	57.5	12.3	1.16	10.6	0.3	T1
2015/06/18	Nadorcott ARC	1	55.4	12.1	1.13	10.7	0.3	T1
2015/07/08	Nadorcott ARC	1	48.9	12.4	1.16	10.7	0.2	T1
2015/06/18	Nova ARC	2	51.7	15.0	1.45	10.4	0.0	T1
2015/07/08	Or 4	1	52.2	14.2	1.17	12.1	0.1	T1
2015/08/14	Or 4	2	58.0	14.9	1.09	13.6	2.8	T1
2015/07/08	Shasta Gold	1xxx	46.0	11.4	1.39	8.2	0.2	T1
2015/04/28	Sonet	3	61.7	11.4	0.79	14.5	1.3	T6
2015/04/28	Sonet 2	1	61.1	10.8	0.53	20.3	0.8	T5
2015/04/01	Sonet	2	60.6	10.4	0.98	10.6	1.3	T7
2015/04/01	Sonet 2	2	61.0	10.4	0.91	11.4	0.0	T6
2015/06/18	Tango	1	57.4	12.8	1.2	10.4	0.0	T1
2015/07/08	Tanor Late	1xxx	45.9	10.9	1.17	9.4	0.2	T1
2015/08/14	Tanor Late	1xxx	41.9	10.6	0.76	14.0	0.0	T1
2015/07/08	Winola	2	50.6	13.0	1.88	6.9	2.2	T1
2015/08/14	Winola	1	57.7	14.2	1.52	9.3	0.0	T1

5.5.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)

Project 998B by S. Meeding and Z. Zondi (CRI)

Opsomming

Die vroeë nawel proef is gevestig in die Addo area van die Sondagsrivier vallei. E-Nawel is ŉ mutasie wat gevind is en word direk vergelyk en ge-evalueer met ander vroeë Nawel seleksies. Al hierdie seleksies is gedurende die 2007 seisoen getopwerk. Swingle citrumelo en Troyer citrange word as onderstamme vir hierdie proef gebruik. Die E-Nawel seleksie wat ge-evalueer word is dogter bome en word direk vergelyk met Fukumoto-, Lina-, Newhall-, Palmer-, Tulegold-, en Washington nawel. E-nawel se naam is huidiglik Addo early.

Summary

The early maturing navel trial is based in the Addo area of the Sundays River Valley. E-Navel (new mutation) was included at this trial site and evaluated with other early navel selections. All selections were topworked in the 2007 season. The rootstocks Swingle citrumelo and Troyer citrange were used for the trial. The E-Navel selection evaluated in this trial are daughter trees and are compared with Fukumoto, Lina, Newhall, Palmer, Tulegold and Washington navel. E-navel had a name change and is currently known as Addo early.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from the Sundays River Valley region of the Eastern Cape. The following early to mid maturing selections were evaluated: Fukumoto, Lina, Newhall, Palmer, Tulegold, E-Navel (Addo early) and Washington.

For navels, a ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 5.5.23.1. List of navel selections evaluated at Sundays River Valley (Penhill) during 2015.

Selection	Rootstock	Planted
Addo early (E-Navel)	Swingle	2007
Fukumoto	Troyer	2007
Lina	Troyer	2007
Newhall	Troyer	2007
Palmer	Swingle	2007
Tulegold	Troyer	2007
Washington	Swingle	2007

Results and discussion

Addo early (E-navel)
The selection had a good fruit size and peaked at count 48. Internal quality was good with a high juice percentage of 53.3%. Addo early developed a good external colour up to colour plate T1 at peak maturity with a good crop on the trees. The leaves had a dark green colour and the tree canopy was fairly dense. The fruit had a protruding navel-end.

Fukumoto
Fukumoto was the selection with the highest Brix for this trial site (11.2°). The colour development was delayed, with colour plate T3 at peak maturity. Brix and acid levels remained high for another 2 weeks and the external colour development caught up with the internal quality. Fukumoto produced a good fruit size and peaked at count 56. The navel-end on the fruit was fairly open and protruding, one of the characteristics of the selection.

Lina
Lina had a delayed colour development with a colour plate T4 to T6 at peak maturity. The selection had a very good fruit size and peaked at count 56; Lina Navel is a selection that has a problem with smaller fruit size in general. The fruit shape was more elongated with a large navel-end (fairly open). Lina developed a high juice content (54.7%) compared to the 2014 season.

Newhall
The fruit size peaked at count 56 for this season which is very favorable for navel production and export. Newhall had a delayed colour development (colour plate T4) when the fruit was at peak maturity. Brix at 10.7° was higher compared to the 2014 season; with a fairly high acid percentage of 1.0%. The selection's juice percentage increased (above 50%) for 2015, higher than in the 2014 season.

Palmer
The external colour development of the selection was very good (colour plate T1) at peak maturity. The selection had a good fruit size and peaked at count 56. The acids dropped slowly, indicating that the selection

can hang on the trees for slightly longer periods. The selection had a lower juice percentage compared to the 2014 season.

Tulegold
Tulegold had a delayed colour development with colour plate T4-5 at peak maturity. Most of the fruit on the trees developed a round fruit shape, but some tended to be more elongated. The selection will remain experimental and there are no commercial plantings to date.

Washington
Washington and Palmer were used as controls at this trial site and are well-known selections. The fruit had a round shape with small to medium navel end opening. Fruit size was good and peaked at count 56. The colour development on the fruit was uniform, but was delayed (T5-6) at peak maturity.

Conclusion

The Addo area is well suited for navel production in South Africa. All the selections had a very good fruit size and peaked at count 56, with Addo early on one evaluation peaking at count 48. The delayed external colour development, as well as high acid percentages in some of the selections will be due to the Swingle rootstock that is 2-3 weeks later in maturity compared to Carizzo- and Troyer citrange with delayed external colour development and higher acid levels. Lina had the highest juice percentage with 54.7%. Fukumoto had the highest Brix 11.2° for this trial. The order of maturity was as follows: Fukumoto started the season followed by Lina, Tulegold, Palmer, Fukumoto, Newhall, Washington, and ending with Addo early. Addo early had very low Brix and is also planted on Swingle rootstock that delayed the maturity.

Table 5.5.23.2. Internal fruit quality data for early and mid Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2015 season.

Date	Grower	Selection	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/03/09	Penhill	Addo Early (E-Navel)	56	49.3	8.4	1.04	8.1	0	T6
2015/04/22	Penhill	Addo Early (E-Navel)	56	48.7	9.5	1.01	9.4	0	T5
2015/05/08	Penhill	Addo Early (E-Navel)	48	53.3	8.9	0.85	10.5	0	T1
2015/05/20	Penhill	Addo Early (E-Navel)	56	52.0	9.3	0.83	11.2	0	T1
2015/05/26	Penhill	Addo Early (E-Navel)	56	49.8	8.7	0.71	12.3	0	T1
2015/03/09	Penhill	Fukumoto	56	50.3	10.8	1.17	9.2	0	T5
2015/04/22	Penhill	Fukumoto	56	48.4	11.2	1.00	11.2	0	T3
2015/05/08	Penhill	Fukumoto	64	53.3	11.1	0.95	11.7	0	T1
2015/03/09	Penhill	Lina	56	54.7	10.4	1.00	10.4	0	T6
2015/04/22	Penhill	Lina	56	54.0	10.6	0.92	11.5	0	T4
2015/03/09	Penhill	Newhall	56	52.7	10.1	1.13	8.9	0	T6
2015/04/22	Penhill	Newhall	56	51.5	10.7	1.02	10.5	0	T4
2015/04/22	Penhill	Palmer	56	49.4	10.0	0.93	11.6	0	T6
2015/05/07	Penhill	Palmer	56	50.9	9.8	0.90	10.9	0	T4-5
2015/05/20	Penhill	Palmer	56	53.4	10.1	0.87	11.6	0	T1
2015/05/26	Penhill	Palmer	56	51.9	10.2	0.79	12.9	0	T2-3
2015/03/09	Penhill	Tulegold	64	51.3	10.0	0.99	10.0	0	T5
2015/04/22	Penhill	Tulegold	56	52.5	10.1	0.92	11.0	0	T4
2015/05/07	Penhill	Tulegold	56	52.8	10.7	0.86	12.4	0	T1
2015/04/22	Penhill	Washington	56	50.6	10.9	1.04	10.4	0	T6
2015/05/07	Penhill	Washington	56	53.1	9.6	0.93	10.3	0	T5

5.5.24 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)**

Project 1001B by S. Meeding and Z. Zondi (CRI)

Opsomming

Hierdie proef bestaan uit vier eksperimentele nawel seleksies waar die oorspronklike mutasie of dogterbome ge-evalueer word. Skoon materiaal van die seleksies is in verskillende proefblokke gevestig en Cambria is as kontrole gebruik in die proefperseël. De Wet 1 is ʼn mid-rypwordende nawel met ʼn ronde vrugvorm. Die vrugte het ʼn toe nawel-ent en geen oes manipulasie word benodig vir ‘n goeie set nie. Lazyboy, KS nawel en Suitangi is laat nawel seleksies met goeie eksterne kleur ontwikkeling; Lazyboy kan vir ʼn langer periode aan die boom hang. KS nawel is uit Cambria geselekteer as ʼn natuurlike mutasie wat ronder vrugte geproduseer het. Suitangi is ook geselekteer as ‘n laat nawel wat moontlik ‘n ronder vrug tot gevolg kan hê asook later ryp word as Cambria.

Summary

The trial consists of four experimental navel selections where the original mutation or daughter trees were evaluated. In addition, clean material was added in numerous new trial blocks and Cambria was used as a control at the trial site. De Wet 1 is a mid-maturing navel producing a round fruit shape. The fruit developed a closed navel end without any manipulation to set a good crop on the trees. Lazyboy, KS Navel and Suitangi are all late maturing navel selections with good external colour development; Lazyboy indicated that it can hang on the trees for an extended period of time (matures well). KS Navel was selected from Cambria because of a natural mutation that produced fruit with a rounder fruit shape compared to Cambria. Suitangi was also selected as a late maturing navel with rounder shape, as well as maturing later compared to Cambria.

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from various regions of the Gamtoos River Valley. The following selections were evaluated: De Wet 1, KS navel, Suitangi and Lazyboy, with Cambria as a control.

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 5.5.24.1. List of navel selections evaluated at various sites in the Gamtoos River Valley, Eastern Cape during the 2015 season.

Selection	Rootstock	Planted
De Wet 1	Rough Lemon	Young
Cambria	Rough Lemon	Old
KS Navel	Rough Lemon	Young
Suitangi	Rough Lemon	Old
Lazyboy	Rough Lemon	Young

Results and discussion

Cambria

Cambria is a well-known late navel selection with very good internal quality. The selection was used as control for the late maturing navel trial in the Gamtoos River Valley. The fruit shape was more elongated compared to other navel selections (Palmer etc.). Cambria had a good fruit size and peaked from count 56. The selection had a better colour development compared to the 2014 season, being at colour plate T1 to 2 at peak maturity.

De Wet 1

De Wet 1 is a mid-maturing navel that has produced a good crop consistently every year. Manipulation is necessary to control fruit size because over cropping results in smaller fruit. The selection developed a fairly soft rind, one of the characteristics of the De Wet selection. De Wet 1 had a closed navel end on the fruit without having to spray 2,4-D; and developed a small internal navel. However, some of the fruit developed a small opening at the navel end. The selection had good fruit size and peaked at count 56. Fruit shape was round and the colour development was better on the mother tree compared to the daughter trees. The internal quality was good with juice content as high as 54.7%. At peak maturity, the external colour peaked at colour plate T4-5. The Brix remained on the low side at 8.9, the same problem as in the 2014 season.

KS navel

KS navel was selected as a branch mutation on a Cambria tree. The fruit shape appeared more round compared to the standard Cambria selection. Daughter trees were planted in a Cambria orchard and fruit tends to be more round in fruit shape compared to Cambria. The selection peaked at count 56. The selection had a juice percentage of 50.1%.

Lazyboy

Lazyboy was selected at Cambria, close to Patensie, and developed round fruit on the trees with good internal quality. The tree had a round “bushy” shape and bore most of the fruit on the outside of the tree. The fruit remained firm and can hang on the tree for an extended period. In the 2015 season the selection had low juice percentages, below 50%., Brix of 12° and low acids (0.65%).

Suitangi

Suitangi was one of the late maturing navel selections evaluated, with very good external colour development. The selection had a deep orange rind colour with a fairly small navel end. There was a good crop on the trees this season. Suitangi peaked from count 56 to count 64. Internally Suitangi produced good quality with high juice content; every sample tested was over 54% juice. High Brix levels of over 11 with acids of 0.85%, assured good tasting fruit with good flavour.

Conclusion

The season started with De Wet 1 navel (mid to end of June) and this selection developed the lowest Brix content this season. After De Wet 1 navel, Lazyboy matured with lower Brix:acid ratios in the 2015 season compared to the 2014 season. KS Navel, Cambria and Suitangi ended the navel season. These three selections matured in the same time frame with Suitangi being the latest of the three selections to mature. Low Brix and acid levels were observed in the fruit this season. The Rough Lemon rootstock could have had an affect; Rough Lemon is known to produce fruit with lower internal quality fruit compared to Carizzo and Swingle.

Table 5.5.24.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2015 season.

Date	Grower	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/07/09	K Scheepers	Cambria	RL	56	52.3	9.0	0.68	13.2	0	T1-2
2015/05/28	E Dupreez	De Wet 1	RL	56	54.7	8.8	0.75	11.7	0	T4-5
2015/06/25	E Du Preez	De Wet 1	RL	56	46.1	8.9	1.12	7.9	0	T5
2015/07/09	K Scheepers	KS Navel	RL	56	50.1	9.6	0.61	15.7	0	T1
2015/07/09	P Streso	Lazyboy	RL	40	48.3	10.3	0.80	12.9	0	T6
2015/07/09	P Streso	Lazyboy	RL	56	49.0	10.3	0.69	14.9	0	T5
2015/08/04	P Streso	Lazyboy (Daughter)	RL	56	46.8	12.0	0.65	18.5	0	T1
2015/06/25	I Ferreira	Suitangi	RL	64	54.1	11.1	0.91	12.2	0	T4-5
2015/07/09	I Ferreira	Suitangi	RL	64	56.8	11.4	0.85	13.4	0	T5
2015/08/04	I Ferreira	Suitangi	RL	64	55.2	12.4	0.74	16.8	0	T1
2015/08/19	I Ferreira	Suitangi	RL	56	56.5	12.6	0.64	19.7	0	T1

5.5.25 PROGRESS REPORT: Evaluation of Valencia selections in a semi-desert production area (Kakamas)

Project 964B by S. Meeding and J. Joubert (CRI)

Opsomming

Die Valencias wat bespreek word in hierdie proef was in die 2010 seisoen getopwerk. Die bome het hulle tweede drag in die 2015 seisoen gehad. Die vrugtevlieg populasie was onder beheer gehou vir die 2015 seisoen. As gevolg van die hoë druk van kruisbestuiwing in hierdie proef blok het die meeste seleksies saad ontwikkel. Die Valencias hou die suur persentasies langer as die Nawel seleksies.

Summary

The Valencias discussed in this trial were top worked in the 2010 season. The trees produced their second crop in the 2015 season. The fruitfly population was better controlled for the 2015 season. Due to the high pressure of cross pollination in this variety block, most of the selections produced seed. The Valencias tend to keep their acids longer compared to the navel selections.

Objective

- To find suitable Valencia selections with superior characteristics for the semi-desert production area (Kakamas).

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha 2, Bennie 2, Du Roi 2, Henrietta, Jassie, Kobus du Toit Late, Lavalle, Gusocora (G5), Louisa, McClean, McClean SL, Midknight 1, Moosrivier late 1, Moosrivier late 2, Rhode Red, Ruby Red, Skilderkrans, Valencia late, Weipe.

Table 5.5.25.1. List of Valencia selections evaluated at Mosplaas (Kakamas) during 2015.

Selection	Rootstock	Topwork
Alpha 2	X639	2010
Bennie 2	X639	2010
Du Roi 2	X639	2010
Henrietta	X639	2010
Jassie	X639	2010
Kobus du Toit	X639	2010
Lavalle	X639	2010
Gusocora	X639	2010
Lavalle 2	X639	2010
Louisa	X639	2010
McClean	X639	2010
McClean SL	X639	2010
Midknight 1	X639	2010
Moosrivier late 1	X639	2010
Moosrivier late 2	X639	2010
Rhode Red	X639	2010
Ruby Red	X639	2010
Skilderkrans	X639	2010
Valencia late	X639	2010
Weipe	X639	2010

Results and discussion

There was a better crop on the trees for the 2015 season. Colour development on most of the selections was very good (T1) before peak maturity. Moosrivier late 2 and Weipe had the most delayed colour development of all the selections. Kobus du Toit late, Louisa, McClean SL, Midknight 1, Moosrivier late 2, Valencia late and Weipe are all selections that did not reach an internal juice percentage of 50% and more; Weipe being the lowest after the second evaluation (39.4%).

Lavalle 2, Louisa, Moosrivier late 2, Skilderkrans and Weipe were the only selections that were completely seedless at this trial site. Moosrivier late 1 had the highest number of seeds on average per fruit (12 seeds). Du Roi 2 and McClean SL developed the most fruit splitting of all the selections.

Conclusion

Bennie, Weipe and Moosrivier late 2 were the only selections with a delayed external fruit colour development. Moosrivier late 1 was the selection with the most seeds per fruit on average (12.0). Lavalle 2, Louisa, Moosrivier late 2, Skilderkrans and Weipe were the only selections that were completely seedless.

Table 5.5.25.2. Internal fruit quality data for Valencia selections at Mosplaas (Kakamas) during the 2015 season.

Date	Cultivar	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2015/07/28	Alpha 2	64	51.2	10.5	1.17	9.0	0.7	T1
2015/05/13	Bennie	64	43.7	9.5	1.44	6.6	2.7	T5
2015/06/09	Bennie	72	50.9	9.8	1.24	7.9	3.7	T2
2015/07/28	Du Roi 2	88	52.3	11.0	1.47	7.5	3.1	T1
2015/07/28	Henrietta	64	51.2	9.9	1.31	7.5	2.6	T1
2015/07/28	Jassie	72	52.7	11.7	1.30	9.0	4.1	T1
2015/07/28	Kobus Du Toit	88	45.4	10.9	1.28	8.5	4.6	T1
2015/07/28	Lavalle 2	64	51.8	11.2	1.60	7.0	0.0	T1
2015/07/28	Louisa	72	49.9	10.5	1.19	8.8	0.0	T1
2015/07/28	McClean	72	50.4	11.6	1.34	8.6	2.5	T1
2015/07/28	McClean SL	72	46.6	11.0	1.01	10.9	4.3	T1
2015/07/28	Midknight 1	72	46.2	11.5	0.95	12.1	0.1	T1
2015/07/28	Moosrivier Late	72	56.3	11.1	1.30	8.6	12.0	T1
2015/07/28	Moosrivier Late 2	56	46.7	12.5	1.08	11.6	0.0	T3
2015/07/28	Rhode Red	88	52.4	10.7	1.33	8.1	2.1	T1
2015/07/28	Ruby Red	72	52.2	10.9	1.23	8.9	6.3	T1
2015/07/28	Skilderkrans	72	51.4	11.0	1.17	9.4	0.0	T1
2015/07/28	Valencia Late	72	49.6	10.7	1.42	0.5	2.7	T1
2015/04/15	Weipe	72	42.1	9.2	0.95	9.7	0.0	T5
2015/05/13	Weipe	72	39.4	10.3	0.86	11.9	0.0	T3
2015/06/09	Weipe	72	40.7	10.9	0.79	13.7	0.0	T1

5.5.26 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental Navel oranges in a semi-desert region (Kakamas)

Project 964C by S. Meeding and J. Joubert (CRI)

Opsomming

Die 2015 seisoen was die tweede jaar van evaluasies vir die Nawel seleksies by hierdie proef perseël. Die vrugtevlieg populasie was beter beheer vir die 2015 seisoen en ‘n swaarder drag was sigbaar op die bome. Die semi-woestyn area is nie bekend as ‘n Nawel produksie area nie. Die nawels is geneig om te granuleer voor optimal rypheid en die suur persentasies val teen ‘n vinnige tempo wat die vrugte droog en afgewater laat proe.

Summary

The 2015 season was the second year of evaluations for the Navel selections at this trial site. The fruit fly population was better controlled for the 2015 season and a heavier crop was seen on the trees. This semi-desert area is not known as a Navel production area. The Navels tend to granulate before peak maturity and the acid percentages drop at a fast rate leaving the fruit dry and tasteless.

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 a semi-desert region.

Materials and methods

For navel selections, a ratio of 9:1 is considered to be the build-up towards peak maturity. 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.

Field evaluations and laboratory analyses were conducted on navel selections from a semi-desert region of the Northern Cape. The following selections were evaluated.

Table 5.5.26.1. List of Navel orange selections evaluated at Kakamas during 2015.

Selection	Rootstock	Topwork
Autumn Gold	X639	2010
Barnfield	X639	2010
Cambria	X639	2010
Chislett	X639	2010
Clarke	X639	2010
Dairy Lina	X639	2010
De Wet 1	X639	2010
De Wet 2	X639	2010
Early Lina	X639	2010
Fischer	X639	2010
Fukumoto	X639	2010
Glen Ora late	X639	2010
Hutton	X639	2010
Lane Late	X639	2010
M7	X639	2010
Powell Summer	X639	2010
Robyn 2	X639	2010
Summer Gold	X639	2010
Witkrans	X639	2010

Results and discussion

M7 and De Wet 1 were the only navel selections that reached a juice percentage of 50% and higher; M7 (54.8%) and De Wet 1 (53.3%). Barnfield (38.7%), Clarke (39.7%) and Fukumoto (36.5%) had the lowest juice percentages. Cambria and M7 had the smallest fruit size and peaked at count 72. One sample of De Wet 1 also measured count 72. The selections with the largest fruit size were Autumn Gold, Barnfield, Chislett, Clarke, Dairy Lina, De Wet 2, Fischer, Hutton, Lane late, Powell Summer, Robyn 2 and Summer Gold with count 56. With the high pressure due to cross pollination, some selections developed seeds in the fruit. The selection with the highest seed count for this trial site was Clarke (0.7 seed per fruit). The other selections that developed seeds were De Wet 1, Hutton, Summer Gold and Witkrans. M7 had the highest Brix content (11.9°).

Conclusion

The late maturing navels tend to set more fruit on the trees compared to the early selections and acid levels seem to drop quickly due to high temperatures early in the production season. The early selections were more prone to fruit drop than the later selections. In the 2015 season the acids dropped in the late selections even before peak maturity, and the fruit became granulated.

Table 5.5.26.2. Internal fruit quality data for experimental navel selections from the semi-desert (Kakamas) region of the Northern Cape during the 2015 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/06/09	Autumn Gold	56	44.8	11.0	0.65	17.0	0.0	T2
2015/06/09	Barnfield Summer	56	38.7	10.7	0.69	15.6	0.0	T2
2015/06/09	Cambria	72	46.9	10.3	0.69	15.0	0.0	T5
2015/06/09	Chislett	56	37.8	10.7	0.58	18.4	0.0	T3
2015/06/09	Clarke	56	39.7	10.9	0.72	15.2	0.7	T1
2015/05/13	Dairy Lina	56	41.8	11.1	1.00	11.1	0.0	T4
2015/04/15	De Wet 1	72	50.7	9.5	0.70	13.5	0.2	T6
2015/05/13	De Wet 1	64	50.4	10.1	0.64	15.9	0.0	T4
2015/06/09	De Wet 1	64	53.3	10.9	0.67	16.2	0.0	T1
2015/05/13	De Wet 2	56	41.3	9.8	0.68	14.5	0.0	T5
2015/05/13	Early Lina	64	48.6	11.3	0.90	12.6	0.0	T2
2015/05/13	Fischer	56	45.2	10.2	0.63	16.1	0.0	T4
2015/04/15	Fukumoto	64	36.5	10.6	0.70	15.2	0.0	T5
2015/06/09	Glen Ora Late	64	44.8	10.8	0.69	15.8	0.0	T2
2015/06/09	Hutton	56	41.6	11.0	0.66	16.8	0.1	T3
2015/06/09	Lane Late	56	46.0	10.3	0.61	16.8	0.0	T2
2015/04/15	M7	72	54.8	11.9	0.68	17.5	0.0	T4
2015/06/09	Powell Summer	56	44.5	10.8	0.62	17.3	0.0	T1
2015/06/09	Robyn 2	56	44.7	10.6	0.71	14.8	0.0	T2
2015/06/09	Summer Gold	56	45.0	11.1	0.58	19.3	0.3	T1
2015/06/09	Witkrans	64	45.2	10.9	0.66	16.5	0.2	T3

5.5.27 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a semi-desert production region (Kakamas)

Project 964D by S. Meeding and J. Joubert (CRI)

Opsomming

Hierdie mandaryn seleksies was getopwerk op X639 in die 2010 seisoen. Daar was ŉ ernstige probleem met vrugtevlieg in die 2014 seisoen en vrugval het plaasgevind lank voor optimale rypheid. Die probleem was goed beheer vir die 2015 seisoen. Geen van hierdie seleksies is onder net nie en sonbrand is ŉ probleem by sekere van die variteite.

Summary

These mandarin selections were topworked onto X639 rootstock in 2010. There was a serious problem with fruit fly at the trial site during the 2014 season and fruit started dropping from the trees long before it reached peak maturity. The problem was controlled during the 2015 season. None of these mandarin selections is under nets and sunburn was a problem for some of the cultivars.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).

- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in semi-desert regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the semi-desert (Kakamas) region of the Northern Cape. A range of new mandarin hybrids have been added to this area and should bear fruit in the 2015 season.

For mandarin hybrids, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over mature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

Table 5.5.27.1. List of mandarin hybrid selections in the semi-desert region (Kakamas) during the 2015 season.

Selection	Rootstock	Topwork
Furr	X 639	2010
Etna	X 639	2010
Gold Nugget	X 639	2010
Hadas	X 639	2010
IRM 1	X 639	2010
IRM 2	X 639	2010
Nadorcott ARC	X 639	2010
Nadorcott SL	X 639	2010
Or 4	X 639	2010
Phoenix	X 639	2010
Samba	X 639	2010
Shasta Gold	X 639	2010
Sirio	X 639	2010
Tahoe Gold	X 639	2010
Tango	X 639	2010
Tanor late	X 639	2010
Tasty 1	X 639	2010
Valley Gold	X 639	2010
Winola	X 639	2010
Yosemite Gold	X 639	2010

Results and discussion

Furr
Furr is used as a control for mid maturing mandarin selections. The selection has a very good rind colour development (deep orange) and very good juice percentages, which peaked at 62.3%. Furr peels easily and has a very good eating quality with good flavour. The selection had a very high Brix (15.3°) this season considering the specific production area. Due to the high cross pollination pressure, Furr produced the most seed: 22.5 seeds per fruit on average. The trees had a very good yield and fruit size peaked at count 1x.

Etna
Etna is an early mandarin that matures end of April to middle of May. The tree had a very good yield and shows no sign of alternate bearing. The fruit size peaked at count 1 to 1x with high juice percentages; above 60%. The selection will produce seeds in the fruit under cross pollination conditions. At this trial site Etna produced 4.6 seeds per fruit on average.

Gold Nugget
In the 2015 season Gold Nugget had a very large crop on the trees and this may be the reason for the smaller fruit size compared to the 2014 season. The fruit size peaked at count 2; juice percentages were high: above 57% for a hot production area; Brix was high (12.9°) with a good acid percentage of 1.06% resulting in a good quality fruit and flavour. The selection had a low seed count of 0.1- 0.3 seeds per fruit on average and good

colour development with colour plate T2 just before peak maturity. Tree canopy was less dense in comparison to the Western- and Eastern Cape.

Hadas

Hadas is a late mandarin with very high acid percentages. The selection will be seedless in solid blocks but under these heavy cross pollination conditions, the fruit produced 9.3 seeds per fruit on average. The external rind colour is a light yellow colour with a smooth rind texture. Hadas had a very good yield for the medium tree size. The Brix was 16° with acid levels at 1.5%, and juice percentages were very high: over 60%.

IRM 1

The IRM 1 selection is one of two irradiated Murcott selections from Australia. IRM 1 has a slightly less intense external rind colour than the IRM 2 selection; has more ribbing and the fruit is firm and peels easily. The IRM 1 selection had very low juice percentages, just over 44%; Brix was high at 15.3° and the acid remained at 1.02%. External colour was a bit delayed (T2) at peak maturity and the selection had 3.3 seeds per fruit on average.

IRM 2

The selection is low seeded with good internal and external colour. IRM 2 developed some ribbing on the fruit, but less than IRM 1, and the fruit is firm and peels easily and cleanly. The fruit has a very good eating quality with a high Brix:acid ratio of 14.0. In the 2015 season there was not a heavy crop on the trees.

Nadorcott ARC & Nadorcott SL

Nadorcott ARC and Nadorcott SL are induced Nadorcott mututions to minimise the average seeds per fruit. Both selections have the same growth habit and charactaristics as the Nadorcott, and fruit size peaked at count 1. The juice percentages were over 50%; Brix of 12.5, with acid of 0.91%. The seed count per fruit on average ranged from 0.5 to 1.8 seeds per fruit, which is very good in a combined variety block like this. The fruit was fully coloured (T1) at peak maturity.

Or 4

Or is a late maturing mandarin selection that is currently planted commercially. The fruit has a smooth rind with less ribbing than Mor. Or developed a very firm fruit and peels easily. There was a poor yield on the trees for the 2015 season and only enough fruit for a single evaluation. Fruit size peaked at count 2 and juice percentages were high (57.9%) at peak maturity. The selection had high Brix (14.5°) with an acid of 1.03%, giving a very good eating quality fruit for this hot production area. In this variety block the average seed count per fruit was 0.8 (low). The trees are very vigorous and Or 4 is likely to be susceptible to Grey mite in this hot production area.

Phoenix

Phoenix produced a poor to fair crop on the trees, and rind texture was smooth with minimal sunburn. The internal quality indicates that Phoenix may not be suited for this semi-desert production area. The juice percentages were very low at 38.7%; Brix was moderate for this selection (11.8°) and acid levels were 0.57%. The acids dropped very fast before peak maturity which caused the fruit to be granulated. The selection had 0.9 seeds per fruit on average.

Samba

Samba is an early maturing mandarin selection that was one of the best performing soft citrus selections in this semi-desert production area. The selection had minimal to no sunburn and the external rind colour was excellent for this hot area (deep orange). Samba produced a very good crop on the trees and fruit size peaked at count 2. Brix levels peaked at 14.6, with an acid percentage of 0.97%. The juice percentages were high for all three completed evaluations, being over 60%. The selection will produce seed if cross pollinated and under these heavy cross pollination conditions, Samba produced 9.2 seeds per fruit.

Shasta Gold

Shasta Gold had a small tree size with a poor to fair crop on the trees. The fruit size was large (count 1-1X) due to the light crop, with a very coarse rind and ribbing on the fruit, typical characteristics of younger Shasta trees. Trees were thorny on the vigorous main branches but the thorns will disappear when the trees mature, as well as when the crop load increases. The fruit did not develop to a deep orange or red external colour as seen in the colder production areas. The average seed count per fruit was much higher in this trial (high cross pollination pressure) compared to the Western- and Eastern Cape with 2.2 seeds per fruit. The selection had very high acid percentages which tend not to drop low enough to comply to the minimum standards. Shasta developed good juice percentages, over 60%, however closer to peak maturity the juice percentage dropped

to 52%.

Sirio
Sirio is a mid maturing mandarin and the appearance of the fruit in this semi-desert area, compared to the cooler production areas in South Africa, indicated that this area may be too hot for the cultivation of Sirio. The selection produced a very coarse rind texture and the rind is thicker than in the cooler areas. Rind oil levels are very high, and fruit size peaked at count 1. Sirio had very high juice percentage of 59.3%; Brix was 11.9° and the acid level was 1.26%, resulting in a Brix:acid ratio of 9.5; too low for export standards. Under cross pollination the selection produced 8.9 seeds per fruit on average. The fruit was fully coloured (T1) before peak maturity.

Tahoe Gold
Tahoe Gold produced a good yield on the trees, taking the small tree size into consideration. The fruit size peaked from count 1 to count 2. External colour development was very delayed with colour plate T4 before peak maturity. The internal quality was good with high juice percentages, peaking at 63.5%. The selection had a low seed count of 0.7 seeds per fruit on average, which is very low for a mixed trial block like this. Tahoe Gold had a very high acid content and the acid levels did not drop below 1.8%, when the fruit became puffy and fruit dropped.

Tango
Tango had good colour development for this semi-desert production area; the selection was at colour plate T1 at peak maturity. Juice levels were good with percentages as high as 56% for this trial site. Tango had a lower seed count compared to the 2014 season (2.3 seeds per fruit), although this is higher when compared to the Western- and Eastern Cape trial sites due to possible cross pollination pressure (mixed trial blocks). The acid levels remained high during the season and the Brix level reached 12.7°. Fruit size was on the smaller side and peaked between count 2 to count 3.

Tanor late
Tanor late is an ultra late Mandarin selection that matures at the end of August to middle of September in the cold production areas. The selection has a very vigorous tree and an upright growth habit if not pruned, and will have to be manipulated to control the vegetative growth habit. The selection has large thorns that tends to disappear as the tree gets older and as the production improves. Tanor late had a very good juice percentage of 61% before peak maturity. The fruit granulated and the juice percentage dropped to 43.1% before peak maturity. Brix peaked at 12.6°, with an acid percentage of 1.2%; and being a triploid, the fruit developed only 0.7 seeds per fruit on average. Tanor late had a fair crop considering the tree size and age, producing large to extra-large fruit on the trees (count 1-1xx).

Tasty 1
Tasty 1 is an experimental mandarin selection from the Eastern Cape. There was a very good crop on the trees with a uniformfruit size (count 1). The external colour development was good and peaked from T1 to T2. Tasty 1 developed a deep yellow rind colour; juice percentage was high: over 54%. Brix peaked at 11.9°, and the selection developed 4.3 seeds per fruit on average

Valley Gold
Valley Gold had a very good fruit set but there was a 95% fruit drop a month before peak maturity because of severe fruit splitting. The fruit that was left on the trees developed severe creasing. The fruit size peaked from count 3 to count 1. Juice percentage was at 56.9% before peak maturity and dropped to 46.2% at peak maturity; Brix peaked at 13.0 and the ratio reached 9.3 which did not comply with export standards. Under these heavy cross pollination conditions, the selection produced 3.3 seeds per fruit on average.

Winola
Winola is a late mandarin selection that has very high acid percentages. The selection is prone to alternate bearing patterns. Winola developed a deep orange-red external colour before peak maturity. The selection had very good juice percentages, reaching over 50%; Brix peaked at 14.4° and the acids were at 1.67%, giving a ratio of 8.8 that is too low for export. Winola produced 0.8 seeds per fruit on average. The selection had a uniform fruit size development ranging from counts 1 to count 1x.

Yosemite Gold
Yosemite Gold had no crop on the trees in the 2014 season. For the 2015 season the selection had a good crop considering the tree size and age. The trees are less vigorous than in the colder production areas, possibly due to the fact that X639 is a semi-dwarfing rootstock. The selection had very good juice percentages of over

55% in all three evaluations; and a Brix:acid ratio with the Brix peaking at 14° and the acid remaining at 1.16%. Yosemite Gold was at colour plate T1 before peak maturity. The fruit does not develop the deep orange-red rind colour in this semi-desert area seen in the cold production areas.

Conclusion

This semi-desert production area is a marginal growing area for Mandarin hybrids and only certain selections will be profitable under these growing conditions. Furr was the selection with the highest seed count at 22.5 seeds per fruit on average. Hadas had the highest juice percentage of 63.5% and also the highest Brix (16°). Valley Gold had severe fruit splitting: 95% of the crop split and the remainder had severe creasing. Etna and Sirio are the two selections most affected by these harsh growing conditions. Both selections developed large fruit with very coarse rinds as well as severe sunburn problems.

Table 5.5.27.2. Internal fruit quality data for Mandarin hybrid selections from the semi-desert region (Kakamas) during the 2015 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2015/05/13	Furr	1x	59.4	12.8	1.05	12.2	18.0	T2
2015/06/08	Furr	1	62.3	13.6	1.01	13.4	22.5	T2
2015/07/27	Furr	1x	54.3	15.3	0.93	16.4	16.1	T1
2015/04/15	Etna	1	60.7	10.2	1.06	9.7	2.8	T5
2015/05/13	Etna	1x	54.7	10.1	0.80	12.7	4.6	T1
2015/05/13	Gold Nugget	2	55.1	11.6	1.25	9.2	0.1	T5
2015/06/08	Gold Nugget	2	50.6	11.4	1.13	10.1	0.1	T3
2015/07/27	Gold Nugget	2	51.6	12.9	1.06	12.2	0.3	T2
2015/06/08	Hadas	2	51.9	11.4	2.48	4.6	9.3	T3
2015/07/27	Hadas	1	62.6	16.0	1.49	10.7	6.6	T2
2015/06/08	IRM 1	3	44.6	12.5	0.96	13.0	3.3	T3
2015/07/27	IRM 1	2	44.5	15.3	1.02	15.0	1.8	T2
2015/06/08	IRM 2	2	54.7	12.0	0.83	14.4	2.6	T2
2015/07/27	IRM 2	2	56.4	14.0	0.78	17.9	3.0	T1
2015/04/15	Nadorcott ARC	2	59.0	9.7	1.11	8.7	1.8	T6
2015/05/13	Nadorcott ARC	1	51.9	10.9	0.89	12.2	0.9	T3
2015/06/08	Nadorcott ARC	1	52.3	12.3	1.05	11.7	1.3	T1
2015/04/15	Nadorcott SL	2	58.2	9.9	0.94	10.5	0.5	T6
2015/05/13	Nadorcott SL	1	54.9	10.7	0.82	13.1	1.3	T3
2015/06/08	Nadorcott SL	1	57.3	12.5	0.91	13.8	0.8	T1
2015/07/27	Or 4	2	57.6	14.5	1.03	14.1	0.8	T1
2015/06/08	Phoenix	1	38.7	11.8	0.57	20.8	0.9	T2
2015/04/15	Samba	3	60.9	11.9	0.91	13.0	0.8	T5
2015/05/13	Samba	2	60.3	12.4	0.90	13.8	9.2	T1
2015/06/08	Samba	2	61.2	14.6	0.97	15.0	4.3	T1
2015/05/13	Shasta Gold	1x	61.0	11.1	2.41	4.6	2.2	T3
2015/06/08	Shasta Gold	1	60.5	12.2	0.88	13.9	1.1	T2
2015/07/27	Shasta Gold	1x	52.4	13.7	1.70	8.1	0.6	T1
2015/04/15	Sirio	2	59.3	10.5	1.50	7.0	4.8	T5
2015/05/13	Sirio	2	53.7	11.6	1.36	8.5	4.5	T1
2015/06/08	Sirio	1	43.9	11.9	1.26	9.5	8.9	T1

2015/04/15	Tahoe Gold	2	63.5	10.2	2.23	4.6	0.7	T6
2015/05/13	Tahoe Gold	1	61.2	11.3	1.78	6.4	0.7	T4
2015/04/15	Tango	3	56.2	10.0	1.15	8.7	1.4	T6
2015/05/13	Tango	2	56.1	10.8	1.07	10.1	2.3	T4
2015/06/08	Tango	3	56.9	12.7	1.06	12.0	2.3	T1
2015/06/08	Tanor Late	1	60.9	11.8	1.55	7.6	0.7	T1
2015/07/27	Tanor Late	1xx	43.1	12.6	1.19	10.6	0.3	T1
2015/05/13	Tasty 1	1	54.5	11.7	0.85	13.7	4.3	T2
2015/06/08	Tasty 1	1	53.8	11.9	0.81	14.8	1.6	T1
2015/05/13	Valley Gold (B17)	3	56.9	12.3	1.59	7.7	3.3	T3
2015/06/08	Valley Gold (B17)	1	46.2	13.0	1.39	9.3	2.3	T1
2015/05/13	Winola	1	57.6	11.4	1.84	6.2	0.8	T2
2015/06/08	Winola	1x	57.1	12.4	2.00	6.2	0.6	T1
2015/07/27	Winola	1	52.8	14.4	1.67	8.6	0.0	T1
2015/05/13	Yosemite Gold	1x	55.0	11.5	1.33	8.6	2.7	T5
2015/06/08	Yosemite Gold	1x	57.8	12.7	1.24	10.3	1.4	T1
2015/07/27	Yosemite Gold	1	58.0	14.0	1.16	12.1	0.8	T1

5.5.28 PROGRESS REPORT: Cultivar characteristics and climatic suitability of lemons in a semi-desert production region (Kakamas)

Project 964E by S. Meeding and J. Joubert (CRI)

Opsomming

Die 2015 seisoen was die tweede oes vir hierdie suurlemoen proef. Daar was ʼn baie swak vrugset op al die suurlemoen variteite. Dit kan toegeskryf word aan baie hoë temperature gedurende die blom tydperk. Van al agt die seleksies het Limoneira 8A die beste vrugset op die bome gehad. Limoneira 8A het ook die grootste boom ontwikkel en is baie doringagtig. All die suurlemoen seleksies se blare was ʼn ligte geel kleur gewees, asook minder dig en bosagtig soos ‘n normale suurlemoen boom moet wees.

Summary

The 2015 season was the second year of harvest for this lemon trial. There was a very poor fruit set on all the Lemon varieties. This could be due to very high temperatures during the flower period. Out of all eight varieties, Limoneira 8A had the best fruit set on the tree. Limoneira 8A had the largest tree size with very big thorns. All the lemon trees had a light yellow leaf colour and were not as dense and bushy as most lemon trees

Objectives

- To find Lemon selections suitable for the semi-desert production area.
- To produce lemon selections with Eureka like fruit shape, high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted on Eureka, Genoa, Limoneira 8A, Lisbon, Lisbon Yen Ben, Plaat B (Roos), Willow Tree Long and 2PH Lemon.

Table 5.5.28.1. List of Lemon selections evaluated at Kakamas during 2015.

Selection	Rootstock	Topworked
Eureka	X639	2010
Genoa	X639	2010
Limoneira 8A	X639	2010
Lisbon	X639	2010
Lisbon Yen Ben	X639	2010
Plaat B (Roos)	X639	2010
Willow Tree Long	X639	2010
2 PH Lemon	X639	2010

Results and discussion

2 PH seedless Lemon had the lowest number of seeds per fruit. In the third and last evaluation, the selection had 0.5 seeds per fruit. Lisbon was the selection with the most seeds with an average of 15.3 seeds per fruit. For the second season in a row, Plaat B (Roos) had the lowest juice percentage of 34.1%. Genoa, Lisbon and Limoneira 8A had the largest fruit size and peaked at counts 88. Plaat B (Roos) had the smallest fruit and peaked at count 216.

Conclusion

There was a smaller crop on the trees compared to the 2014 season. The lemon selections were not that vigorous and tree canopy was less dense. High temperatures can affect the fruit set as well as the juice percentages. Cultivation of Lemons in the semi-desert area could be a risky choice, but nets could be used to create a more suitable micro-climate.

Table 5.5.28.2. Internal fruit quality data for Lemon selections from the semi-desert region (Kakamas) during the 2015 season.

Date	Cultivar	Count	Juice (%)	Avg. Seed	Colour
2015/04/15	2PH Eureka SL	138	50.2	0.0	T4
2015/05/13	2PH Eureka SL	113	53.6	0.0	T5
2015/07/27	2PH Eureka SL	113	50.9	0.5	T2
2015/04/15	Eureka	138	46.9	6.0	T3
2015/05/13	Eureka	100	46.0	10.9	T5
2015/04/15	Genoa	100	44.7	10.0	T4
2015/05/13	Genoa	88	46.6	14.2	T5
2015/04/15	Limoneira 8A	100	46.3	8.3	T4
2015/05/13	Limoneira 8A	88	46.3	14.8	T3
2015/05/13	Lisbon	189	43.9	2.3	T5
2015/05/13	Lisbon	88	41.8	15.3	T6
2015/04/15	Lisbon	100	35.6	8.6	T4
2015/04/15	Lisbon YB	189	45.9	2.8	T3
2015/04/15	Plaat B	138	39.7	4.5	T4
2015/05/13	Plaat B	216	34.1	0.5	T6
2015/04/15	Willow Tree long	113	47.1	8.5	T3
2015/05/13	Willow Tree long	113	45.1	11.7	T5

5.6 **Climatic Regions of Southern Africa and cultivars being evaluated**

CLIMATIC REGION	AREA	PLACE	CULTIVARS
Hot-Dry	Limpopo	Tshipise	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Musina	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Letsitele	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Hoedspruit	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Hot-Humid	Mpumalanga	Malelane	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Komatipoort	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
	KwaZulu-Natal	Pongola	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Nkwaleni	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
	Swaziland	Lowveld	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
	Mozambique	Southern	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Intermediate	Limpopo	Tom Burke	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
		Letaba	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
		Levubu	Navels (Mid/Late)
			Valencias
			Mandarin Hybrids (Mid/Late)

			Lemons
		Marble Hall	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)

5.7 Approximate maturity periods

5.7.1 Approximate Clementine Maturity Periods in the Cape region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Early Clementine (Exp)																												
Oronules																												
Marisol																												
Early Oroval																												
SRA63																												
Oroval																												
Nules																												

Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

5.7.2 **Approximate Grapefruit Maturity Periods in the Northern region of South Africa**

		March				April				May				June				July				Aug				Sept			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Star Ruby																												
	Marsh / Nartia																												
	Jackson																												
	Ray Ruby																												
	Henderson																												
	Rosé																												
	Flamingo																												
	Star Ruby late																												

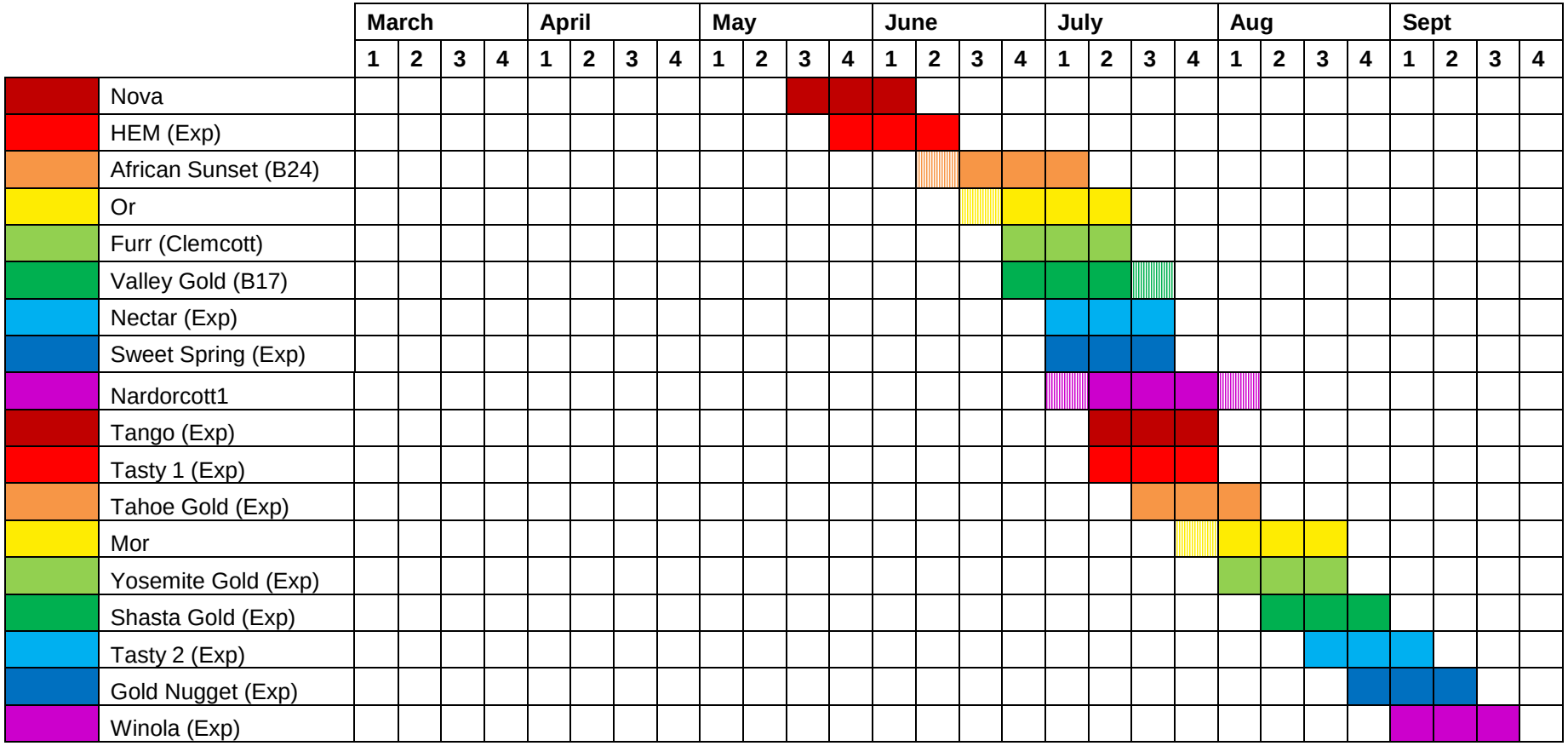
Exp = Experimental Cultivar

5.7.3 Approximate Lemon Maturity Periods in the Cape region of South Africa

		March				April				May				June				July				Aug			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Eureka																								
	Eureka SL (Exp)																								
	Genoa																								
	Lisbon																								
	Limoneira																								

Exp = Experimental Cultivar

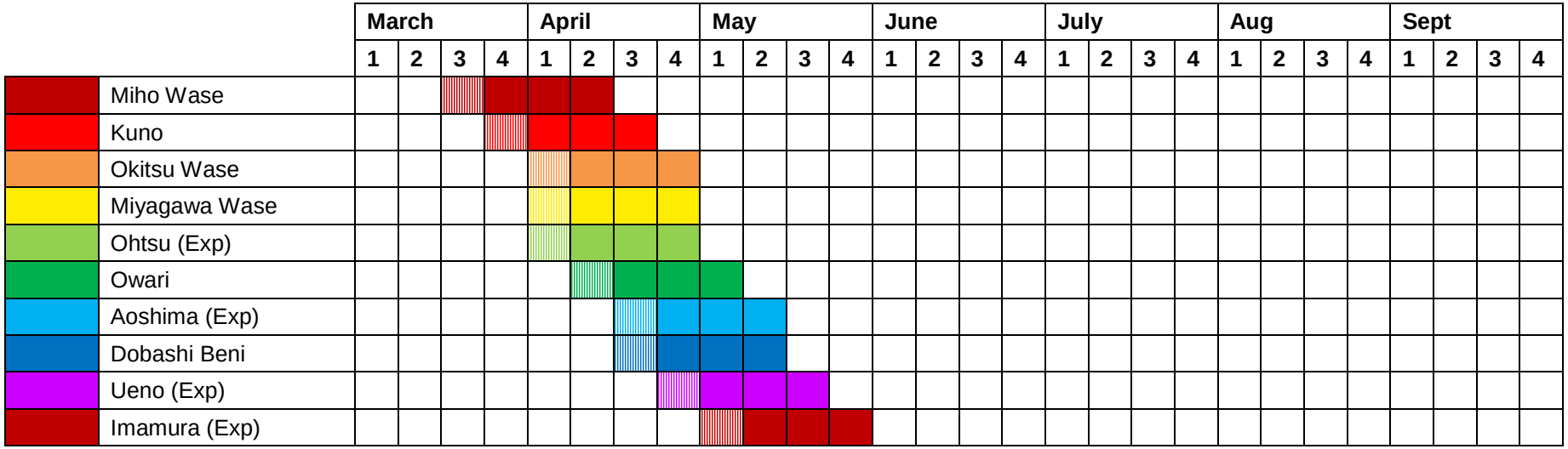
5.7.4 **Approximate mandarin hybrid Maturity Periods in the Cape region of South Africa**



Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

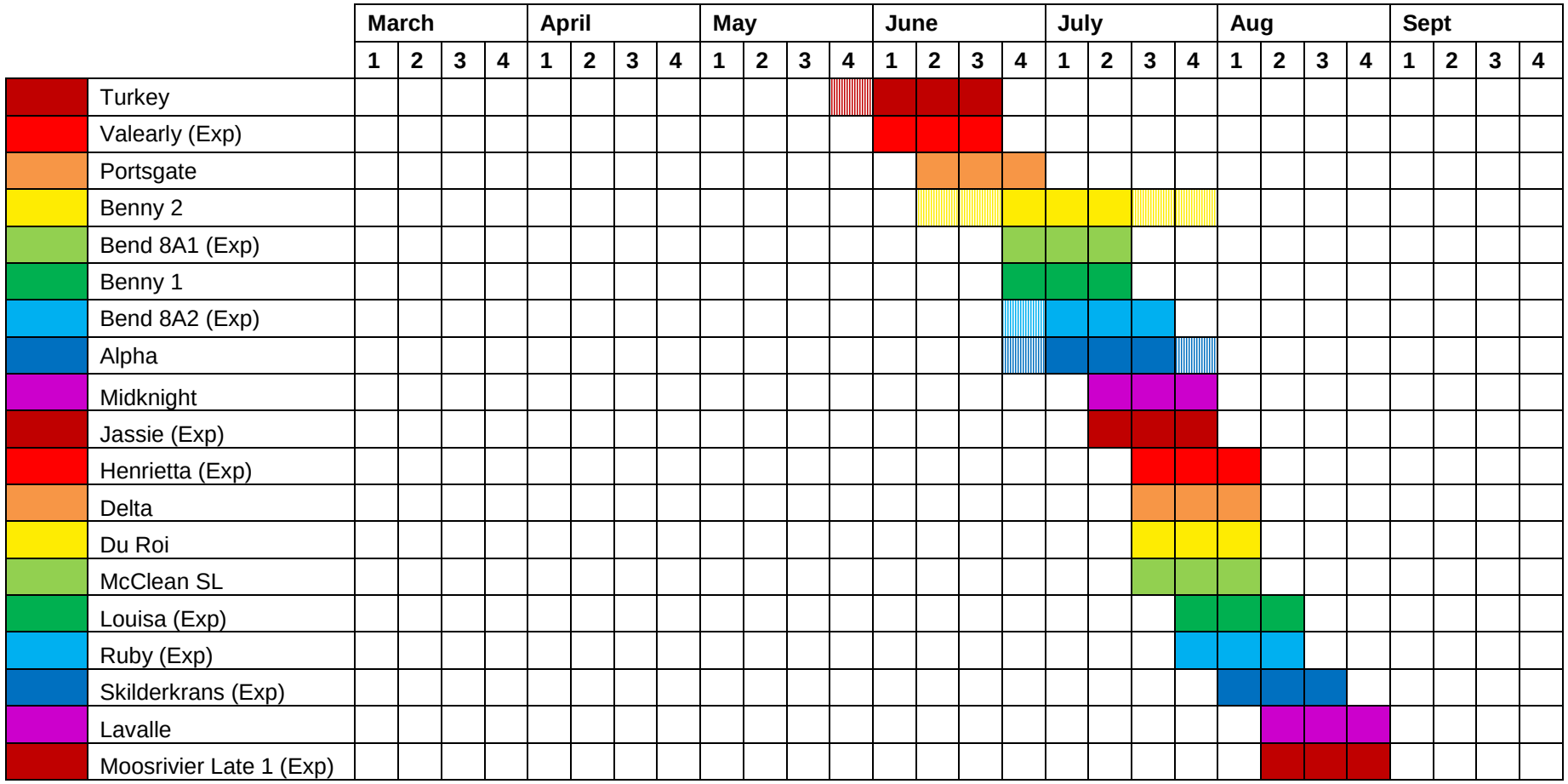
5.7.5 Approximate Satsuma Maturity Periods in the Cape regions of South Africa



Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

5.7.6 Approximate Valencia Maturity Periods in the Northern region of South Africa

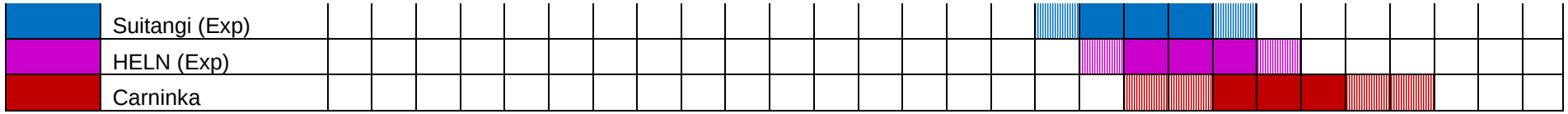


Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

5.7.7 **Approximate Navel Maturity Periods in the Cape region of South Africa**

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Fukumoto																												
Newhall/Navelina																												
HAEN (Exp)																												
Fischer																												
DRN (Exp)																												
EHN (Exp)																												
Bahianinha																												
Palmer																												
Washington																												
Cara Cara																												
EDPN (Exp)																												
Autumn Gold																												
Barnfield Summer																												
Summer Gold																												
Powell Summer																												
Witkrans																												
Lane Late																												
KSEN (Exp)																												
Cambria																												
Glen Ora Late																												
KSN (Exp)																												
Robyn																												
Lazy Boy (Exp)																												



Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

6 CITRUS IMPROVEMENT SCHEME (CIS)

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6.1 Summary

The purpose of the CIS is to enhance the standard of the South African citrus industry by ensuring that only horticulturally superior plants, which are free of viruses, diseases and pests, are supplied to producers and certified. The Citrus Growers Association of southern Africa (CGA) is responsible for the CIS and delegated its authority to CRI. In order to achieve this objective, close co-operation is required between CRI, the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC), DAFF's Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities. The organisations and committees, as well as all participating role players in the CIS are represented on the CIS Advisory Committee (CISAC), which advises CRI on the CIS operations as specified in its Procedural Guide. The 2015/16 season was again a record budwood supply year, with 4.94 million certified buds supplied to nurseries. This is 10% more buds than in 2014/15 and 38% more than in 2013/14. Supply was mostly driven by unprecedented demand for lemon and mandarin buds. A record 2.1 million lemon buds were supplied in 2015/16, compared with 1.27 million in 2014/15 and 0.72 million in 2013/14. Aside from lemons, demand for mandarins was also unprecedented with a rolling average of 1.64 million buds for the past three seasons. A slight increase in Clementine bud demand was also observed, while demand for other citrus types declined marginally. The CFB currently holds 354 cultivar lines of which 65% is privately owned or managed. This creates an additional challenge to meet the budwood demand as the roughly 50% of total budwood supply is of private cultivars, which must be coordinated with the owner or agent. Despite these challenges, the CFB continues to improve in its central supply objectives by reducing the percentage of certified buds cut in nurseries from 40% in 2012/13 down to 27.6% in 2015/16. CFB also supplies 79% of the industry's rootstock seed demand, and surplus quantities seed of most cultivars are stored as contingency measure. However, lemon demand dramatically increased the demand for Eureka compatible rootstock cultivars and these were in short supply. Rough Lemon and Volckameriana seed had to be imported from USA to meet demand. The CFB's rootstock orchards were expanded by 51% and first crops will be harvested next season. With the high demand for citrus trees, a 50% increase in nursery capacity was observed, with 25 nurseries presently certified by the CIS. The phytosanitary status of the CIS is ensured by virus-elimination and diagnostic services prior to CIS introduction and was again confirmed through routine re-indexing of mother trees as well as multiplication blocks. Citrus biosecurity in southern Africa is safeguarded through the CIS activities and the industry's support and use of CIS certified plant material. Initiatives to promulgate the CIS as a compulsory statutory scheme under the Plant Improvement Act are ongoing.

Opsomming

Die doel van die SVS is om die standaard van die Suid-Afrikaanse sitrusbedryf te verbeter deur te verseker dat slegs bome van die beste hortologiese gehalte, wat vry is van virusse, siektes en peste, aan produsente gelewer en gesertifiseer word. Die Sitruskwekersvereniging van suider Afrika (CGA) is verantwoordelik vir die SVS en delegeer sy gesag aan CRI. Ten einde hierdie doelwit te bereik, word noue samewerking tussen CRI, die Landbounavorsingsraad se Instituut vir Tropiese en Subtropiese Gewasse (LNR-ITSG), DAFF se Direktoraat van Plantgesondheid (DPH) en die sitruskwekerye verteenwoordig deur die Suid-Afrikaanse Sitrus Kwekers se Vereniging (SACNA) vereis. Daarbenewens koördineer die Kultivar en Patologie komitees die relevante SVS-aktiwiteite. Hierdie organisasies en komitees, asook al die deelnemende rolspelers in die SVS, word op die SVS Advieskomitee (CISAC) verteenwoordig en adviseer CRI oor die SVS-bedrywighede soos gespesifiseer in die operasionele riglyn. Die 2015/16 seisoen was weer 'n rekord jaar met betrekking tot okuleerhout verskaffing, waar 4.94 miljoen gesertifiseerde ogies aan kwekerye voorsien is. Dit is 10% meer ogies as in 2014/15 en 38% meer as in 2013/14. Die verskaffing is meestal gedryf deur ongekende aanvraag na suurlemoen en mandaryn ogies. 'n Rekord van 2.1 miljoen suurlemoen ogies is in 2015/16 voorsien, in vergelyking met 1.27miljoen in 2014/15 en 0.72 miljoen in 2013/14. Afgesien van suurlemoene, was die aanvraag na mandaryne ook ongekend met 'n rollende gemiddeld van 1.64 miljoen ogies vir die afgelope drie seisoene. 'n Effense toename in Clementine aanvraag is ook waargeneem, terwyl die aanvraag na ander sitrus variteitstipes effens afgeneem het. Die CFB het tans 354 kultivar lyne waarvan 65% in privaat besit is en so bestuur word. Dit skep 'n bykomende uitdaging siende dat sowat 50% van die totale okuleerhout verskaffing, dié van private kultivars is wat met die eienaar of agent gekoördineer moet word. Ten spyte van hierdie uitdagings, het die CFB daarin geslaag om steeds hul doelwit van sentrale verskaffing te verbeter. Die behoefte vir die kwekery om self te sny het van 40% in 2012/13 tot 27.6% in 2015/16 verminder. CFB verskaf ook 79% van die bedryf se aanvraag vir onderstam saad, en die surplus saad van meeste kultivars word gestoor as deel van 'n gebeurlikheidsplan. Die suurlemoen aanvraag het egter die aanvraag na Eureka-verenigbare onderstam kultivars drasties verhoog en tekorte is daarvoor ervaar. Growweskilsuurlemoen en Volckameriana

saad is vanaf die VSA ingevoer om in die aanvraag te probeer voldoen. Die CFB se onderstamboorde is 51% vergroot en die eerste drag sal aanstaande seisoen geoes word. Met die hoë aanvraag na sitrus bome, is 'n verhoging van 50% in kwekery kapasiteit waargeneem, met 25 kwekerye wat tans gesertifiseer is deur die SVS. Die fitosanitêre status van die SVS word verseker deur virus-reiniging en diagnostiese dienste voor introduksie tot die SVS en word weer bevestig deur roetine her-indeksering van die moederbome en vermeerderingsblokke. Sitrus biosekuriteit in suider Afrika word beveilig deur die SVS-aktiwiteite en ondersteuning deur die bedryf, asook die verskaffing van SVS-gesertifiseerde plantmateriaal. Inisiatiewe om die SVS as 'n verpligte statutêre skema onder die Plantverbeteringswet te promulgeer, word voorgesit.

6.2 Budwood

This report summarises the seasonal supply of budwood from June 2015 to May 2016. A total of 4,934,236 buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries. This is 10% more buds than in the same period of 2014/15 and 37.7% more buds than in the same period of 2013/14. During this period 9,490 buds were exported to neighbouring countries. Increased demand was mostly from Northern Cape (147.3%), Mpumalanga (31.4%), Eastern Cape (20.0%) and Western Cape (12.9%), whereas a decrease in demand was experienced in North West (-30.0%), Limpopo (-8.3%), and KwaZulu Natal (-2.5%). Lemon (42.5%) was the most popular citrus type, followed by mandarin (32.0%), Valencia (8.86%) and Clementine (6.6%); in 2014/15 this proportion was 25.8%, 36.6%, 11.5% and 5.7%, respectively (Tables 6.2.1 and 6.2.2). The top 30 varieties comprised 91.7% of total number of buds supplied. Eureka Lemon (2nd most popular cultivar in 2013/14 and most popular cultivar in 2012) was the most popular cultivar in 2014/15 and 2015/16, followed by Nadorcott 1 mandarin and Tango mandarin (Table 6.2.3). The need for authorised cutting in nurseries has decreased from 40.0% in 2012/13 to 31.0% in 2013/14 to 27.7% in 2015/16 (Figure 6.2.1).

Table 6.2.1. Buds supplied during the period July to June 2013/14-2015/16.

Budwood supply	2013/14	2014/15	2015/16
Local supply			
Eastern Cape	768 210	944 832	1 133 973
KwaZulu Natal	24 400	38 500	37 530
Limpopo	1 217 663	1 304 141	1 196 186
Mpumalanga	265 757	237 560	312 100
North West Province	46 500	167 900	117 600
Northern Cape	188 600	142 530	352 500
Western Cape	1 059 234	1 580 285	1 784 347
Total	**3 570 364**	**4 415 748**	**4 934 236**
International supply			
Angola		43 014	
Botswana	7 000		
Dem. Rep. Congo	12 150	12 150	8 490
Zambia	565		
Zimbabwe		23 800	1 000
Swaziland		450	
Total	**19 715**	**79 414**	**9 490**

Table 6.2.2. Buds supplied during the period July to June 2013/14-2015/16.

Area	2013/14	%	2014/15	%	2015/16	%	Growth on 2014/15
Eastern Cape	768 210	21.4%	944 832	21.0%	1 133 973	22.9%	20.0%
KwaZulu Natal	24 400	0.7%	38 500	0.9%	37 530	0.8%	-2.5%
Limpopo	1 217 663	33.9%	1 304 141	29.0%	1 196 186	24.2%	-8.3%
Mpumalanga	265 757	7.4%	237 560	5.3%	312 100	6.3%	31.4%
North West Province	46 500	1.3%	167 900	3.7%	117 600	2.4%	-30.0%
Northern Cape	188 600	5.3%	142 530	3.2%	352 500	7.1%	147.3%
Western Cape	1 059 234	29.5%	1 580 285	35.2%	1 784 347	36.1%	12.9%
Other African States	19 715	0.5%	79 414	1.8%	9 490	0.2%	20.0%
Total	**3 590 079**	**100%**	**4 495 162**	**100%**	**4943726**	**100%**	**11.7%↑**

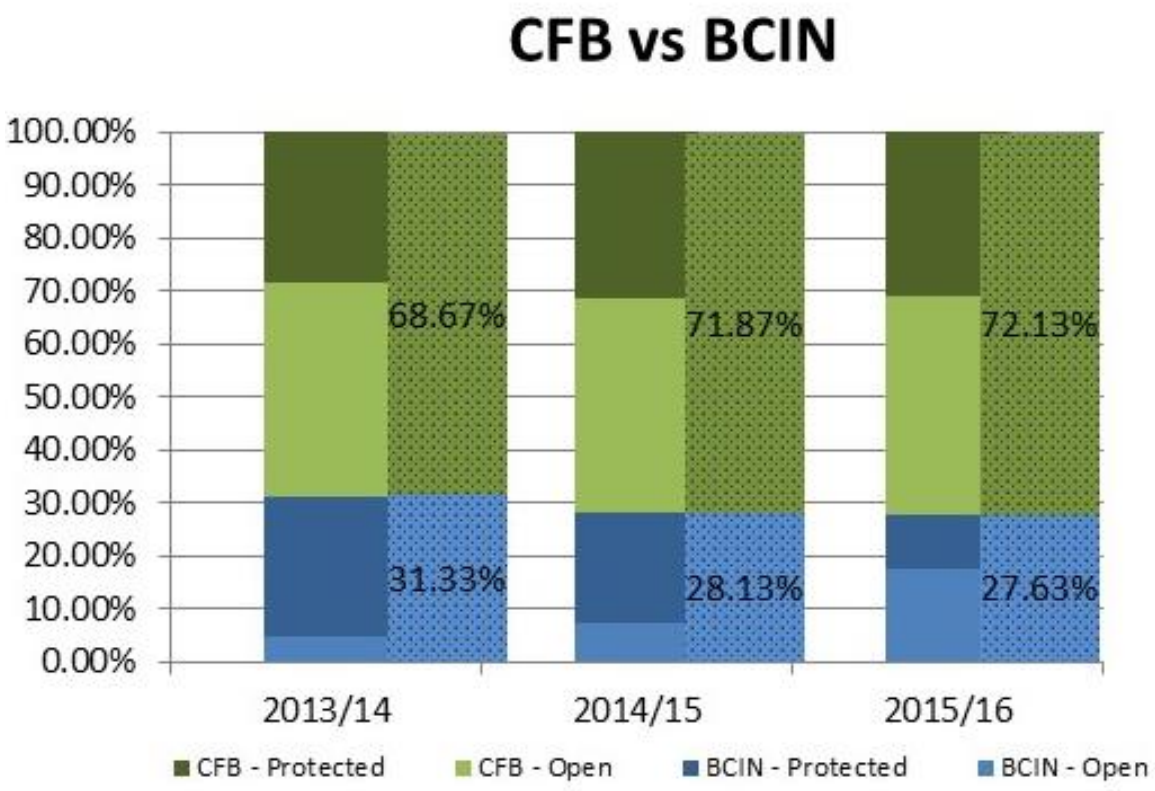


Figure 6.2.1. Budwood (% of total) of open and protected cultivars supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2013/14 to 2015/16.

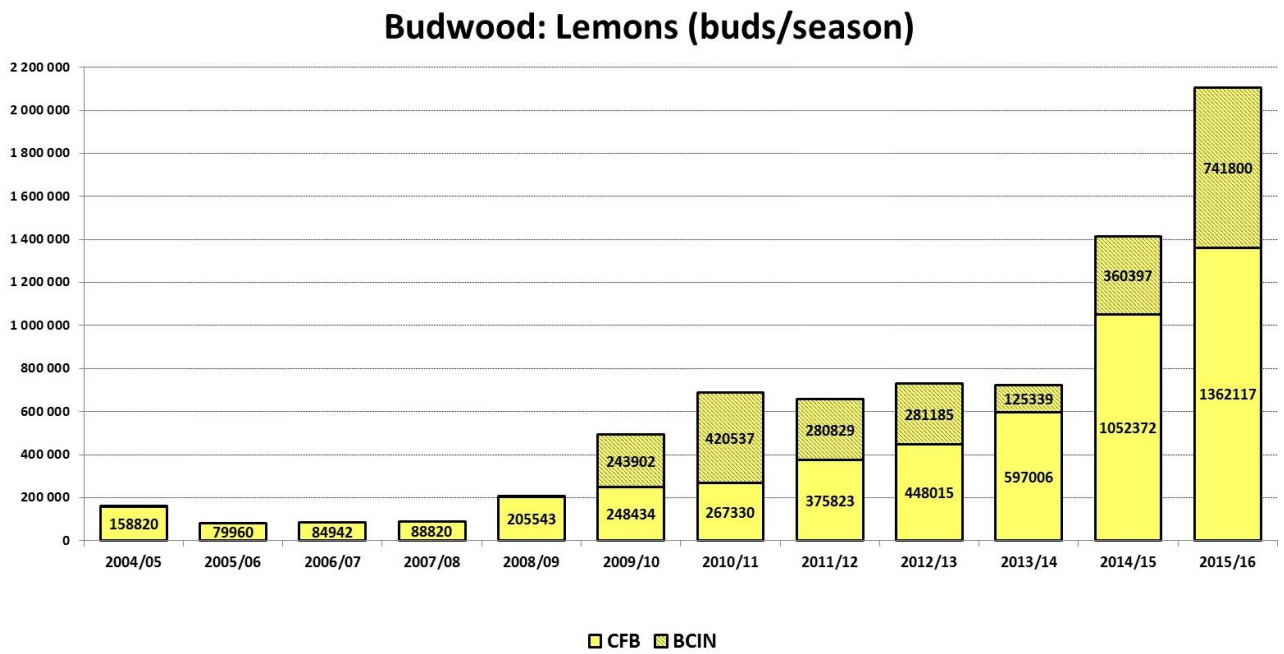


Figure 6.2.2. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods June to May from 2004/05-2015/16.

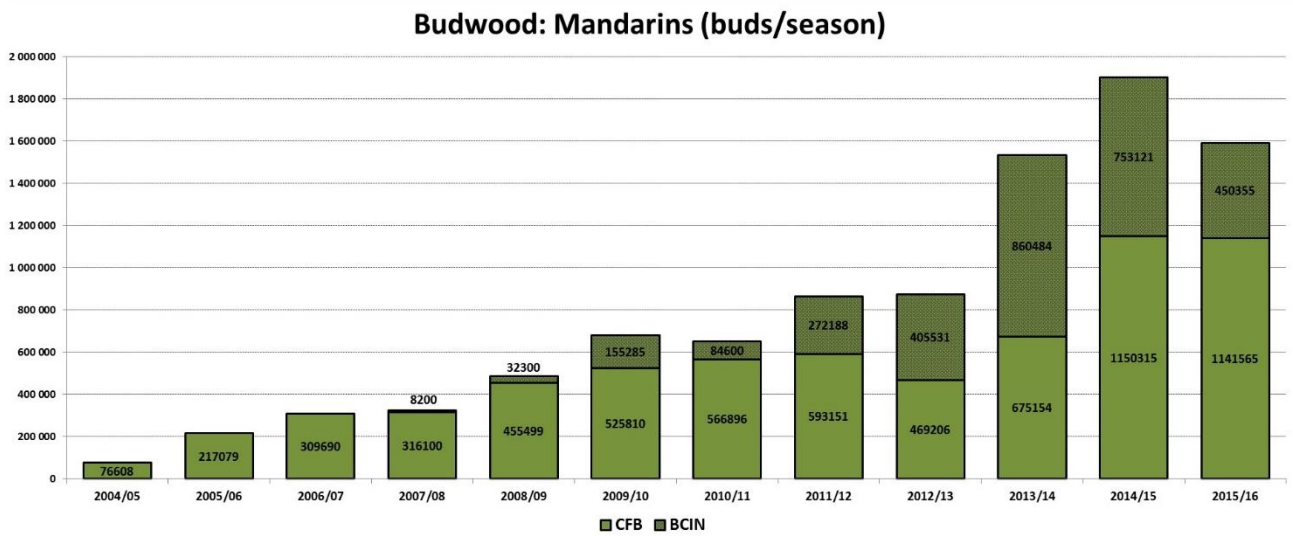


Figure 6.2.3. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2004/05-2015/16.

Table 6.2.2. Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2013/14 – 2015/16.

Variety Type	Year	EC	KZN	LIM	MPU	NWP	NC	WC	Africa	Total
Clementine	2013/14	19 952		7 500	85		5 500	47 973	1 850	82 860
	2014/15	37 759		29 350	330		7 000	183 981	6 550	264 970
	2015/16	56 601		28 920	1 010	150	26 200	211 566	1 100	325 547
Diverse	2013/14	275	1 750	3 000	120	1 200	200	520		7 065
	2014/15	80			450	800		2 035	100	3 465
	2015/16	100		2 600	890	2 000		1 640	240	7 470
Grapefruit	2013/14		1 400	35 870	41 600		49 550	270	1 455	130 145
	2014/15		1 500	21 050	14 600	3 500		700	1 200	42 550
	2015/16	660	3 000	31 770	49 650			2 092	500	87 672
Kumquat	2013/14		2 000	3 400	1 400	3 100	1 000	1 050		11 950
	2014/15			8 300	3 100	4 000		2 500	1 000	18 900
	2015/16	10		3 600	4 400	5 000		1 200		14 210
Lemon	2013/14	264 768	3 750	271 300	31 545	5 200	28 300	116 147	1 335	722 345
	2014/15	431 061	17 500	358 345	54 490	63 800	56 260	290 626	1 000	1 273 082
	2015/16	733 291	16 000	487 986	120 835	57 000	181 000	503 305	1 500	2 100 917
Lime	2013/14	20		2 800	3 460	0		6 150	365	12 795
	2014/15	10		7 400	1 000			5 940	14 050	28 400
	2015/16	2 150	1 500	1 280	2 000			8 683	100	15 713
Mandarin Hybrid	2013/14	300 717	7 500	374 663	50 690	23 900	83 200	693 658	1 290	1 535 618
	2014/15	330 685	5 000	406 586	66 285	20 500	47 450	931 797	600	1 808 903
	2015/16	225 686	2 500	248 980	65 380	17 300	109 100	914 224	750	1 583 920
Midseason	2013/14	215			40	700		5 535		6 490
	2014/15	20						4 958		4 978
	2015/16				15			3 368		3 383
Navel	2013/14	143 764	1 000	72 200	15 475	7 100	13 450	117 877	6 260	377 126
	2014/15	113 999	6 000	123 150	22 050	19 950	14 500	82 022	24 336	406 007
	2015/16	74 288	8 500	109 815	31 225	29 650	9 900	46 180	3 900	313 458
Pummelo	2013/14			3 500					15	3 515
	2014/15			2 400				20		2 420
	2015/16			1 760	15					1 775
Satsuma	2013/14	4 635	5 000	6 800	225	900		21 306	1 000	39 866
	2014/15	8 825	1 000	4 700	3 510	1 000	6 700	48 269		74 004
	2015/16	6 852	3 000	1 900	685	500	1 400	35 776	1 400	51 513
Valencia	2013/14	33 864	2 000	436 630	121 117	4 400	7 400	48 748	6 145	660 304
	2014/15	22 393	7 500	342 860	71 745	54 350	10 620	27 437	30 578	567 483
	2015/16	34 335	3 030	277 575	35 995	6 000	24 900	56 313		438 148
Total		**2847 015**	**100 430**	**3 717 990**	**815 417**	**332 000**	**683 630**	**4 423 866**	**108 619**	**13 028 967**

Table 6.2.3. Top 30 cultivars based on total number of buds supplied for seasons July to June from 2013/14 – 2015/16.

	2013/14	BCIN	CFB	2014/15	BCIN	CFB	2015/16	BCIN	CFB
1	Nadorcott 1 Man	433088	191909	Eureka Lem	192173	554767	Eureka Lem	659875	792271
2	Eureka Lem	60750	380790	Nadorcott 1 Man	276805	294737	Tango Man	131000	214930
3	Tango Man	305440	112468	Tango Man	293746	163335	Lisbon Lem	55325	233566
4	Late Val	44000	168220	Midknight Val		243515	Nadorcott 1 Man	8500	232512
5	ARCCIT1614 (B17) (Valley Gold) Man	81456	92637	Lisbon Lem	27100	188122	ARC Nadorcott LS Man	194755	40352
6	Midknight Val		170605	Witkrans 3 Nav	54303	111111	Midknight Val	10000	197662
7	Limoneira 8A Lem	53800	75466	2PH Eureka SL Lem	87912	69335	Or 4 (2) Man	23100	143576
8	Witkrans 3 Nav	39798	69740	ARCCIT1614 (B17) (Valley Gold) Man	25660	130421	ARCCIT1614 (B17) (Valley Gold) Man		165025
9	Star Ruby Grapefruit		108710	Nova Man		139324	Nova Man		150131
10	Lisbon Lem	9000	94146	Nules Cle	64221	68175	Limoneira 8A Lem	8200	140975
11	Nova Man		83157	Late Val		116178	2PH Eureka SL Lem	14500	127110
12	Or 4 Man	32300	39515	Or 4 (2) Man		101805	Witkrans 3 Nav	9600	114085
13	Cambria 3 Nav	13300	41574	Limoneira 8A Lem		84024	Nules Cle	31475	72473
14	Gusocora (G5) Val	8700	42595	RHM (Royal Honey) Man	82200	1460	Nules (2) Cle	61947	36030
15	Mor 26 Man		48457	Mor 26 Man		77985	Andes 1 Clemenluz Cle	21600	75138
16	Delta Val		47989	Genoa Lem		62743	Late Val		75210
17	Alpha Val		47006	Or 4 Man		59530	Genoa Lem	3900	62448
18	Nules Cle		35934	Nules (2) Cle	26740	32785	Leanri Man	41100	24391
19	Chislett M7 Nav		33911	Alpha Val	7200	48065	Gold Nugget Man	4100	43904
20	Empress Man		33800	Andes 1 - Clemenluz Cle	23469	29850	Delta Val		40081
21	Bennie 2 Val		31095	Cambria 3 Nav		46873	Cara Cara Nav	13800	25115
22	Lane Late (Cal.) Nav		30403	IR M2 (QDPI #283) Man	38800	850	FE 1 (Jackson 1) Gra		35135
23	Glenora Late Nav		26303	Bahianinha Nav		35950	Mor 26 Man		30201
24	Nules (2) Cle		25520	Washington Nav		35515	Cambria 3 Nav		29418
25	Carninka Late Nav		25296	Empress Man	3000	30900	Star Ruby Gra		28825
26	Bahianinha Nav		24735	Autumn Gold Nav	23219	7436	Turkey Val		28415
27	2PH Eureka Seedless Lem		23951	Delta Val		30323	Belalate Sat		26360
28	Or 4 (2) Man		23435	Gusocora (G5) Val		28385	Palmer Nav		24276
29	Genoa Lem		20211	Belalate Sat		28330	Tanorlate (2) (MH7 (2)) Man	200	23360
30	Benny 2 Val		19400	Bearss Lim		27825	Miho Wase Sat		20452
	Top 30	**1081632**	**2168978**	**Top 30**	**1226548**	**2849654**	**Top 30**	**1292977**	**3253427**
	All other Cultivars	43189	296280	All other Cultivars	37800	381160	All other Cultivars	73031	324291
	Total	**1124821**	**2465258**	**Total**	**1264348**	**3230814**	**Total**	**1366008**	**3577718**

6.3 Seed

The unprecedented increase in demand for Eureka compatible rootstocks in 2014/15 and 2015/16 has necessitated the need to import seed from the USA. During May 2015 to April 2016, 3624 litres of seed were supplied locally by the CFB and 341 litres of seed were exported (Table 6.3.1). Carrizo citrange remains the most popular rootstock (46.9%), followed by C35 citrange (13.8%), X639 (8.6%), Rough Lemon (8.5%) and Swingle citrumelo (8.5%) (Table 6.3.2).

Table 6.3.1. Seed (litres) supplied by the CFB and Seed Produced by Nurseries during the periods May to April 2013/14 – 2015/16.

Seed supply	2013/14	2014/15	2015/16
Local supply			
Eastern Cape	802	697	570
Gauteng		0	40
KwaZulu Natal	10	43	34
Limpopo	1 663	2 170	1 451
Mpumalanga	77	84	128
North West Province	29	98	125
Northern Cape	199	172	252
Western Cape	941	1 084	1 024
Total	**3 721**	**4 350**	**3 624**
International supply			
Australia	33	15	
Botswana			2
Chile	41	103	126
Congo	9	25	-9
Morocco	9		
Mozambique		31	
Portugal	80	140	213
Reunion		10	
United Arab Emirates		6	
Zambia	27	13	8
Zimbabwe		2	1
Total	**199**	**345**	**341**

Table 6.3.2. Seed (litres) supplied by the CFB during the periods May to April 2013/14-2015/16.

Variety Type	2013/14	%	2014/15	%	2015/16	%
Experimental		0.0%	4	0.1%	0	0.0%
Benton Citrange		0.0%		0.0%	19	0.5%
C35 Citrange	987	25.2%	677	14.4%	548	13.8%
Carrizo Citrange	1589	40.6%	1988	42.4%	1858	46.9%
Cleopatra Mandarin	12	0.3%	14	0.3%		0.0%
Flying Dragon	120	3.1%	140	3.0%	213	5.4%
Minneola x Trifoliata	98	2.5%	146	3.1%	162	4.1%
Rough Lemon	490	12.5%	572	12.2%	336	8.5%
Swingle Citrumelo	383	9.8%	551	11.7%	339	8.5%
US812 (Sunki X Benecke)		0.0%		0.0%	12	0.3%
Troyer Citrange	44	1.1%	10	0.2%	5	0.1%
Volckameriana	75	1.9%	168	3.6%	133	3.4%
X639	82	2.1%	414	8.8%	339	8.6%
Yuma Citrange	36	0.9%	10	0.2%		0.0%
Total	**3920**	**100.00%**	**4695**	**100.00%**	**3965**	**100.00%**

6.4 Production

With multiplication trees in production, the CFB presently carries a potential budwood stock of >7 million buds per year of 354 cultivar lines. As the top 30 varieties comprise 91.7% of demand, multiplication tree stocks are being managed in order for CFB to be able to timeously supply demand of the sought-after varieties. During April to March 2016, 21550 increase trees were budded. These consisted of 15 new releases from the ARC-

ITSC and re-multiplication of 58 existing cultivars (Table 6.4.1). The insulated tunnels allowed for faster multiplication of a much higher concentration per surface area of increase trees. Thus far this initiative seems to be successful as active growth was observed into winter and budwood was already harvested in the summer from the small increase trees. The 6705 trees in 2-L and 230-ml pots that were budded last season in the rapid multiplication tunnel were replanted in 10-L bags in Greenhouse 4 C. A further 7248 seedlings in 230-ml polytubes and 7776 seedlings in 2-L bags are available for budding in autumn and spring of the next reporting period. Fifteen different rootstock varieties (total of 1205 trees) were planted in two new seed source orchards with a high density planting distance of 6 × 1.5 m to increase early yields; alternate trees will be removed at a later stage to reduce the planting density.

Table 6.4.1. New cultivar introductions from 2013/14 – 2015/16.

Area	**2013/14**	**2014/15**	**2015/16**
New introductions from ARC's STG laboratory	17	17	15
New introductions from CRI's STG laboratory	11	18	
Re-multiplication of existing cultivars	64	13	58

6.5 Tree Certification

There were 2,764,719 trees certified during April 2014 to March 2015. This is 724,108 more trees than in the same period of 2014/15 and 1,840,186 more than in 2013/14 (Table 6.5.1). Of the applications received, the tree certificates not meeting the certification requirement were 54805, 20742 and 25235 for the last three consecutive years (Table 6.5.2). This was mostly because of the Phytophthora status or tree age that exceeded 30 months after budding. The trees not meeting the certification requirements were reduced from 5.6%, to 1% to 0.9%.

6.6 Nursery Certification

Twenty-four nurseries were visited during the May 2015 audits. Twenty-three nurseries retained their certification status, while one nursery was provisionally certified and another has postponed their inspection. Upon completion of the outstanding requirements, the provisionally certified nursery may be fully certified.

Twenty-seven nurseries were visited during the November 2015 audits. Twenty-three nurseries retained their certification status, two were provisionally certified as new nurseries and another two were provisionally certified. The increase in Phytophthora infestation in certain nurseries is of concern and additional support is given to assist these nurseries to correct the problem. Table 6.6.1 lists the CIS certified nurseries.

Table 6.5.1. Trees certified during the period April to March from 2013/14-2015/16.

Variety Type	Year	EC	GP	KZN	LIM	MPU	NW	NC	WC	NWP	Exported	Total
Clementine	2013/14	1200	5560		20699	6820			34043			68322
	2014/15	3780	310	2200	670	20	2334		24711		3647	37672
	2015/16	19353			10215	8710			35519			73797
Diverse	2013/14						1435		425			1860
	2014/15		300		19							319
	2015/16				2500				1200			3700
Grapefruit	2013/14				3340	9080	970					13390
	2014/15	626		1112	4874	9014			500		100	16226
	2015/16	2300	13370		17930	17270					6460	57330
Lemon	2013/14	101014		3250	12923	26800	6900		15924		6500	173311
	2014/15	151698		11520	126672	39403	12407	600	63664		14940	420904
	2015/16	363833	8000	5200	109820	113980	15380		109478		7935	733626
Lime	2013/14				1500				435			1935
	2014/15			1500	504	2103			500		300	4907
	2015/16	2355		200	1300	596						4451
Mandarin Hybrid	2013/14	44061		6000	35414	29105	33435		167823		500	316338
	2014/15	207534		5150	135623	153123	59460	22247	128686		21064	732887
	2015/16	227831		1020	149772	335931	13380	6320	420957	3330	13640	1172181
Navel	2013/14	86781	50	1500	2492	64632	13860	5847	14650		3100	192912
	2014/15	105957			59290	45556	6520	3600	99455		19825	340203
	2015/16	98937		2000	62435	34475	5826	11500	83333	1300	2175	301981
Pumelo	2013/14						6					6
	2014/15					430						430
	2015/16					845						845
Satsuma	2013/14	9900	230		1153	1720	940	400	14274		1930	30547
	2014/15	20240	4010		5703	1458			18957		717	51085
	2015/16	7429			2744	2760		10000	19602			42535
Valencia	2013/14	12770	4560	1600	64795	24167	8300		8020		1700	125912
	2014/15	18213	330	520	238875	79530	21305	6410	33500		37295	435978
	2015/16	79228	19970	300	165916	78850	5179		23530	1300		374273
Total		**1565040**	**56690**	**43072**	**1237178**	**1086378**	**207637**	**66924**	**1319186**	**5930**	**141828**	**5729863**

Table 6.5.2. Trees not meeting the certification criteria during the period April to March from 2013/14-2015/16

Tree Certification	Year	EC	GP	KZN	LIM	MPU	NW	NC	WC	NWP	Exported	Total
Certified	2013/14	255726	10400	12350	142316	162324	65846	6247	255594		13730	924533
	2014/15	508048	4950	22002	572230	330637	102026	32857	369973		97888	2040611
	2015/16	801266	41340	8720	522632	593417	39765	27820	693619	5930	30210	2764719
Non Certified	2013/14						750		44025			54805
	2014/15				470				7476			20742
	2015/16								1110			25235
Total		**1565040**	**56690**	**43072**	**1237648**	**1086378**	**208387**	**66924**	**1371797**	**5930**	**141828**	**5830645**

Table 6.6.1. CIS Certified Nurseries in November 2015

Nursery	Town	Province	Contact Person	Tel	Fax	Cell	Email
Apapanzi Kwekery	Kirkwood	Eastern Cape	Nellis Meiring	042 230 1483	042 230 0923	082 550 6210	nellis@srvalley.co.za
Atwell Citrus Nursery**	Kirkwood	Eastern Cape	Wayne Attwell	042 230 1560	086 674 3312	072 463 7118	attwellcitrus@srvalley.co.za
Augsburg Kwekery	Clanwilliam	Western Cape	Alta Laing	082 952 8127	086 661 4372	079 527 0316	admin@augsburgnursery.co.za
BF Joubert Kwekery	Kirkwood	Eastern Cape	Francois Joubert	042 230 0309	042 230 0280	084 951 1922	bfjkweek@srvalley.co.za
Casmar Kwekery	Mooinooi	North West	Neville Wenhold	014 574 3152	014 574 3798	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	Citrusdal	Western Cape	Patricia Willemse	022 921 3526	022 921 3957	076 622 7007	info@cederbergtreenursery.co.za
Du Roi Kwekery	Letsitele	Limpopo	Felix Hacker	015 345 1650	015 345 1414	082 879 5923	felix@duroibugs.zo.za
Esselen Kwekery	Malelane	Mpumalanga	Leon Esselen	013 790 0160	013 790 0492	083 325 0565	esselenk@mweb.co.za
Gamtoos Kwekery	Patensie	Eastern Cape	Keuler Engela	042 283 0506	042 283 0978	072 260 9813	keuler@rikusld.co.za
H J Joubert Kwekery	Montagu	Western Cape	Herman Joubert	023 614 2237	023 614 2237	082 578 5747	hopewell@breede.co.za
Henley Citrus	Letsitele	Limpopo	Charles Boyes	015 386 0211	015 386 0248	082 264 9916	charles@bigday.co.za
Letsitele Kwekery	Letsitele	Limpopo	Barend Vorster	015 345 1600	015 345 1601	083 259 5590	barend@mahela.co.za
Loskop Kwekery	Marble Hall	Limpopo	Jan Odendaal	082 040 0014	086 623 0912	082 491 0538	loskopkwekery@ctecg.co.za
Mistkraal Nursery	Kirkwood	Eastern Cape	Tyna Ferreira	042 230 0614	042 230 1461	082 789 5150	beans@srvalley.co.za
Namakwaland Sitrus	Clanwilliam	Western Cape	Tobias Basson	027 482 2503	027 482 1562	082 784 4123	tobias@namakwalandsitrus.com
Ngwenya Kwekery	Malelane	Mpumalanga	Milanie v/d Merwe	013 790 3004	013 790 3480	082 418 7693	milanie@riversidefarm.co.za
Oase Sitrus Kwekery	Hartswater	Northern Cape	Gerrit Schlebusch	053 474 2080	053 474 2080	082 907 1562	oasekwekery@lantic.net
Oranjerivier Sitrus Kwekery	Kakamas	Northern Cape	Blom Rossouw	054 441 0183	086 544 9691	083 306 0622	osk@vodamail.co.za
Paksaam Kwekery*	Patensie	Eastern Cape	Pieter Lamont	042 283 0201	042 230 1461	072 575 4471	paksaam@gamtoos.co.za
Rietvlei Kwekery	Tzaneen	Limpopo	Lucas McLean	083 630 3236	086 672 8450	083 630 3236	rietvlei@global.co.za
Sondagsrivier Hillside Kwekery	Kirkwood	Eastern Cape	Willem Truter	042 230 0349	042 230 0510	083 227 6655	willem@srvalley.co.za
Stargrow Kwekery	Citrusdal	Western Cape	Marco du Toit	022 921 2232	022 921 2747	082 563 0795	stargrowcitrus@alazon.co.za
Sundays' River Citrus Nursery **	Kirkwood	Eastern Cape	Riaan Slabbert			072 184 8726	srcnursery@igen.co.za
Tulbagh Kwekery	Tulbagh	Western Cape	Bredell Roux	023 230 0694	023 230 1353	082 214 2520	admin@tulbaghnursery.co.za
Tweeling Kwekery	Kirkwood	Eastern Cape	Jan Potgieter	042 230 1408	042 230 1408	082 560 2179	tweeling@srvalley.co.za
Waterfall Nursery*	Adelaide	Eastern Cape	Rudi van der Meulen	046 684 0738	046 684 1451	082 695 3433	waterfall@intekom.co.za
Witkrans Kwekery	Boshoek	North West	Linda Grobler	014 573 3036	014 573 3036	082 414 4739	witkrans1@mweb.co.za

*Provisionally certified **Provisionally certified – new Nursery

6.7 Statutory Improvement Scheme

The statutory CIS proposal was extensively discussed and debated in meetings with all participating citrus nurseries, a retail nursery, cultivar management companies and growers. A status document stating the benefits and detriments of a voluntary or compulsory statutory improvement scheme, including summarised feedback and inputs from all stakeholders, was discussed at a public workshop facilitated by the NAMC on 9 April 2014. The workshop was attended by 38 persons representing stakeholders, including growers, SACNA, nurserymen, cultivar managers, CGA, CRI and DAFF representatives. The workshop debated matters arising from the consultation process on which more clarity or consensus was required. The NAMC meeting concluded, as was reported in 2013/14, that a compulsory scheme offered the most advantages as well as protection from biosecurity risks for the citrus industry in South Africa, but that the needs of all role players including those not supportive of a compulsory scheme should be considered. Subsequently, meetings were also held with private cultivar managers and SACNA, of whom certain members opposed a compulsory scheme, as well as the ARC who did not attend the workshop. The issues raised by the ARC in its initial opposition of the proposal have been resolved on operational level, but official notification from ARC management is still pending. This issue is receiving ongoing attention.

6.8 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5 km around the CFB as a citrus free area, was published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DAFF. Most residents have removed their citrus trees. DAFF has made several follow-up visits to owners refusing to remove trees, and is still addressing the matter with the remaining two owners.

6.9 Shoot tip grafting (STG), pre-immunisation and nucleus block management

Project 790 by J.H.J. Breytenbach S.P. van Vuuren and G. Cook (CRI)

Opsomming

Groeipunt enting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is vier nuwe seleksies ingedien vir GPE en ŉ verdere 34 seleksies van vorige introduksies is in verskeie fases voor vrystelling. Virusvrye boompies van verskillende kultivars en seleksies word as ŉ genebron in ŉ insekvrye tonnel by CRI in Nelspruit bewaar. Virusvrye materiaal word met ŉ toepaslike *"Citrus tristeza virus"* bron gepreïmmuniseer voordat dit aan die Sitrus Grondvesblok (GVB) by Uitenhage vrygestel word. Vier nuwe seleksies is aan die GVB voorsien en is ook by die CRI genebron gevoeg. ŉ Kultivar agent het 48 van hulle seleksies bygevoeg tot die CRI genebron, wat afkomstig is van die LNR genebron as deel van die duplikasie van die twee genebronne. Die genebron bestaan tans uit 354 kultivars en seleksies. Virusvrye materiaal word aan verskeie kultivar eienaars op versoek verskaf wat deur hulle gebruik word vir uitvoer of eksperimentele doeleindes. Hierdie projek word gesluit maar die aktiwiteite word geïnkorporeer ŉ nuwe projek 1144.

Summary

Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before introduction into the Citrus Improvement Scheme. During the current year four new selections were received for STG and a further 34 submissions, from previous years, are at various stages in the process before release. A virus-free gene source is maintained in an insect-free tunnel at CRI. Virus-free material is pre-immunised with a suitable *Citrus tristeza virus* source before it is supplied to the Citrus Foundation Block (CFB) at Uitenhage. Four new selections were supplied to the CFB and added to the CRI gene source. A cultivar agent submitted 48 selections to be maintained at the CRI facility as part of the duplication of the gene sources at the CRI and ARC facilities. The gene source at CRI comprises of 354 cultivars and selections at present. Virus-free material is supplied to various cultivar owners on request for either export or trial purposes. This project is closed but the activities will be incorporated in project 1144.

6.10 Diagnostic services for graft transmissible diseases

Project 796 by J.H.J. Breytenbach, S.P. van Vuuren and G. Cook (CRI)

Opsomming

Die sukses van die Sitrusverbeteringskema (SVS) berus op ŉ fitosanitêre program wat op ŉ diagnostiese opsporing van die teenwoordigheid van skadelike patogene gebaseer is. Die SVS behels die eliminering van die patogene en die onderhou en verspreiding van gesonde voortplantingsmateriaal. Biologiese en molekulêre indeksering word gedoen op nuwe toevoegings tot die SVS voordat die materiaal aan die Grondvesblok

verskaf word asook herindeksering van moederbome wat by die Grondvesblok (GVB) gehuisves word. Daar word hier verslag gelewer op die voortdurende aktiwiteite van die SVS. Die moederbome by die GVB word op ŉ rotasie basis elke tweede jaar geherindekseer om te bepaal of enige strawwe CTV rasse, of enige sitrus viroïede, in die moedermateriaal voorkom. Die biologiese evaluasie van die CTV virulensie in 224 moederbome is gedurende die jaar begin, die resultate is egter nog nie bekend nie. Viroied indeksering van dieselde 224 moederbome is ook gedoen en geen besmetting is opgespoor nie. Sitrus viroïed indeksering van vermeerderingsblokke in twee kweekhuise by die GVB is voltooi. Algemene diagnostiese dienste en ondersoeke na probleme t.o.v. ent-oordraagbare siektes in die industrie word op ŉ *ad hoc* basis gedoen en verslag word ook in hierdie projek gelewer. Enkel viroïed spesies is vir sewe viroïede verkry deur die vervaardiging van besmette klone vir elke viroïed spesie. Hierdie klone dien as maklik beskikbare bronne vir studies van simptoom uitdrukking van enkel of spesifieke kombinasies van viroïed besmettings. Hierdie projek word getermineer maar die aktiwiteite word geïnkorporeer in Projek 1144.

Summary

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, the elimination thereof, and the maintenance and distribution of healthy propagation material. Biological and molecular indexing is done on new introductions prior to release to the Citrus Foundation Block (CFB) as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents are present or have been inadvertently introduced. The ongoing activities of these CIS functions are reported. The mother trees maintained at the CFB are indexed every two years on a rotating basis for the presence of severe CTV strains and for the presence of citrus viroids (CVd). The biological evaluation of CTV severity in 224 mother trees was initiated and the results are still pending. Biological and molecular indexing for viroids on the same 224 mother trees was concluded. No viroids were detected on these mother trees. Viroid indexing of the first two greenhouses of the CFB multiplication blocks was completed. General diagnostics and investigations into *ad hoc* industry problems and concerns relating to graft transmissible diseases are also reported within this project. Single viroid species sources for seven viroids were obtained by producing infectious clones for each viroid species. These clones serve as readily available inoculum sources for studying symptomology of single or specific combinations of viroid infections. This project is terminated but the activities will be incorporated in Project 1144.

7 INTERNATIONAL VISITS

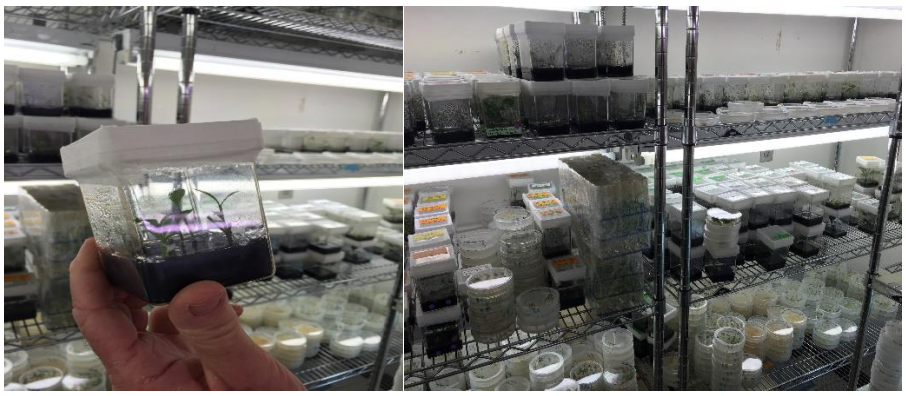
7.1 J. JOUBERT
Report on visit to the USA (Florida and California) to update our knowledge on cultivars and rootstocks (Jan/Feb 2016)

Florida (UF Lake Alfred)

My first introduction to Florida's Citrus industry was at the University of Florida's campus (IFAS) for citrus research and education centre at Lake Alfred. Bill Castle was the first person on my list, being a world leader in citrus rootstock development and breeding. We discussed the latest rootstock trials and the new selections available for evaluations with numerous scions. The need for smaller tree size as well as adaptability of the new rootstocks to replant soils were some of the focus points. Better crop production, precocious bearing, optimal fruit size and optimal internal quality were important factors during the development process.

Fred Gmitter and Jude Grosser (cultivar breeders) joined Bill and myself later that day to discuss future scion and rootstock trial work. They were in the process of planting a combination trial that included all their latest

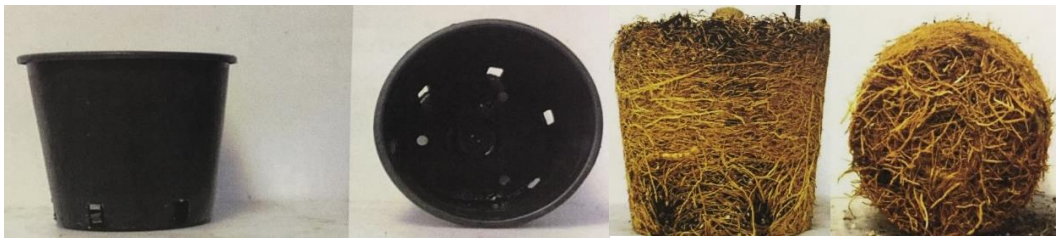
selections and a wide range of the new rootstocks, including some of Kim Bowman's new rootstock selections. Jude arranged a special visit to his lab and breeding facilities, discussing new selections in the pipeline. He was using tissue culture methods to establish and multiply the new bud-wood material for testing purposes, as illustrated below.



Fred accompanied me to their mother material facility where trees were kept in different pots to protect their selection source. They used different pot types to keep their trees healthy and in good condition for optimal root development and growth.

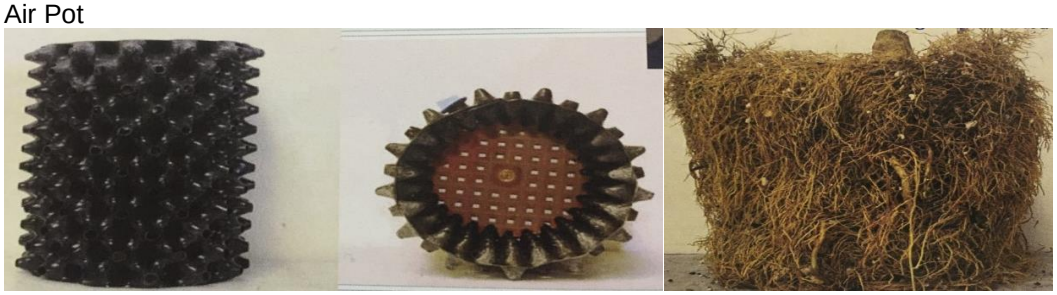
<u>Root development - different pot types</u>

Traditional smooth sides



Root routing

Air Pot



They have a facility called CUPS (Citrus Undercover Production System) where the cultivar trial trees were kept in pots to protect them from HLB infection for evaluation purposes. A fully automated spray machine was used inside the CUPS facility to control pests etc. Data loggers were used to monitor minimum and maximum temperature, humidity in the soil and soil temperature.

CUPS Facility

Part of the CUPS facility was the Thermotherapy - heat treatment (42°C) process, where they steam-treated the scion and roots in pots for HLB (Asian greening). This treatment was only possible in a controlled environment (pots in under-cover structure or pots in an open environment) with the limitation of not being able to treat citrus trees in the ground (citrus orchard).

Thermotherapy - heat treatment facility

There are a wide range of new cultivars, including sweet red grapefruit and easy peeling mandarins with very high sugar levels in the pipeline that will be imported to South Africa for evaluation purposes to expand the current selection range.

Florida (USDA Fort Pierce)

Kim Bowman, situated at the USDA facility in Fort Pierce is a rootstock breeder that was involved with the development of the US-812 (Sunki x Benecke) rootstock imported to South Africa for replant conditions and

more specifically high pH soils. Kim has a number of new promising rootstocks in the pipeline for evaluation purposes in South Africa, including dwarfing selections for more compact trees. Kim showed me around their facility, glasshouses and new rootstock trial in open ground orchards.

Kim Bowman at rootstock trial

They also make use of tissue culture practises to increase their rootstock material for trial purposes. The rootstock seeds were planted in testubes to germinate. When the rootstocks are ready they bud the scion cultivars onto the rootstocks to produce citrus trees for evaluation purposes. These trees will then be planted into open-air trial blocks for evaluation purposes to determine crop production, fruit size, internal quality, colour development and compatibility qualities.

Propagating rootstocks for trial purposes in the glasshouse facilities

California (UCR - Riverside)

Tim Williams, breeder at the University of California, Riverside, accompanied me to their breeding and evaluation facility at Riverside. I met the breeding and development team at the Riverside campus. They are working on numerous new low-seeded and seedless mandarin cultivars, as well as low naringin (bitter taste) grapefruit options.

Ventura area is cool and close to the coast with large lemon plantings, mainly Eureka and Lisbon. Tim took me to a trial site where they were looking at seed content between the original cultivar and the irradiated version. They do physical seed counts on the fruit as a comparison. Brokaw is one of the nurseries in the area producing very good quality citrus trees to the growers.

The “WORLD AG EXPO” was at Bakersfield in the Central valley with displays of equipment and agricultural practices, including pruning and spray machines. Nurseries had trees on the display and orders for new plantings were made by citrus growers.

WORLD AG EXPO

California (UCR – Lindcove)

At Lindcove Field Station there is an open foundation block with all the different cultivar selections included to evaluate throughout the citrus season. The breeding team can experience the potential of the cultivar and decide to develop a source to include in different trial blocks for evaluation purposes. Included was a seedless lemon selection with very promising characteristics and developing smaller thorns compared to the original cultivar (improved selection).

Seedless lemon (small thorns)


The last stop was at Mulholland Citrus nursery near Orange Cove, owned and managed by Tom Mulholland. They have a tunnel facility with no interior frame structure, only supported by two electric fans. There is a solar back-up system in case of power failures, as well as an additional electric fan in case of mechanical failure.


The nursery is world renowned for quality citrus tree production and well known for excellent citrus tree propagation to the growers, as illustrated below.


8 EXTENSION / VOORLIGTING April 2015 - Maart 2016

By/Deur Hennie le Roux, Hannes Bester, M.C. Pretorius, Keith Lesar, Dawid Groenewald, Andrew Mbedzi en Melton Mulaudzi (CRI)

8.1 VOORLIGTINGOORSIG

8.1.1 Die 2015 Seisoen

Die skatting vir 2015 vir die totale uitvoervolumes was aanvanklik 113.1mil kartonne, maar is later afwaarts aangepas na 111.8mil kartonne, hoofsaaklik a.g.v nawels en pomelo's wat effens laer was. Aan die begin van die jaar is 'n baie moeilike seisoen voorspel, maar die uiteinde was ŉ uitstekende uitvoerseisoen met ŉ rekord 118.1mil kartonne wat verpak is. Behalwe vir nawels, wat 1.5 miljoen kartonne minder as die 2014 seisoen was, was al die ander sitrustipes se volume hoër as die vorige jaar.
Vir sommige produsente was dit hul beste jaar ooit, wat inkomste aan betref. Goeie verkoopspryse en die swak wisselkoers het tot goeie verdienste vir produsente gelei. Dis net weereens ŉ bewys van hoe vloeibaar die uitvoermark kan wees. Verlede seisoen was veral vir pomelo-produsente besonder moeilik en die vooruitsig was duister vir 2015, maar die samewerking onder die pomeloprodusente om die markte beter te bestuur het ook daartoe bygedra dat beter pryse behaal is as die vorige seisoen.

Behalwe vir Satsumas was die terugvoer oor die gahalte van die vrugte algemeen goed. Foto's was wel van tyd tot tyd vanaf uitvoerders ontvang met tekens van bederf en skildefekte. Dis duidelik uit boordbesoeke, wat wyd oor die produksiegebiede gedoen is, dat produsente oor die algemeen heeltemal te lig snoei en nalaat om somersnoei te doen, praktyke wat belangrik is vir goeie gehalte, effektiewe siekte- & plaagbeheer, en skil-integriteit. Omdat die markte sterk was, is daar egter nie groot gehalteprobleme gerapporteer nie. Een van die meer kommerwekkende probleme wat ervaar is, was swamgroei op palette, wat toenemend voorgekom het. Dit kan wesenlike probleme vanuit 'n marktoegangsoogpunt skep indien dit nie betyds uitgesorteer word nie. Koue-steri 'failures' is ook gerapporteer, veral na China. Die Cooling Working Group is besig met inisiatiewe om dit op te volg.

8.1.2 Die 2016 Seisoen

Die huidige seisoen het uiterste uitdagings en aanpassings tot gevolg gehad weens die verlammende droogte en bogemiddelde hoë temperature wat in meeste produksiestreke voorkom. Werkswinkels oor droogtebestuur is in Letsitele en Hoedspruit gehou, asook individuele produsent-besoeke om produsente by te staan met droogtebestuur-strategië. Wydverspeide reën het wel in Maart in heelwat gebiede in die noorde voorgekom, maar watervlakke in die damme het nog nie noemenswaardig gestyg nie.

In meeste van die Noordelike streke word laer opbrengste verwag weens die voordurende effek van die droogte, asook uitermatige hoë temperature. Dele in die noordelike produksiestreke het erg onder hael deurgeloop en 'n afname van etlike miljoen kartonne word vir hierdie dele voorspel. Dit wil voorkom of sekere witluis-spesies in van die produksie areas besig is om toe te neem. Groblersdal/Marble Hall, Letsitele en Hoedspruit het hoër as normale witluis-besmetting in boorde gerapporteer.

Die totale oesskatting van 11.2 miljoen kartonne is ongeveer sewe miljoen kartonne laer as verlede jaar, hoofsaaklik a.g.v pomelo's en Valencias wat gesamentlik 'n afname van 10 miljoen kartonne toon. Nawels, sagte sitrus en suurlemoene is gesamentlik drie miljoen kartonne hoër as verlede jaar. Vruggrootte is oor die algemeen kleiner en produsente is tydens die CMF vergadering in Maart gewaarsku om weereens met groot omsigtigheid te bemark, ongeag die feit dat die markvooruitsigte belowend lyk.

8.1.3 CRI-PTF

Gedurende 2014 is baie suksesvolle proewe op A15C kartonne met die volgende papierkombinasies gedoen: 200g/m² Kraftpride (KP) en 165g/m² Ultraflute (UF). Hierdie kombinasie is dan ook op grootskaal geïmplementeer. Gedurende die eerste kwartaal van 2015 is eksperimentele A15C kartonne met 175g/m² "High Kappa" KP en 150g/m² UF vervaardig. Die stapelsterkte resultate volgens die laboratorium-toetse was uitstekend. Dit het gewissel tussen 703 en 730Kg, terwyl die minimum vereiste 600kg is. Gebaseer op die huidige lysprys van papier is die verskil in prys tussen bogenoemde kombinasies R0,95 per A15C karton.

Die WCCPF het die aanbring van ŉ datum van vervaardiging op die palet wat hulle na die VSA gebruik verpligtend gemaak. Gerugte dat dit verpligtend is vir alle palette, het versprei. CRI-PTF kon daarin slaag om die status quo vir 2015 op die standaard sitruspalette te handhaaf. Vanaf 1 Januarie 2016 is die aanbring van ŉ datum van vervaardiging egter verpligtend gemaak op alle sitruspalette. Die spesifikasie is

dienooreenkomstig aangepas. Groot hoeveelhede oordra-voorrade sonder die datum het baie onsekerhede veroorsaak. DAFF het egter toegestem om dispensasie toe te staan.

In samewerking met Premier Pallets en die Schoeller Allibert Groep in Duitsland is ʼn proef met ʼn plastiese palet by Schoeman Boerdery gedoen. A15C kartonne (Suurlemoene – "Hi-cube") is op die palet gepak. ʼn Rak is gesimuleer en die deurbuiging van die plastiese palet is vergelyk met die standaard sitruspalet. Deurbuiging op die plastiese palet was aansienlik meer en die vervaardiger, wat teenwoordig was, het toegegee dat hulle palet sterker gemaak sal moet word.

Daar is goedkoop hoekstukke van baie swak gehalte in omloop. Die huidige spesifikasie dui slegs die afmetings en water-absorpsie van die materiaal, wat aan die buitekant gebruik word, aan. A.g.v probleme met swak kwaliteit hoekstukke, het daar ʼn behoefte vir ʼn meer omvattende spesifikasie ontstaan. Spesiale toerusting is laat maak en in samewerking met Sappi se SANAS geakkrediteerde laboratorium is ʼn reeks toetse gedoen. Die gevolgtrekking is dat die toevoeging van defleksie onder ʻn sekere lading sinvol sal wees. Die besluit om hoekstukke te gebruik wat aan die voorgestelde defleksie standaarde voldoen lê by die produsent.

Die toerusting om oop kartonne op te maak (vou en lym) is geweldig duur en palettisering is moeilik en tydrowend. Wanneer klagtes oor onstabiele palette en kartonne wat inmekaar sak, ontvang word, is dit in die meeste gevalle oop vertoon-kartonne wat ter sprake is. Op versoek van heelwat produsente is daar begin met werk om te bepaal of oop vertoon-kartonne nie met T64 kartonne vervang kan word nie. Die grootste uitdaging is om die "UK Multiples" te oortuig. Palettisering van T64 kartonne is makliker en vinniger. T64 kartonne se buitestukke kan net verwyder word sodat die binnestukke met die vrugte daarin dan as oop vertoon-kartonne gebruik kan word.

Gedurende die tweede kwartaal is daar weer proewe gedoen met die volgende papier kombinasies nl. Kraftpride Linerboard (KP) en Ultraflute (UF) in g/m²:

Kontrole kartonne: Buitestukke 175KP/165UF/175KP.
Binnestukke 175KP/165UF/175KP/165UF/175KP.
Eksperimentele kartonne: Buitestukke 175KP/150UF/175KP.
Binnestukke 175KP/150UF/175KP/150UF/175KP.

Kartonne is per pad vanaf Letsitele na Durban vervoer en na voor-verkoeling in "Hi-cube Containers" na Rotterdam verskeep. Soortgelyke proewe is in die Oos-Kaap gepak en na die M/O en Europa verskeep. Al die kartonne het in ʻn baie goeie toestand op die oorsese markte aangekom. Sappi is tans besig met die ontwikkeling van ʻn 170g/m² KP en proewe sal gedoen word sodra 170KP beskikbaar is.

Sporadiese probleme met onstabiele palette op oorsese markte is op versoek van uitvoerorganisasies en individuele produsente ondersoek. Met die hulp van die betrokke uitvoerorganisasies is kartonne na die STC laboratorium gestuur vir toetse om te bepaal of kartonne aan die voorgestelde spesifikasies voldoen. In een geval was dit duidelik dat die pakhuis nie die palettiserings protokolle gevolg het nie.

Ontleding van Sappi se SANAS geakkrediteerde laboratorium se resultate tov stapelsterktes van kartonne, van 2011 tot 2015, toon duidelik dat die stapelsterkte ʻn opwaartse kurwe toon, terwyl die basiese massa van die "linerboard" en die "fluting" afgekom het op A15C kartonne van 200KP/175SF twee jaar gelede, na 175KP/165UF in 2015 en die nuutste proewe met 175KP/150UF. Die 175KP is ook ʻn nuut ontwikkelde 175 Hoë Kappa KP. Dit het meegebring dat jaarlikse prysverhogings aansienlik laer was.

Vertikale lugvloei deur sekere van die uitvoerkartonne is ondersoek en die mening is dat slegs die 600x400mm en 500x300mm oop vertoon-kartonne se ventilasie gate op die bodem dalk nie na wense is nie. Vertikale lugvloei proewe met E15D kartonne met twee gate op die bodem is vergelyk met kartonne met ses gate. Die verkoeling van die vrugte in die kartonne met die ses gate was betekenisvol beter as die vrugte in die kartonne met die twee gate. Die plan is om die kartonne te verander van twee na ses gate op die bodem.

Swamgroei op palette was gedurende 2015 ʻn wesenlike probleem. Die betrokke vervaardigers is gekontak en hulle het die versekering gegee dat palette wel met SOPP behandel is. SOPP is egter nie ʻn voldoende nie, en navorsing is aangevra om hierdie ernstige probleem op te los.

Volgens ʻn plaaslike vervaardiger word hy oorval met navrae oor "Automatic Place Packers" (APP) en bestellings vir APP. Sy versoek is dat alle oop vertoon-kartonne meer APP vriendelik gemaak word. Die probleem is die flappe wat terugvou om driehoeke in die hoeke van die kartonne te vorm. Die kartonne is so ontwerp om stapelsterkte te verhoog en om te verhoed dat kartonne in mekaar in-nes. Hierdie versoek gaan

geweldige hoë kostes vir kartonvervaardigers meebring, en stapelsterkte gaan ook nadelig beïnvloed word. Dus is dit nog ‘n rede om ernstig te kyk na die verandering van oop vertoon-kartonne na teleskopiese kartonne.

‘n Na-seisoen vergadering is met Sappi gehou oor 2015 se geakkrediteerde laboratorium toetse. Die kwaliteit van die kartonne gedurende 2015 was goed. 118,1 miljoen kartonne is gepak en geen probleme met voorsiening is ondervind nie. Gedurende November is daar weer samesprekings met Sappi se senior personeel gevoer en Sappi het toegestem het om al die 2016 toetse weer op hulle kostes te doen.

Sommige produsente wil die keuse hê om goedkoper kartonne van ‘n laer gehalte te kan koop, en indien kartonvervaadigers die kartonne nie verskaf nie, kan hulle besigheid verloor. Die volgende is voorgestelde veranderings aan die akkreditasie-stelsel vir kartonvervaardigers.

Kartonne wat aan die spesifikasies voldoen

- Vervaardiging van kartonne wat aan die spesifikasies voldoen gaan voort
- ‘n Spesiale merk wat aandui dat die kartonne aan die spesifikasies voldoen sal op al hierdie kartonne gedruk word
- Die toets van hierdie kartonne sal net soos in die verlede voortgaan
- Meer kartonne sal by pakhuise getrek word.

Kartonne wat nie aan die spesifikasies voldoen nie

- Val buite die akkreditasie-stelsel
- Sal nie getoets word nie
- Spesiale merk sal nie op hierdie kartonne gedruk word nie
- Probleme met hierdie kartonne sal nie deur CRI opgevolg word nie
- Kartonvervaardigers sal geen verantwoordelikheid vir moontlike eise aanvaar nie.
  Die wysigings sal eersdaags in ‘n nuwe Pakmateriaal Spesifikasies dokument opgeneem en versprei word.

8.1.4 **Na-oes voorligting**

Pakhuise regoor die land is weer op ‘n een tot een basis besoek en die houding en terugvoering is in die meerderheid pakhuise weereens baie positief, met goeie interaksie en samewerking met die pakhuise. Dis steeds kommerwekkend om te sien hoe sekere van die pakhuise nog te min aandag aan pakhuissanitasie en waksaanwending gee. Daar is nog te veel pakhuise wat nie voorsortering doen nie, en die groen sporulerende-besmette vrugte word eers na die chloor vrugwasstelsels verwyder. Bederf is op uitskotvrugte waargeneem as gevolg van vrugte wat te lank in ontgroening gestaan het, en ook van die aantal groen/blouskimmel vrugte, na ontgroening, wat in die vrugwas-stelsels gedompel word. Hierdie is steeds ’n groot probleem in verskeie pakhuise. Die miljoene swamspore in die was-stelsels, veral die dompelbaddens, kan duidelik gesien word. Dit bly ’n resep vir bestandheid. Chloorvlakke, wat in hierdie stelsels gemeet word, bly laag, en soms kon geen konsentrasie gemeet nie, en die pH vlakke is ook verkeerd. Hierdie probleme dra almal by tot swak pakhuissanitasie.

Kraakskil op nawels, Clementines en ander kultivars was ook ’n geweldige probleem wat hierdie seisoen waargeneem is. Somige pakhuise het tussen 12 en 15% uitskot op hulle nawel-oes a.g.v kraakskilvrugte gehad. Die waarneming is dat kraakskil vir die afgelope drie seisoene toegeneem het. Hierdie seisoen se verhoogde kraakskil kan waarskynlik toegeskryf word aan die laer reënval wat waarskynlik gelei het tot laer kalsium-opnames.

Sporadiese bederf, veral suurvrot, was meer van ’n probleem, in sekere van beide die Noordelike en Suidelike streke, veral op die vroeë kultivars (Satsumas en Clementines) en op die nawels, weereens as gevolg van swaar reënval voor die plukseisoen, en wisselvallige reënval tydens pluk. Haelskade in een of twee van die streke het ook hoë bederf op die vroeë kultivars veroorsaak, en baie van die wondpatogeen-infeksies, veral suurvrot, is in die uitskot-vrugte waargeneem. Min latent patogeen-infeksies het tot dusvêr voorgekom.

Swak paletisering is steeds ’n probleem in van die pakhuise. Van die palette staan netjies en regop en dit lyk asof daar gepoog is om dit reg te doen, en dan sal daar ander palette wat soos die “toring van Pisa” lyk. Party palette se hoekstukke loop van bo af tot heel onder die palet tot op die vloer. Ander palette se hoekstukke loop tot in die middel van die palet se voetstuk, ander hoekstukke eindig by die onderste karton en ander raak aan die begin van die voetstuk van die palet. Te veel palette met gebuigde hoekstukke is ook waargeneem. Daar is geen eenvormigheid nie. Paletisering lyk in te veel pakhuise nog slordig.

Baie pakhuise poog om hulle kritiesebeheerstelsels deeglik te bestuur, veral sanitasie, maar dit is nog steeds ’n groot bekommernis om in pakhuise te stap en te sien hoeveel pakhuise die groen/blou sporulerende-

besmette vrugte nogsteeds in die vrugwas-stelsels dompel. Baie pakhuise het meer “klinies” geword, wat pakhuissanitasie betref, maar daar is nog te veel pakhuise wat nie voorsortering toepas nie, en dan kla hulle van bederf-terugvoer uit die markte of die besmette vrugte in die retensie monsters.

Tydens die pakhuisbesoeke is ʼn punt daarvan gemaak om swamspoor pluise van besmette uitskot vrugte, retensie monsters en van besmette vrugte in die vrugwasstelsels te neem en die monsters by die Diagnostiese laboratorium te ontleed vir moontlike bestandheid. Die eerste tekens van bestandheid het al voorgekom. Hierdie praktyk sal meer gereeld by die pakhuise toegepas word en die pakhuise sal aanbeveel word om hulle eie monsters in te stuur vir moontlike bestandheid-ontleding.

Waksaanwending is ook ʼn kritiese-beheerpunt wat baie wisselvalig in pakhuise is agv. nat vrugte in die waksaanwending. Daar is duidelik min waks op die vrugte te sien na aanwending, en dit is heel waarskynlik die oorsaak van koueskade en vogverlies simptome te sien in fotos oor terugvoering van die markte. Die vogverlies simptome lyk soos skil afbraak in en om die blomkelk weefsel waar daar heelwat minder waks aangewend word. Heelwat koueskade simptome op vrugte in fotos uit die markte is hierdie seisoen waargeneem.

Die Patensie-area in die Oos Kaap het geweldig baie reënval en oorstromings in die later gedeelte van die seisoen gehad. Heelwat Phytophthora bruinvrot op Nadorcotts is waargeneem, veral waar produsente versuim het om vir moontlike besmetting te spuit. Sporadiese gevalle van suurvrot en groenskimmel is ook waargeneem. Indien die EU besluit dat die toekomstige gebruik van guazatine gestaak gaan word, sal die moontlike sporadiese gevalle van suurvrot ‘n groot probleem vir die bedryf word.

Tydens die pakhuisbesoeke is gekyk na die plukpraktyke van die vrugte in twee verskillende boorde. Dit was skrikwekkend om die aantal plukbeserings op die vrugte te sien. Dit is ʼn baie groot oorsaak van bederf. Skilprobleme op sekere kultivars het die afgelope seisoen uitgestaan. Daar was ‘n noemenswaardige toename in koueskade op spesifieke sensitiewe-skil kultivars, veral na koue-steri markte toe.

Pakhuis “check lists” is die afgelope jare vir elke pakhuis ingevul met al die nodige besonderhede en veranderlikes van al die aanbevole behandelings van elke kritiese beheerpunt. Die doel van al hierdie lyste is om elke pakhuis te oudit en na te gaan om seker te maak dat hul aan CRI se aanbevelings voldoen. Die lyste word tans elektronies gelaai en daar is, tot datum, omtrent 50 lyste gelaai. Dit is ‘n voortgaande proses en die gegewens van elke pakhuis kan verander word, indien nodig, soos wanneer opgraderings of veranderings aangebring word.

Die 2016 sitrus seisoen in die Noordelike- en Suidelike produksiegebiede het teen ‘n baie stadige pas begin, as gevolg van die uitermatige hoë temperature, algemene droogtetoestand en watertekort in die meerderheid produksiegebiede. Haelskade in Hoedspruit in die Noorde en in Citrusdal in die Suide het ook groot skade aan die bome en vrugte veroorsaak. As gevolg van al hierdie skade was daar ‘n geweldige afname in volume van die betrokke kultivars in die Hoedspruit gebied, soveel so dat ʼn paar produsente gerapporteer het dat geen vrugte in sekere boorde uitgevoer kan word nie.

Gepaard met hierdie wisselvallige toestande en ‘n algemene beperking aan die besproeiing van die sitrusbome, was daar ook ʼn kort tydperk van swaar reënval en oorstromings in die Komatipoort gebied, aangrensend aan die Kruger Wildtuin, waar die Sabie- en Sandriviere oorstroom het.

Die oorsaak van al die toestande, veral die hoë temperature en tekort aan water het die sitrusbome onder geweldige stremming geplaas. Dit het heelwat endokserose op suurlemoene vanaf Tshipise veroorsaak, asook blom-ent afbraak op Tahiti lemmetjies vanaf Paarl. Die algehele gehalte van die meerderheid Satsuma besendings uit die Wes-Kaap was baie belowend tydens aankoms in die markte. Geen na-oes bederf is tot dusver gerapporteer of waargeneem nie.

8.1.5 Noordelikke produksiegebiede – (Groblersdal/Marble Hall, Burgersfort en Ohrigstad, Letsitele, Tshipise en Weipe asook Hoedspruit)

Die laer reënval tendense het produsente in veral die Letsitele-area genoop om drastiese maatreëls te ondersoek en te implementeer om groot verliese te voorkom, veral met die oog op die komende 2016 seisoen. Gedurende hierdie tydperk was hul waterkota reeds verminder tot 60% met verdere beperkings wat in die vooruitsig gestel is voor die nuwe seisoen ‘n aanvang neem.

Heelwat meer witluis infestasies as gewoonlik is in meeste van die produksiestreke aangemeld. Die rooidopluis populasies was ook meer opsigtelik in vergelyking met vorige seisoene.

Hierdie plae is spesifiek aangespreek tydens die produksiewerkswinkels. Heelwat nuwe aanplantings word in meeste van die streke beplan. Aanplantings wissel van hoofsaaklik manderyn tipes in sekere areas, suurlemoen asook lemoen kultivars.

Die droogte in die Noordelike produksie gebied gedurende die tweede helfte van 2015 het wel krisis afmetings begin aanneem met sekere plase in sekere areas wat, indien dit nie dringend sou reën nie, geen water beskikbaar sou hê in die nuwe jaar nie. Indien opgaardam vlakke nie dramaties styg nie, kan hierdie situasie wesenlike gevolge vir die volgende seisoen inhou. Die meeste streke het vêr onder die gemiddelde jaarlikse reënval tot op hede ontvang.

Heelwat skade is aangerig in van die ergste haelbuie in Hoedspruit area waar miljoene kartonne vir die 2016 seisoen verlore is. Daar was wel ook skade in die Marble Hall, Groblersdal, Ohrigstad en Burgersfort areas maar wel in ‘n mindere mate. ‘n Toename in windskade is ook duidelik sigbaar in meeste van die produksie areas. Goeie reën het wel vroeg in 2016 in groot dele van die noorde uitgesak en verligting gebring, maar sekere areas loop steeds gebuk onder die knellende droogte.

Daar is besonderse mooi Clementine en suurlemoen oeste in die Mvurwi gebied in Zimbabwe. Die gebied beloof om in die toekoms weer opgebou te kan word as ‘n uitstekende sitrusproduserende area. Groot gedeeltes van Mazoe is oorgeneem deur mev Mugabe. Uitstekende suurlemoene is net so laat staan wat ‘n groot tekort aan suurlemoene op die vars mark veroorsaak het. Dit is opvallend dat *Pseudocercospora angolensis* steeds nie op grootskaal in ‘n probleem ontaard het vandat die plase in die noorde van Zimbabwe onteïen en verwaarloos is nie. Die enigste verklaring hiervoor is dat die verwaarloosde boorde feitlik geen nuwe lote vorm nie en daar gevolglik min vatbare blaarmateriaal is vir infeksies. Slegs een vrug met letsels is gevind by ‘n stalletjie langs die pad. Die vrug is moontlik van Glendale afkomstig.

8.1.6 **Sitrus Koueketting**

Verskeie versoeke om die probleme in die koueketting te bespreek, het gelei tot ‘n baie goeie vergadering wat vir die Cooling Working Group gereël is. Verskeie kundiges op die gebied van verkoeling het die vergadering bygewoon en skitterende insette gelewer. Terugvoer oor navorsingswerk wat gedoen word, is gelewer. Verder is heelwat ontstellende foute en gebreke in die koueketting bespreek. Dis duidelik beklemtoon dat heelwat verliese as gevolg hiervan deur die bedryf gelei word. ‘n Operasionele handleiding vir die hele koueketting is in die proses om vir al die verskillende rolspelers opgestel te word, vanaf die boord tot in die mark. Daar is ‘n baie sterk gevoel dat ‘n kundige aangestel moet word as operasionele bestuurder vir die koueketting om operasionele navorsing te doen en verantwoordelikheid vir die operasionele bestuur van die koueketting moet neem.

8.1.7 **Sitrusverbeteringskema**

Die Sitrusverbeteringskema slaag nie tans in sy doel om die sitrusbedryf teen minderwaardige plantmateriaal te beskerm nie. Daar is bloot net te veel swak bome wat in nuwe aanplantings geplant word. Veral wortelvrot en kinkelwortel kan as die grootste probleme uitgesonder word. Daar is op die oomblik so groot vraag na bome dat produsente enige gemors sal plant wat beskikbaar kom. Ten spyte van die feit dat een van die probleemkwekerye onlangs van die lys van geakkrediteerde kwekerye verwyder is, is produsente nie van die gevolge daarvan ingelig nie, naamlik dat die bome wat op bestelling by hierdie kwekery staan, nie gesertifiseer kan word nie, en meer nog, dat die gehalte bome wat daar staan totaal onaanvaarbaar is. Die produsente se belange word nie hierdeur op die hart gedra nie en is teenstrydig met CRI se visie en aanbevelings. Die riglyne vir sertifisering sal indringend hersien of strenger toegepas moet word, asook tegniese ondersteuning/advisering aan kwekerye op ‘n gereelde grondslag.

8.1.8 **CRI Na-oes Werkswinkels**

Die jaarlikse CRI na-oes werkswinkels is weer soos die vorige jare gedurende Januarie en Februarie in die grootste produksiestreke aangebied. Twee werkswinkels is in Limpopo aangebied, een in Tzaneen en een by Loskopdam. Verder is daar een werkswinkel in elk van Mpumalanga (Nelspruit), KZN & Swaziland (Durban), Oos-Kaap (Jeffreysbaai) en Wes-Kaap (Stellenbosch) aangebied. Die werkswinkels is met rekordgetalle deur verskeie rolspelers in die sitrusbedryf, waaronder hoofsaaklik pakhuispersoneel en produsente, tegniese adviseurs, verpakkingsmateriaal vervaardigers, kundiges in die chemiese bedryf, PPECB, DAFF en ander na-oes rolspelers bygewoon.

Vir die tweede agtereenvolgende jaar het DST-PHI fondse beskikbaar gestel wat aangewend is om die kostes rakende die werkswinkels gedeeltelik te help dek. Wenkem was die vierde agtereenvolgende jaar die hoofborg, en ICA die platinum borg. Goue borge was Yeager Corporation en ArcAqua. Verskeie silwer (Label Pro,

Technidex) en brons (Citrashine, Corruseal, Houers Koöperatief, M'Pact, Neopak, Omega Bins, Sunnypacks, Winmar) borge was ook betrokke. Groot dank en waardering word uitgespreek teenoor al die borge, asook DST-PHI wat dit help moontlik maak het om die werkswinkels aan te bied.

'n Wye verskeidenheid onderwerpe is aangebied om almal op hoogte te bring met die nuutste inligting rakende onder andere marktoegang, sanitêre en fitosanitêre aangeleenthede, uitvoerregulasies, kwaliteit- en bederfbeheer, voedselveiligheid, verpakkings-aangeleenthede en logistieke projekte.

Tabel 8.1.8.1. Opsomming van aktiwiteite deur Hennie le Roux, Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode April – Junie 2015.

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes / Sprekers**
7 Apr 15	Corruseal, Wadeville	Bulk bin vergadering om probleme wat Hortex gehad het op te los.	Mark Fry. Rajiv Mehta. Dawid Groenewald.
7 – 9 Apr 15	Letsitele Hoedspruit	Opening van Gubitz pakhuis; Mahela NuDawn landgoed – problem boorde	MC Pretorius Gubitz; Eddie Voster Ivan – Nu Dawn
8 Apr 15	Ryton Landgoed pakhuis Ngodwana	Besoek/konsultasie	Keith Lesar Arno Erasmus
8 Apr 15	Joubert en seuns pakhuis Schoemanskloof	Besoek/konsultasie	Keith Lesar Arno Erasmus
10 Apr 15	Patensie: Grant Du Preez	Probleme met nuwe aanplanting	Hannes Bester Thys Du Toit
13 Apr 15	Waterberg studiegroep	Zebediela – Kultivar beplannings vergadering Bufland boerdery - Naboomspruit	MC Pretorius Rod Ford Johan Joubert Danie Janse van Rensburg
13 Apr 15	Brits	Swartvlekproewe	Tiaan Schutte C. Kotze Hennie le Roux
14 Apr 15	Waterberg studiegroep	Rolemsha – Kultivar proef Vivo - haelskade	MC Pretorius Johan Joubert Pietman – Limpopo sitrus
15 Apr 15	Waterberg studiegroep	Sirkel N boerdery – kultivars en boordbesoeke	MC Pretorius Johan Joubert Rudie – Sirkel N
14 Apr 15	Lynwood Bridge.	Projek beplannings vergadering.	Hannes Bester. Dawid Groenewald.
15 Apr 15	Skilderkrans Bdy	Snoei op suurlemoene	Hannes Bester
16 Apr 15	SASSCON Vergadering	Agenda	Hennie le Roux Hannes Bester MC Pretorius
17 Apr 15	Sappi Technology Centre.	Hoekstuk laboratorium toetse	René v/d Westhuizen Dawid Groenewald
20-23 Apr 15	Forresters pakhuis Mvurwi Zimbabwe	Besoek/konsultasie Harare Studiegroepvergadering	Keith Lesar Hennie le Roux John Perrott
22 Apr 15	Sappi Technology Centre.	"Edge Crush" en papier toetse op Nampak Fluting	Jason Knock. Dawid Groenewald
24 Apr 15	Magalies , Brits	Ondersteuning aan sapprodusente wat begin uitvoer	Hennie le Roux
5 Mei 15	Groblersdal	Area plaas besoeke Schoonbee Landgoed Piet Engelbrecht trust	MC Pretorius Ista Upton Piet Engelbrecht
6 Mei 15	Groblersdal	DeWagendrift boerdery Roslee Boerdery	MC Pretorius Pieter Nel

			Charles Rossouw en Koos de Wet
7 Mei 15	Groblersdal	Afri-fert boordprobleme	MC Pretorius Donovan Chrisjan Blighnaut
6 Mei 15	Schoeman Boerdery.	Proef met plastiese palet Beplanning van KP/UF proewe.	Schoeller Allibert Groep. Christo de Jonge Dawid Groenewald. Frikkie van Wyk
6 Mei 15	Oudtshoorn: Manie Du Plessis Bennie Terblanche Kerneels Nortje	Nuwe sitrus-ontwikkelings	Hannes Bester
7 Mei 15	Smart Agri vergaderings Riebeeckkasteel Citrusdal	Climate Change	Hannes Bester
8 Mei 15	Sappi Technology Centre.	Karton akkerditasie.	Jason Knock. Dawid Groenewald
13 Mei 15	Nelspruit	CIS siektebestuur vergadering	MC Pretorius Paul Fourie Jan van Niekerk Elaine Basson
14 Mei 15	Hoedspruit	Plaasbesoeke – Haelskade Unifruitti Richmondt Estates Essex Blyderus Overburg	MC Pretorius Fanie Meyer Hannes Booyens Diederick Fourie Cobus Redelinghuys Sybrand van Vuuren Johan Kellerman
14 Mei 15	Brits	Proewe met FMC masjiene om effek op CBS te sien	Hennie le Roux Ryno Prins
14 Mei 15	Sappi Technology Centre.	Opvolg laboratorium toetse op hoekstukke	Dawid Groenewald.
17-19 Mei 15	Stellenbosch	DAFF/CIS stakehoulder meeting - Stellenbosch	MC Pretorius Elma Carstens DAFF
19 Mei 15	Burgersfort	Studiegroep vergadering	MC Pretorius
19 Mei 15	Vaalharts: Saamfarm Bdy	Boordbesoeke Pakhuisbesoek	Hannes Bester
20 Mei 15	Britz: Transformation Growers	Snoeidemonstrasie & boordbesoeke	Hannes Bester Hennie le Roux
20 Mei 15	Nelspruit	Symposium en navorsings vergadering met Stoller SA	MC Pretorius
22 Mei 15	Nelspruit	BASF – tegniese vergadering/ symposium vergadering	Hennie le Roux MC Pretorius Tim Grout Tian Schutte
25 Mei 15	Letsitele: Mahela Bdy	Snoeiwerkswinkel	MC Pretorius Hannes Bester
25 Mei 15	Laeveld pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
26 Mei 15	Gubitz pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
26 Mei 15	Rooister pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
26 Mei 15	Henley pakhuis	Besoek/konsultasie	Keith Lesar
26 Mei 15	Sappi Technology Centre	Karton akkreditasie aangeleenthede en papier toetse	Jason Knock. Dawid Groenewald.
26 Mei 15	Letsitele: Groep 91 Bosveld Sitrus	Snoeiwerkswinkels	MC Pretorius Hannes Bester

	CLB Bdy		
27 Mei 15	Letsitele: Laeveld Sitrus Letaba Estates	Pakhuisbesoek Blaarmonsters: vergroening	MC Pretorius Hannes Bester
	Ohrigstad Studiegroep	Snoeiwerkswinkel	MC Pretorius Hannes Bester
	Burgersfort Studiegroep	Snoeiwerkswinkel	MC Pretorius Hannes Bester
	Paul Kruger Hek Protea Hotel	Openingspreker tydens SANA konferensie om hulle in te lig oor die gevare wat HLB inhou vir die sitrusbedryf	Hennie le Roux
27 Mei 15	Schoeman Boerdery Frans Smit Boerdery	Voorlopige werk op vertikale lugvloei proewe en palet inspeksies	Frikkie van Wyk Dawid Groenewald
27 Mei 15	Groep 91 pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
27 Mei 15	Mabarisa pakhuis (Bosveld Sitrus) Letsitele	Besoek/konsultasie	Keith Lesar
27 Mei 15	The Plains pakhuis (Groep 91) Letsitele	Besoek/konsultasie	Keith Lesar
27 Mei 15	Mahela pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
28 Mei 15	Nouvelle la Cotte pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
28 Mei 15	Merite pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
28 Mei 15	Christie Landman pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
28 Mei 15	CP Minnaar pakhuis Letsitele	Besoek/konsultasie	Keith Lesar
29 Mei 15	Vaalharts: Retha Greyling Lemoenkop Sitrus	Boord- en pakhuisbesoeke	Hannes Bester
1 – 12 Jun 15	Nelspruit	Eksp 1092 – veldproef beplanning en uitleg	MC Pretorius Charl Kotze
1 Jun 15	Malelane	BEE besoek aan La Rochell boerdery re. herplantopsies	Andrew Mbedzi Hennie le Roux
8 Jun 15	Sappi Technology Centre.	Ondersoek swak kwaliteit kartonne op versoek van Letaba Est.	Dawid Groenewald
9 Jun 15	Schoeman Boerdery	Vertikale lugvloei proef	Frikkie van Wyk. Dawid Groenewald
9 Jun 15	Swaziland Ngonini	Besoek saam met J. Robberts aangaande nuwe aanplantings	J. Robberts Hennie le Roux
9 Jun 15	Mvurwi: Forresters Estate	Studiegroep snoei-werkswinkel	Hannes Bester
10 Jun 15	Nelspruit	Vergader met Argenti Lemon aangaande stand van suurlemoene in Argentinië	Hennie le Roux Tiaan Schutte
12 Jun 15	Winterveldt	Bespreking tussen die Nederlandse ambassadeur, Winterveldt en CRI rakende hul problem en oplossings	Hennie le Roux Andrew Mbedzi Nederlandse ambassadeur
12 Jun 15	Schoeman Boerdery	Einde van lugvloei proef. Haal palette uit koelkamer en verwyder “I” Buttons.	Frikkie van Wyk Dawid Groenewald
15 Jun 15	Mooinooi	Besoek nuut gevestigde BEE produsente	Hennie le Roux
17 Jun 15	Corruseal	Vergadering oor ernstige probleme wat ons met Corruseal ondervind	Rajiv Mehta Dawid Groenewald
17 Jun 15	Swellendam: Klipbult Bdy	Transformasie-projek	Hannes Bester
17 – 25 Jun 15	Nelspruit	Eksp 1092	MC Pretorius

		Monsterneming en voorlegging voorbereiding van PhD studie	Charl Kotze
17 Jun 15	Gaberone	Besprekings met Botswana se Departement van Landbou aangaande nuwe moontelike sitrus ontwikkelings in Noord Botswana en SA uitvoere	Hennie le Roux
18 Jun 15	Magalies	Winterveldt en Magalies se voortgesette betrokkenheid	Hennie le Roux
18 Jun 15	Paarl: Wenkem opleiding	Aanbieding oor Suid-Afrikaanse Sitrusbedryf	Hannes Bester
	JBT: Jaco Theron	Na-oes & borgskap	Hannes Bester
22 Jun 15	Clanfresh pakhuis Clanwilliam W. Kaap	Besoek/konsultasie	Keith Lesar
22 Jun 15	Namakwaland Sitrus pakhuis Clanwilliam W. Kaap	Besoek/konsultasie	Keith Lesar
22 Jun 15	Wespak (Radyn – Suiderland) sitrus pakhuis Clanwilliam W. Kaap	Besoek/konsultasie	Keith Lesar
21-22 Jun	Queens Town/ Whittle Sea	BEE besoek vir vestiging van nuwe BEE sitrusprojekte	Hennie le Roux Elrita Venter
23 Jun 15	Patrysburg pakhuis Clanwilliam W. Kaap	Besoek/konsultasie	Keith Lesar
23 Jun 15	Mouton Sitrus pakhuis (was Visser Sitrus) Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
23 Jun 15	Groenkloof pakhuis (ALG) Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
23 Jun 15	CRI Nelspruit	CRI-PTF: Akkreditasie-stelsel	Vaughan Hattingh Hannes Bester Dawid Groenewald Hennie le Roux
		IPM Research Workshop	IPM Researchers Vaughan Hattingh Hennie le Roux Hannes Bester MC Pretorius
24 Jun 15	Cederpack Citrus pakhuis Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
24 Jun 15	Quattro Citrus pakhuis Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
24 Jun 15	ALG pakhuis Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
24 Jun 15	Bergendal pakhuis Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
24 Jun 15	Piekenierskloof Vrugte Verpakkers pakhuis Citrusdal W. Kaap	Besoek/konsultasie	Keith Lesar
25 – 28 Jun 15	Mopanie KNP	NSSA – Editorial committee member – NSSA book on nematodes	MC Pretorius
25 Jun 15	Imibala pakhuis Franschhoek W. Kaap	Besoek/konsultasie	Keith Lesar
25 Jun 15	Suiderland Sitrus pakhuis Swellendam W. Kaap	Besoek/konsultasie	Keith Lesar
25 Jun 15	Swellenfruit Sitrus pakhuis Swellendam W. Kaap	Besoek/konsultasie	Keith Lesar
25 Jun 15	Thornlands Citrus pakhuis Swellendam W. Kaap	Besoek/konsultasie	Keith Lesar
29 Jun 15	Augrabies:	Boordbesoeke	Hannes Bester

	Oseiland Bdy Kakamas: Karstens Boerdery		Hennie le Roux
29 – 30 Jun 15	Stellenbosch	US Departement plantpatologie PhD voorlegging	MC Pretorius
30 Jun 15	Kakamas: JH Retief Bdy	Boordbesoeke Snoeidemonstrasie Cultivar-opsies Vrugset Bederfbeheer	Hannes Bester
	Zwartbooisberg	Snoeidemonstrasies Vrugset Alternatiewe drag Bederfbeheer	Hannes Bester

Tabel 8.1.8.2. Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode Julie-September 2015

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes/ Sprekers
1 Jul 15	Kakamas:Karstens Bdy	Pakhuisbesoek Vrugset & vruggrootte Snoei	Hannes Bester
	Groenheuwel Bdy	Kultivars Snoei Vrugset	Hannes Bester
	Zwartbooisberg	Bemesting Kultivars	Hannes Bester
3 Jul 15	Sappi Technology Centre (STC)	Inligtingsessie oor die verkoop van Enstra en Cape Kraft deur Sappi	Dawid Groenewald
6 – 8 Jul 15	Peru besoekers	Peru besoekers Nelspruit, Letsitele	MC Pretorius
13 Jul 15	Schoeman Boerdery	Vertikale verkoelingsproef	Dawid Groenewald Frikkie van Wyk Christo de Jonge
14-15 Jul 15	Jon Pinker	PHI Begroting	Hannes Bester Jon Pinker
16 Jul 15	Sondagsrivier Studiegroepvergadering	Bactrocera dorsalis	Hannes Bester Aruna Manrakhan
16 Jul 15	Schoeman Boerdery	Beëindiging van Vertikale verkoelingsproef	Dawid Groenewald
17 Jul 15	Glenfair Sakesentrum	Bespreek 175KP en 150UF proewe met Mpact.	Dawid Groenewald Flip Welman, Mpact
20 Jul 15	Swellendam: Klipbult Bdy	Assessering vir CPAC Vrugset Snoei	Hannes Bester Jakkie Stander
20 – 23 Jul 15	CRI - Grondvesblok	CIS evaluasies	MC Pretorius
21 Jul 15	Stellenbosch: Waitrose Fresh Produce Technical Conference	Agenda	Hannes Bester
21 Jul 15	Waterval (Bosveld) Sitrus pakhuis Burgersfort	Besoek/konsultasie	Keith Lesar
22 Jul 15	STC	Strategie vergadering met Sappi	Dawid Groenewald Wimpie Mostert, Houers en Craig Zorab en Jason Knock, Sappi
22 Jul 15	Naranja Sitrus pakhuis Burgersfort	Besoek/konsultasie	Keith Lesar
23 Jul 15	Morone Sitrus pakhuis Burgersfort	Besoek/konsultasie	Keith Lesar

23 Jul 15	PLM Sitrus pakhuis Burgersfort	Besoek/konsultasie	Keith Lesar
28 – 29 Jul 15	Waterberg studiegroep	Studiegroep vergadering en boord en pakhuis besoeke	MC Pretorius Keith Lesar
30 Jul 15	Bufland Sitrus pakhuis Naboomspruit	Besoek/konsultasie	Keith Lesar MC Pretorius
30 Jul 15	Groblersdal	Wagendrift boerdery pakhuis besoek	MC Pretorius Keith Lesar
29 Jul 15	Stellenbosch: Thys Stoltz	Solar-energiestelsels vir sitruspakhuise	Hannes Bester
30 Jul 15	Kalie Kirsten	Snoeidemonstrasie	Hannes Bester
31 Jul 15	STC	Toets van 175KP/150UF kartonne	Dawid Groenewald
4 Aug 15	Marble Hall sitrus pakhuis Marble Hall	Besoek/konsultasie	Keith Lesar
5 Aug 15	Schoonbee sitrus pakhuis Groblersdal	Besoek/konsultasie	Keith Lesar
5 Aug 15	Goldport (Goedehoop) sitrus pakhuis Marble Hall	Besoek/konsultasie	Keith Lesar
5 Aug 15	Piet Engelbrecht Trust sitrus pakhuis Marble Hall	Besoek/konsultasie	Keith Lesar
3 – 5 Aug 15	Weipe en Tshipese	Bertus Dillman, Peter Nicholson Boord en pakhuis besoeke	MC Pretorius Johan Joubert
6 Aug 15	Elephant Citrus Exporters pakhuis Groblersdal Stofberg pad)	Besoek/konsultasie	Keith Lesar
6 Aug 15	Rosle Boerdery sitrus pakhuis Groblersdal	Besoek/konsultasie	Keith Lesar
6 Aug 15	Diphale sitrus pakhuis Groblersdal	Besoek/konsultasie	Keith Lesar
6 – 7 Aug 15	Houers, Letsitele	175KP/150UF proewe en ontleding van vertikale verkoelingsproef se resultate	Dawid Groenewald Wimpie Mostert Frikkie van Wyk
11–14 Aug 15	Durban	CIS vergroenings opname beplanning	MC Pretorius DAFF
11 Aug 15	Sun Valley Estates: Weenen	Snoei Kultivars Vrugset	Hannes Bester
12 Aug 15	Gluckstad: Millbrook Oils	Kultivars Vestiging Transformation	Hannes Bester
14 Aug 15	Glenfair Sakesentrum	“ Automatic Place Packer” Moontlike veranderinge aan oop vertoon-kartonne	Dawid Groenewald Wimpie Mostert, Houers
17-21 Aug 15	Durban en omgewing	CIS vergroenings opname in KZN	MC Pretorius DAFF verteenwoordigers
20 Aug 15	Kirkwood	CGA CBS vergadering	Hannes Bester Deon Joubert (CGA)
20 Aug 15	Pretoria Mark	Ondersoek deurbuiging op kartonne	Dawid Groenewald
24 Aug 15	Oranjevier Studiegroep	Navorsings prioriteite	Hannes Bester
25 Aug 15	Irene Winkelsentrum	Kry kartonne by Sunnypacks vir vergelykende toetse op vertikale lugvloei	Dawid Groenewald Justin Arpin, Sunnypacks
25 Aug 15	Moletele Citrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
25 Aug 15	Rederberg (Redlinghys) Sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar

25 Aug 15	Soleil (Glencoe)Sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
25 Aug 15	Southhampton Citrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	Moriah Citrus Estate pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	BJ Blyde Sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	Olifant River Estate sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	Canyon Pakkers sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	Ambrosia Processing sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
26 Aug 15	Blyde Pakkers (Unifrutti) sitrus pakhuis Hoedspruit	Besoek/konsultasie	Keith Lesar
27 Aug15	Vergadering	Tommy Landman en Siegfried du Preez ivm. nuwe sitrus pakhuis	Keith Lesar
27 Aug 15	ETP meeting	Agenda	Hannes Bester Dawid Groenewald
31 Aug 15	Moreleta Park	Koueketting vergadering met John Mc Glashan	Dawid Groenewald
2 Sep 15	Cookhouse	Beplanning van Transformation Grower Technical workshop	Hannes Bester Melton Mulaudzi
2-4 Sep 15	Plettenbergbaai	CIS vergroenings opname in die Buffersone	MC Pretorius DAFF verteenwoordigers
7-10 Sep 15	Stellenbosch	Soilborne symposium US Adele McLoed Aalwurm kontrak werk vergadering met nie toksiese middel	MC Pretorius
7 Sep 15	Hankey sitrus pakhuis OosKaap	Besoek/konsultasie	Keith Lesar
7 Sep 15	Mandaryn sitrus pakhuis Patensie OosKaap	Besoek/konsultasie	Keith Lesar
7 Sep 15	Patennsie Ko-op sitrus pakhuise (**twee pakhuise**) OosKaap	Besoek/konsultasie	Keith Lesar
8 Sep 15	Riverside citrus pakhuis Katrivier Fort Beaufort OosKaap	Besoek/konsultasie	Keith Lesar
8 Sep 15	Kat-co citrus pakhuis Katrivier Fort Beaufort OosKaap	Besoek/konsultasie	Keith Lesar
8 Sept 15	Eden Fruit citrus packhouse Katrivier Fort Beaufort OosKaap	Besoek/konsultasie	Keith Lesar
8 Sep 15	Katrivier Studiegroep	Navorsings prioriteite	Hannes Bester
10 Sep 15	Irene Winkelsentrum	Vergaderings met Corruseal en New Era i.v.m. veranderinge aan akkreditasie stelsel.	Dawid Groenewald Rajiv Mehta Nick Engelbrecht en André Cuturi
14 Sep 15	Tzaneen: Houers Koöp	Akkreditasie-stelsel	Dawid Groenewald Hannes Bester
15 Sep 15	STC	Toets van Lona/Kaspersnek kartonne.	Dawid Groenewald
15-16 Sep 15	Limpopo CRI Produksie, IPM & DM werkswinkel	Agenda	MC Pretorius Hannes Bester

	(Swadini)		Andrew Mbedzi Jan van Niekerk Tian Schutte Aruna Manrakhan Sean Moore Sean Thackeray Tim Grout Teunis Vahrmeijer Jakkie Stander
17-18 Sep 15	Limpopo CRI Produksie, IPM & DM werkswinkel (Loskop)	Agenda	MC Pretorius Hannes Bester Andrew Mbedzi Jan van Niekerk Tian Schutte Aruna Manrakhan Sean Moore Sean Thackeray Tim Grout Teunis Vahrmeijer Jakkie Stander
18 Sep 15	Irene Winkelsentrum.	Bespreek moontlike veranderinge aan akkreditasie stelsel met Sunnypacks	Dawid Groenewald. Justin Arpin en Gideon Galloway
19 Sep 15	Vaalharts Studiegroep	Navorsings prioriteite	Hannes Bester
22 Sep 15	Hazyview	Vergadering met Thandaza Pallets en kry hout monsters by hulle	Dawid Groenewald Malcolm Easton
22 Sep 15	Nelspruit CRI	Swamgroei proewe	Dawid Groenewald Keith Lesar
22-23 Sep15	Mpumalanga CRI Produksie, IPM & DM werkswinkel	Agenda	MC Pretorius Hennie le Roux Hannes Bester Andrew Mbedzi Jan van Niekerk Tian Schutte Aruna Manrakhan Sean Moore Sean Thackeray Tim Grout Andrew Miller Teunis Vahrmeijer Jakkie Stander
23 Sep 15	M'Pact: Flip Welman	Akkreditasie-stelsel	Dawid Groenewald Hannes Bester
29 Sep 15	Centurion, Pretoria	Vergadering oor veranderings aan die akkreditasie stelsel met Neopak	Dawid Groenewald Johan Nel Adriaan du Buisson

Tabel 8.1.8.3. Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode Oktober - Desember 2015

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes / Sprekers**
1-2 Okt 15	Oos-Kaap CRI Produksie, IPM & DM werkswinkel	Agenda	Hannes Bester Jan van Niekerk Tian Schutte Aruna Manrakhan Sean Moore Sean Thackeray Tim Grout Teunis Vahrmeijer

			Jakkie Stander Paul Fourie
8 Okt 15	Cooling Working Group meeting	Agenda	Hannes Bester Dawid Groenewald Paul Cronje
8 Okt 15	Hoedspruit studiegroep	Studiegroep vergadering en DAFF terugvoering	MC Pretorius
12 Okt 15	Lynnwood Bridge	Chep Pallets	Dawid Groenewald
13 Okt 5	CLFQ Committee meeting	Navorsings voorleggings	Hannes Bester Tim Grout Paul Cronje Teunis Vahrmeijer Jakkie Stander
15 Okt 15	Sappi Tech.Centre	Na Seisoen vergadering Craig Zorab/Jason Knock	Dawid Groenewald.
14 Okt 15	Cultivar Evaluation Committee meeting	Evaluasie proewe	Hannes Bester Tim Grout Johan Joubert Stephen Meeding
20 Okt 15	IPM Research Committee meeting	Navorsings voorleggings	Hannes Bester Tim Grout Hennie le Roux Sean Moore
21 Okt 15	DM Research Committee meeting	Navorsings voorleggings	Hannes Bester Tim Grout Hennie le Roux Paul Fourie
22 Okt 15	Villa: John Mac Intyre & Nolene	Navorsingsimposium 2016 borgskap	Hennie le Roux Hannes Bester
22- 23 Okt 15	Ohrigstad	Studiegroep vergadering en plaas besoeke: Smit le Roux, Kobus Beetge, Marnus van der Walt	MC Pretorius
23 Okt 15	Sappi: Andre Oberholzer & Madelaine Fourie	Navorsingsimposium 2016 borgskap	Hennie le Roux Hannes Bester Dawid Groenewald
	BASF: Rita vd Merwe & André Pretorius	Navorsingsimposium 2016 borgskap	Hennie le Roux Hannes Bester Dawid Groenewald
2 Nov 15	Letsitele	Bosveld sitrus: Pierre Smit	MC Pretorius
3 Nov 15	Letsitele Hoedspruit	Mahela: Eddie Voster en Hugo Endeman – bespuitings proef in probleem boord Ambrosia – Hoedspruit problem boord besoek Letsitele studiegroep vergadering – Skilprobleem terugvoering	MC Pretorius MC Pretorius, Jan v Niekerk, Charl Kotze MC Pretorius, Paul Cronje
4 Nov 15	Letsitele	Skilprobleem en wortelvrot bestuur besprekings: Groep 91 Letaba landgoed Bosveld sitrus CP Minnaar	MC Pretorius, Paul Cronje, Burgert van Rooyen, Henk van Rooyen MC Pretorius, Paul Cronje, Harry Grove, Andries en tegniese span op Letaba MC Pretorius, Paul Cronje en Pierre Smit MC Pretorius, Paul Cronje en Carel Minnaar

5 Nov 15	Letsitele	Skilprobleem en wortelvrot bestuur besprekings: Laeveld sitrus	MC Pretorius, Paul Cronje en Gerhard Voster en produksie personeel
3 Nov 15	CRI Na-oes Werkswinkels	Beplanningsvergadering	Hannes Bester Dawid Groenewald Keith Lesar Arno Erasmus Catherine Savage
4 Nov 15	Packaging Working Group meeting	Agenda	Dawid Groenewald Hennie le Roux Hannes Bester
9 Nov 15	Champagne Sports Resort	Navorsingsimposium vergadering	Hennie le Roux Hannes Bester MC Pretorius Jean De Gasperi Christine Stoppel-Grove
10 Nov 15	Drakensberg	NSSA symposium beplanning	MC Pretorius
11 Nov 15	CRI Nelspruit	CIS – Virologie CIS - Grondgedraagd	MC Pretorius Paul Fourie Hennie le Roux LRN CRI Navorsers
17 Nov 15	Pretoria	Universitiet van Pretoria – vergadering en monsters aflewer	MC Pretorius Prof Nico Labuschagne
24 Nov 15	Cooling Working Group meeting	Agenda	Hannes Bester Dawid Groenewald Paul Cronje
24 Nov 15	Karino	Monsterneming exp 1092	MC Pretorius Charl Kotze Jan van Niekerk
2 Des 15	Arysta: Bertha Spangenberg	Navorsingsimposium 2016 borgskap	Hennie le Roux Hannes Bester
2 Des 15	Groblersdal/Marble Hall	Produsent besoeke: Dewagendrift boerdery Hertzog boerdery	Pieter Nel Hannes Hertzog
3 Des 15	Groblersdal/Marble Hall	Roslee boerdery Schoeman boerdery – Droogte en haelbestuur strategie praatjie aangebied	Koos de Wet Frans Olivier
4 Des 15	Groblersdal/Marble Hall	Piet Engelbrecht trust	Pieter Engelbrecht
7- 8 Des 15	Katrivier	Katco – Probleem boord besoeke – 3 Plase en boorde besoek	MC Pretorius Isabel Sparks Produsente
8 Des 15	CRI Board meeting	Agenda	Vaughan Hattingh Tim Grout Hannes Bester Jon Pinker
9 Des 15	Jeffreys Baai	Hannes voorligtings bespreking en Pakhuis werkswinkel beplanning	MC Pretorius Hannes Bester Corrie Muller
10 Des 2015	Sappi Tech. Centre	Select a Box akkreditasie	Dawid Groenewald

Tabel 8.1.8.4. Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode Januarie - Maart 2016

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
8 Jan 16	Wes-Kaap: Boschendal: Aldo Du Plessis ICA: Wouter Schreuder JBT: Jaco Theron	Werkswinkel venue Werkswinkel & simposium borgskappe	Hannes Bester
18-21 Jan 16	Nelspruit / KNP	CRI Bestuurs vergadering	Hannes Bester MC Pretorius
22 Jan 16	Innovation Hub Pretoria	Select a Box Akkreditasie.	Dawid Groenewald Fernando da Silva.
25-27 Jan 16	Tzaneen: Limpopo 1 CRI Na-oes werkswinkel	Agenda	Vaughan Hattingh Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Paul Cronje Wilma Du Plooy Wouter Schreuder Andrew Mbedzi
28-29 Jan 16	Loskopdam: Limpopo 2 CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Paul Cronje Wilma Du Plooy Wouter Schreuder
2- 3 Feb 16	Letsitele	Studiegroep vergadering: Droogte strategie, vrugtevliegbeheer; Boord en produsent besoeke: Mahela, Groep 91, Bosveld	MC Pretorius Aruna Manrakhan Teunis Vahrmeijer
3 Feb 16	Sappi Technology Centre (STC)	Akkreditasie toetse en skedule beplanning.	Craig Zorab Jason Knock Dawid Groenewald
8-10 Feb 16	Nelspruit: Mpumalanga CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Paul Cronje Wilma Du Plooy Wouter Schreuder
11-12 Feb 16	Durban: KZN & Swaziland CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Wilma Du Plooy Wouter Schreuder
15-16 Feb 16	Patensie	Paksaam kwekery besoek	MC Pretorius Paksaam kwekery personeel

16-17 Feb 16	Jeffreysbaai: Oos-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Paul Cronje Wilma Du Plooy Wouter Schreuder Melton Mulaudzi
16-19 Feb 16	Stellenbosch: Wes-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Sean Moore Catherine Savage Paul Cronje Wilma Du Plooy Wouter Schreuder
24 Feb 16	Nelspruit	CGA Roadshow	MC Pretorius
25 Feb 16	Malelane	CGA Roadshow - Riverside	MC Pretorius Hennie le Roux
1 Mrt 16	Innovation Hub Pretoria	Strategiese beplanning sessie met New Era Packaging	Stuart Esterhuizen Dawid Groenewald
1 Mrt 16	Fort Beaufort: CGA Roadshow	Agenda	Hannes Bester
1 – 3 Mrt	Letsitele	Midknight produksie werkswinkels en kultivar proef blok besoeke asook Chimera opnames by spesifieke boorde: Bosveld, Mahela, Groep 91	MC Pretorius Johan Joubert James Warrington
2 Mrt 16	Kirkwood: CGA Roadshow	Agenda	Hannes Bester
8 Mart 16	Nelspruit	Brondal – produsent en boord besoeke	MC Pretorius Johan Joubert
9 Mrt 16	Onderberg	Chimera opname in spesifieke boorde	MC Pretorius Paul Fourie Faan van Vuuren Hannes Breedt Johan Joubert
10 Mrt 16	Johannesburg	Nematologie Vereeniging bestuursvergadering	MC Pretorius
11 Mrt 16	STC	Inligtingsessie met Sappi. Gee inligting oor tonne papier benodig vir 2016	Danie Jonker Craig Zorab Jason Knock Dawid Groenewald.
14 Mrt 16	Nelspruit	Performance appraisals	Hannes Bester MC Pretorius Keith Lesar Andrew Mbedzi
14–17 Mrt 16	KNP	Nematologie Vereeniging: Outeurs vergadering om Nematologie boek te finaliseer vir druk en bind	MC Pretorius
15 Mrt 16	Nelspruit: Simposium vergadering	Agenda	Hannes Bester Jean De Gasperri Jon Pinker Christine Stoppel-Grove
16 Mrt 16	Pretoria	Performance appraisal en CRI-PTF besprekings	Hannes Bester Dawid Groenewald
17 Mrt 16	Johannesburg: CMF meeting	Agenda	Hannes Bester Dawid Groenewald

18 Mrt 16	Diep in die Berg, Pretoria.	Amptelike bekendstelling van die CGA GDC	Justin Chadwick Dawid Groenewald
22 Mrt 16	STC	Beplanning en inligtingsessie met Corruseal	Rajiv Mehta Dawid Groenewald
29 Mrt 19	Innovation Hub Pretoria	Personeel veranderings by Neopak. Inligtingsessie met hulle nuwe Landbou personeel	Johan Nel. Paul Backhouse Etienne vd Lingen Dawid Groenewald.
29-30 Mrt 16	Nelspruit	Symposium vergadering: Borgskappe te finaliseer	MC Pretorius Jean de Gasperri
31 Mrt 16	Innovation Hub Pretoria	Na-oes merk vereistes op kartonne en "recycling symbols". Verskaffers van toedraaipapier vir vrugte.	Wimpie Mostert Dawid Groenewald.

8.2 TRANSFORMATION EXTENSION OFFICERS' ANNUAL REPORT (April 2015 - Maart 2016)

8.2.1 The Citrus Study Groups

The study group can be one of the best places to get your questions answered about confusing or difficult issues in the farming set up. The study group allows for a good review of issues in the farm. The members of the study group can improve their problem-solving abilities by working together to solve difficult challenges. And if the group does this on a regular basis, all members will learn the skill set required to solve any type of challenges.

The emerging citrus study group sessions went well during the 2015/2016 season in Limpopo, Eastern Cape and Kwazulu Natal provinces. On the 14th of April 2016 another emerging citrus study group was formed in the Brits area in the North West province. The following table indicate the dates, venues, number of attendances, presenters and the topics that were presented in the different study group sessions held in these provinces.

Table 8.2.1.1. The dates, venues, number of attendances, presenters and the topics that were presented in the different study group sessions held in in Limpopo, Eastern Cape and Kwazulu Natal provinces during the 2015/2016 season.

Date:	Study Group Name	Venue	No of People	Presenters	Topics
20 Apr 15	Sundays River Valley	Summerville Packhouse	9	Assistant Packhouse Manager	Fruit receiving point Chlorination and Sorting Hot Water Bath Waxing, Labelling, Grading, Palletizing and PPECB Inspection
21 Apr 15	Patensie	Patensie Packhouse	7	Packhouse Manager	Fruit receiving point Chlorination and Sorting Hot Water Bath Waxing, Labelling, Grading, Palletizing and PPECB Inspection
21 Apr 15	Waterberg	Sunningdale Farm	21	Andrew	Replanting of old Orchards Time to secure seedlings Management of young Orchards Importance of irrigation
24 Apr 15	Vhembe	Musunda Farm	83	Michael Aphane Makhuvha Armstrong	Leaf and Soil Sampling Scouting of Citrus Pests Bactrocera Dorsalis (BD) Fertilization of Citrus
08 Jul 15	Sundays River Valley	Luthando Farm	18	Melton Andrew Combrink	Pruning of Citrus Trees Irrigation Scheduling and Fertilization of Citrus
09 Jul 15	Patensie	Boplaas Farm	7	Melton	Pruning of Citrus Trees Irrigation Scheduling Fertilization of Citrus

14 Jul 15	KAT River Valley	Peddie Riellyvale Farm	16	Melton Melton Susan Zwelonke Lawrence	Pruning of Citrus Trees Fertilization of Citrus Irrigation Scheduling Land Use Management CGAGDC Feedback to farmers
16 Jul 15	Kwazulu Natal	Thulwane Packhouse	7	Melton Melton Melton Mzo	Pruning of Citrus Trees Irrigation Scheduling Fertilization of Citrus CGAGDC Feedback to farmers
30 Jul 15	Mopani	Lulekani Agric. Office	23	Andrew Andrew KD Baloyi	Effects of BD on Social Life Fertilization of Citrus Trees Control of Oriental Fruit Fly
06 Aug 15	Vhembe	Mkharo Farm	76	Teunis (UP)	Water Use and Water Stress Management
27 Aug 15	Waterberg	Gillimburg Farm	23	Farm Manager	Picking of the Citrus Fruit for Export.
03 Sep 15	Sundays River Valley & Patensie	Patensie Boplaas Farm	34	Melton Melton Melton Ngwanda	Control Oriental Fruit Fly Citrus Spring Pests Complex Control of FCM Donation of BD Traps
08 Okt 15	Kwazulu Natal	Nkwaleni Valley Hall	7	Melton Melton Mzo	Control Oriental Fruit Fly Citrus Spring Pests Complex CGAGDC Feedback to farmers
09 Okt 15	Vhembe	Mamusha Boerdery	43	Avcasa CEO Andrew DAFF	Responsible Use of Pesticides Control of Citrus Pests Control of Oriental Fruit Fly
11 Nov 15	KAT River Valley	Cape College	18	Melton Melton Susan Lawrence	Control Oriental Fruit Fly Control of FCM Comments on BD Traps CGAGDC Feedback to farmers
24 Nov 15	Waterberg	Mr. Mashishi's Farm	32	Andrew Andrew	Planting Management Video Care of young Citrus Trees
25 Nov 15	Mopani	Mariveni Farm	36	Andrew Andrew	Control of Fruit Flies and FCM Management of CBS
13 Jan 16	KAT River Valley	Cape College	19	Dr. Phehane	Acquiring of Cultivars from ARC
21 Jan 16	Sundays River Valley	Luthando farm	11	Melton	Fruit fly Control Soil and Leaf Analysis B.I Control and Activities Plan
22 Jan 16	Patensie	Boplaas	06	Melton	Fruit fly Control Soil and Leaf Analysis B.I Control and Activities Plan.
26 Jan 16	Kwazulu Natal	Nkwaleni Valley Hall	05	Melton	Fruit fly Control Soil and Leaf Analysis B.I Control and Activities Plan.
11 Feb 16	Vhembe	Den Staat Farm	77	Farm Manager Andrew	Scouting of Citrus Pests Control of Oriental Fruit Fly
17 Feb 16	Mopani	New Dawn Farm	28	Farm Manager	Citrus Orchard Layout Using of Accredited Nurseries Importance of Scouting
18 Feb 16	Waterberg	Ratanang Project	16	Andrew	Care of young citrus trees Types of irrigation systems

8.2.2 **Information Days**

The Limpopo, Eastern Cape and Kwazulu Natal provinces have started information days. These information days have become traditional events in these three provinces. The aim of these events is to encourage the

developing citrus growers, agricultural officers and other stakeholders in the citrus industry to come together and share citrus production and marketing information. These events are held annually and the host district in these provinces choose the theme of the information day.

8.2.2.1 **The Citrus Field Day Event**

The Citrus Field Day has become a traditional event for the developing citrus growers in the Limpopo Province. The event rotates around the five districts namely; Vhembe, Mopani, Waterberg, Capricorn and Sekhukhune. This event is held annually in one of the above mentioned districts. The district that is going to host the Citrus Field Day chooses the theme. The aim of this day is to encourage the emerging growers, extension officers and other related stakeholders in the citrus industry to come together and share citrus production and marketing information.

During the 2015 season the Citrus Field Day event was held on the 1st of October at the Easy farm in Tshivhilwi under the Thulamela local municipality. The theme of the event was "Promoting the competitiveness of citrus farming in a dynamic economic environment." The following topics were covered under this theme. Importance of irrigating citrus, soil and leaf sampling as the basis of fertilization of citrus, monitoring and control of the Oriental fruit fly, marketing the citrus fruit, importance of a farm business plan and financial support to farmers.

A total of 110 people from all the Limpopo province districts attended the citrus field day event. The event was sponsored by CGA and the Limpopo Department of Agriculture.

8.2.2.2 **Eastern Cape Citrus Transformation Grower Day**

The Grower day is an event that has started in the Eastern Cape Province in 2012. It has become an annual event that rotates amongst the two main districts in the province (namely Amathole and Cacadu districts). The main aim of the event is to address challenges facing BEE citrus growers. This year the event was hosted at Willowtree Masifunde Sonke Training Centre, Addo in the Sundays River Valley. The event was organized by Citrus Research International (CRI), the Citrus Growers Association of Southern Africa (CGA) and the Eastern Cape Department of Rural Development and Agrarian Reform (DRDAR).

A total of 79 delegates attended the grower day although a total of 80 people were anticipated. The delegates were composed of 3 CRI staff members, 1 from Citrus Academy, 28 DRDAR officials, 2 DAFF officials from PE, 1 from Eastern Cape Rural Development Agency (ECRDA), 2 from River Bioscience, 2 from SRCC Technical Division, 1 from Sundays River Local Economic Development (LED), 1 from Riverside, 1 from CGA Transformation, 1 Norusheni Coop member and 35 Farmers. The following companies sponsored the Grower Day:

- River Bioscience, Sundays River Valley also sponsored three shirts which were given to the three candidates nominated from the registers by General manager, Keith Danckwetts.
- 80 x 500ml Water bottles sponsored by River Bioscience Sundays River.
- Fruit for display sponsored by SRCC Production Division, SAFE Packhouse, Ripplemead farm, Rallyvale Farm and Dankbaar farm. The main sponsor for the day was CGA.

8.2.2.3 **KZN Citrus Transformation Grower Day**

This Grower day is an event that has started in the KZN Province on the 10th of March 2016. It will become an annual event that will take place in the KZN Area. The main aim of the event is to address challenges facing BEE citrus growers. The event was hosted at Nkwaleni Valley Processing plant under Umlalazi Municipality. The event was organized by Citrus Research International (CRI), the Citrus Growers Association of Southern Africa (CGA) and the KZN Department of Agriculture under Umlalazi Municipality.

A total of 33 delegates attended the grower day although a total of 40 people were anticipated. The delegates were composed of 2 CRI staff members, 1 from Citrus Academy, 7 DARD officials, 5 DAFF officials from KZN, 1 intern from ARC, 2 Agricultural Economists from Zulu land District Municipality (ZDM), 1 from Lona Marketing Division, 1 from Nkwaleni processing plant. 13 Farmers from NKwaleni area (Umlalazi and Melmoth), Vryheid (Mpangisweni Community Trust) and Uthugela area (SunValley).
The topics discussed were:

- Oriental fruit fly and management by Siyanda from DAFF KZN
- Soil and leaf analysis by Andrew Mbedzi from CRI
- BEE Bursary Support by Ms. Jacomien de Klerk from CA
- Fruit processing by Mr Jan Kotze and showing the participant's the facilities.

- Message of Support by Melton from CRI
- Closing Remarks by Mr Mzo Makhanya Farmer & Chairperson of CGDC

The following companies sponsored the Grower Day:
- Nkwaleni Processing
- CGA
- Citrus Academy

8.2.3 **Workshops**

8.2.3.1 **CRI Citrus Extension Regional Workshops**

Citrus Research International (CRI) hosts three citrus extension regional workshops annually. These workshops are attended by both the small, emerging, developing and commercial citrus farmers in Southern Africa. The aim of these workshops is to prepare the citrus farmers for the citrus growing season. The three workshops are the packhouse, production and pest and disease management:

CRI Citrus Packhouse Workshop
The 2016 CRI Citrus Packhouse workshops were held in January, February and March at the Fairview Hotel in Tzaneen, Loskop Dam in Groblersdal, CRI Boardroom in Nelspruit, Mentors Kraal in Jeffreys Bay and Blue Waters hotel in Durban respectively. The workshops went on very well and the small and developing farmers, Department of Agriculture Forestry and Fisheries (DAFF), officials from Provincial Department of Agriculture (PDA) and Citrus Academy students attended these workshops. These workshops were aimed at preparing the citrus farmers for the upcoming packing season. The CGA transformation section sponsored the citrus growers in Limpopo, Mpumalanga, KZN and Eastern Cape with registration fees and the farmers paid transport and accommodation fees for themselves. A total of 67 developing citrus growers attended the packhouse workshops.

CRI Citrus Production, Pests and Diseases Management
All the CRI Citrus Production workshops were cancelled due to the fact that most of the citrus growers were busy with the harvesting of their crops. The CRI Citrus Production workshop was then merged with the CRI Citrus Pest and Disease Management workshop and the two were done at the same time during August and September 2015 in Limpopo, Mpumalanga, Eastern Cape and Western Cape provinces respectively.

The CRI Citrus Regional Extension Workshops were held in September at Swadini Resort in Hoedspruit, Loskop Dam in Groblersdal, CRI Boardroom and in Nelspruit and in Mentors Kraal in Jeffreys Bay respectively. A total of 56 developing citrus growers attended the workshops.

8.2.3.2 **Citrus Emergent Export Excellence Training Workshops**

The Citrus Academy outsourced funding for the Citrus Emergent Export Excellence training workshop programmes from the Department of Agriculture Forestry and Fisheries (DAFF) Directorate Marketing. The training workshops were facilitated by Mr. Louis Von Broembsen and Sam Louw from the Citrus Academy. In the Northern region these workshops were conducted during the months January, February and March 2015 and they are not going to be part of this report. Only the Mpumalanga workshop will be reflected as it was done during April 2015. The transformation extension officers Mr. Melton Mulaudzi and Andrew Mbedzi supported the facilitators of these workshops. The workshops took place as follows

Table 8.2.3.2. Citrus Emergent Export Excellence Training Workshops

Date	Province	District	Area	Venue	No. Attendance
21-23 Apr 2015	MPU	Mbombela	Nelspruit	CRI Boardroom	9
31 Mrt – 2 Apr 2015	W/CAPE	West Coast	Citrusdal	Citrusdal County Lodge	3
14 – 16 Apr 2015	N/CAPE	ZF Mgcawu	Kakamas	Lake Gappa	7
5-7 May 2015	E/CAPE	Cacadu	Patensie	Patensie Hotel	8
12-14 May 2015	E/CAPE	Amathole	KAT River Valley	Savoy Hotel	16
19-21 May 2015	E/CAPE	Cacadu	Sundays River Valley	Willow Tree Training Centre	20
26-28 May 2015	KZN	uMgugundlovu	P/Maritzburg	Ascot Inn	8

8.2.3.3 **The Solidaridad Rural Horizon Workshop**

Solidaridad Rural Horizons held a workshop from the 3rd to the 5th August 2015 at Lemoenkloof in Paarl area. The CGA sent three people to attend the workshop (thus 2 farmers, 1 from Limpopo Province, Mr. Tompson Makhili, 1 from Eastern Cape, Mr. Lawrence Mgadle and 1 CRI Extension officer, Mr. Melton Mulaudzi).

The workshop started off by welcoming all the participants. Karin Kleinbooi provided an introduction of the global Solidaridad network and followed it up with contextualizing the purpose of the workshop by a project briefing which set out the details of the joint project between Solidaridad and SIZA; what it aims to achieve and which approach will be used.

Pieter Sijbrandij explained the Rural Horizons (RH) value proposition in supporting producers, producer organizations, companies, buyers, retailers and government extension services. It can assist producers in their organizational skills, increase productivity and efficiency and help them to reach certification level. These include propositions of inclusion (producer friendly), bottom up approach (control of development process is held by producers), that involve meaningful engagements (different actors and different parts of the value-chain), continual improvement (stepwise progress), a learning tool and cost effectiveness. He explained how the tool supports the joint Solidaridad/SIZA intervention process and that the tool is part of the project and the project is part of a broader change process. He also explained what outcomes can be expected from farmers at different levels and capabilities.

Hence, RH provides a true reflection of priority needs (what is more urgent), a gap analysis against relevant standards, link producers to support material and what should be put in place to support improvement and sustainability performance. The tool can go as far as calculating the cost of improvements if this information is integrated into the tool. Hence project priorities and agenda is based on self-assessment and starts where the farmer is *located* in terms of sustainability. He also explained that RH is not a certification tool but provides a platform for producers to develop towards certification. It channels technical support in a step by step approach towards producers that is based on relevant benchmarks and that is developed from requirements decided by all the entities involved. Pieter also explained the RH business model (pricing components and use of information).

Violaine Laurens Medeiros facilitated a discussion on the application of RH worldwide. This was followed by a demo (on the basic toolkit). In the session on actors and processes Karin Kleinbooi gave an outline on the entire process starting with the identification of farmers and where it is envisaged which partners and stakeholders will be involved until the farmer is ready for the certification process.

8.2.4 **Female Farmer of the Year Competition**

Two farmers competed in the Eastern Cape for the first Female farmer of the year competition. Mrs. Buyiswa Steri Ndyenga won the Provincial awards and also contested for National awards in Kwazulu Natal Province. The occasion took place in Durban Garden. Mrs. Buyiswa Steri Ndyenga won the national award in the category, Export markets for 2015. The event was sponsored by Total South Africa, Old Mutual and DAFF. The overall winner of the awards was a lady from Mpumalanga Province. She was accompanied by the Mpumalanga MEC of Agriculture.

8.2.5 **The Citrus Growers Development Chamber (CGDC)**

On the 18th of March 2016 the Chamber held a meeting at Diep in die Berg. The purpose of this meeting was to give feedback to the Chamber members on the developments of the CGA Grower Development Company. The GM of the CGAGDC Mr. Lukhanyo Nkombisa informed the Chamber that the company was going to be launched in the evening and that the Minister of the Department of Agriculture Fisheries and Forestry (DAFF) was going to grace the occasion.

The CGA CEO, Mr. Justin Chadwick, informed the Chamber that all the administration issues regarding the transfer of Melton and Andrew from Citrus Research International (CRI) to the CGA Grower Development Company will be concluded by the end of March 2016. Any further issues related to the staff will be discussed at the CGAGDC Board meeting to be held on the 19th of March 2016 at the Diep in die Berg in Pretoria.

Mr. Samson Ntekane Qommondi was introduced to the Chamber as the new CGA Board of Directors for the northern region and that he is also going to be part of the Chamber Executive Committee member.

8.2.6 **Launch of the CGA Grower Development Company**

The CGA Grower Development Company was launched on the evening of the 18th of March 2016 at the Diep in die Berg, Lynwood in Pretoria. The following dignitaries such as the Minister of Department of Agriculture Forestry and Fisheries (DAFF), DAFF Directors and Senior DAFF managers and personnel, CGA Board Members, Citrus Research Trust, Citrus Academy and other stakeholders such as Bank personnel, other Commodities as well as the farmers were invited to attend the launch of the CGA Grower Development Company.

8.2.7 **The CGA Roadshows**

Every second year the CGA holds the roadshows to give feedback to the growers on the past season and what they should expect on the upcoming season. This year the focus of the road-shows was on the increase of the statutory levy. The developing citrus growers did attend the roadshows but not in great numbers due to the timing of some them. They indicated that most of them were starting in the afternoon and finished late.

8.2.8 **Commodity Project Allocation Committee (CPAC)**

The Citrus commodity will be a formally established structure represented by CGA to strategically consider, debate and consult on the allocation of CASP funding in the Commodity Project Allocation Committee (CPAC) in the Western Cape province. The role of the CGA is to enable new and developing farmers to achieve their goals within the broader commodity partnership with Western Cape DoA. The committee must ensure that all recommended applications comply with the applicable selection criteria and that the allocations are prioritized in terms of the key deliverables which were dealt with under the fruit CPAC.

Mr. Piet Smit was voted in as the Citrus CPAC chairperson and his vice chairperson is Ms. Mariette Kotz and Mr. Hannes Bester and Johan Mouton will assist the CPAC with Technical advice.

8.2.9 **Greening Committee**

The greening committee has been formed in the Eastern Cape province to make awareness campaigns about the citrus greening disease. On the 28th of May 2015 the committee held a successful greening awareness meeting at Mbizana and 32 participants from DRDAR, CRI, Mbizana Local Municipality officials responsible for Agricultual Projects, Dept. of Health officials, nursery owners and leaders of farmer organizations in the area attended. A presentation on greening disease was done by Bongani and on oriental fruit fly by DAFF. After the awareness the citrus greening committee deliberated on the issue of training of officers on greening diseases and on the issues of servicing the oriental fruit fly previously known as B.I. The list of extension officers was given as well as training dates. The proposed date for OR Tambo is the 11th June and for the Alfred Nzo is the 10th of June 2015. The trained Extension Officers will be responsible for monitoring the traps on monthly basis. Dr. Hennie Le Roux is also a member of this greening committee.

8.2.10 **CGA Mentorship Programme**

There is still no funding for the CGA mentorship programme from the provincial departments of agriculture (PDAs). The CGA Grower Development Company will continue to engage with the provincial departments of agriculture for the funding of the mentorship programme.

8.2.11 **Government Grant and Recap Funding**

The Eastern Cape citrus growers and the Department of Agriculture held several meetings to discuss the assistance of farmers through Government grant funding for 2015/16 financial year. The Honourable MEC Mlibo Qoboshiyane promised to assist the Ripplemead pack house with an amount of R9,2 million for the rebuilding of the pack house for the (2016) financial year and will secure more finance from the 2017 budget. He indicated that the ruling party has mandated him to deliver this for a period of 5 years. The KAT River Valley farmers will again receive another R4 million for fertilizers and pesticides. The Western District (Cacadu) will also receive R4 million to support selected farms at Patensie and Sundays River valley with inputs and infrastructures.

On the 9th October 2015 Mbuyiselo farm at Kirkwood in the Sundays River Valley received their second and last Recapitalization funding from the Department of Rural Development and Land reform. This will be used for the renovation of staff houses and cloakroom, upgrading of the office and some production inputs for the 2016 crop. The first trench of recapitalization was used for orchard development, planting of lemons in order to improve the variety mix on the farm, the purchase of a bakkie, water cart, Cab tractor and a forklift. The balance was allocated to production cost and upgrading of the irrigation system.

8.2.12 **Challenges**

- Some farmers in the KAT River Valley, North West and Limpopo provinces experienced hail damage to their crops.
- Land transfer issue and tax clearance certificates is causing a delay for some KAT River farmers to access their phase 2 recap funding.
- Poor communication between the responsible government officials and citrus growers with regard to recapitalization funding.
- Lack of budget for the training of the Limpopo Department of Agriculture extension officers who are coordinating citrus in the province.
- Most of the upcoming citrus growers are faced with financial challenges.
- Lack of funding for the CGA Mentorship Programme
- Shortage of credible mentors to assist in the CGA Mentorship Programme.

8.2.13 **Summary of Transformation Extension Officers' Activities. (April 2015 to March 2016)**

Date:	Venue/Place:	Activity/Topics:	Speakers/Extension Officer:
02 Apr 15	Easy Farm, Tshivhilwi	Farm Visit and Dropping-off Packhouse Testing Kit from Lowveld Chemicals	Andrew Mbedzi
07 Apr 15	Depart. Of Agric. Makwarela	Attending a meeting on Vhutsavha New Lemon Development with the Government Extension Officers	Andrew Mbedzi
08 Apr 15	Sunningdale Farm, Mookgopong	Attending a meeting with the Sunningdale Workers Trust over their neglect to their citrus crop	Andrew Mbedzi
08 Apr 15	Letas farm	Monitoring fruit fly traps and FCM together with B.I trap	Melton Mulaudzi
09 Apr 15	Vhutsavha, Vhembe	Attending a meeting with Vhutsavha new lemon farmers, Government Extension Officers and Vhutsavha community	Andrew Mbedzi
10 Apr 15	Tshipise, Vhembe	Visiting Developing citrus growers in Tshipise with the Government Extension Officers	Andrew Mbedzi
10 Apr 15	Battlesden	Monitoring fruit fly traps and FCM control	Melton Mulaudzi
11 Apr 15	Mariveni, Letsitele	Visit Mariveni farm on my way back to Nelspruit from Vhembe district	Andrew Mbedzi
13 Apr 15	Empangisweni & Fakaza Comm. Trusts	Assess progress of the planting of lemon seedlings and farms' status	Andrew Mbedzi Melton Mulaudzi
14 Apr 15	Sesikhona, Thulwane, Umhlabawethu & Inthatakusa	Assess the farms' status and the Implementation of the Recap funding at Umhlabawethu	Andrew Mbedzi Melton Mulaudzi
15 Apr 15	Madzikane & Tala Valley Citrus Farms	Assess the state of neglect of the two farms and what can be done to resuscitate them	Andrew Mbedzi Melton Mulaudzi
16 Apr 15	Sokhela Farm, Cova & Donovale.	Visiting the Sokhela farm on request from ADA and assess status of Cova & Donovale	Andrew Mbedzi Melton Mulaudzi
18 Apr 15	Armsterdam, Mkhondo	Assessing the suitability of planting citrus on the farm in Armsterdam.	Andrew Mbedzi
20 Apr 15	La-Rochelle Farm, Malalane	Farm visit with Raymond to see the status of the farm	Andrew Mbedzi
20 Apr 15	Summerville Pack house Sundays River Valley	Citrus study group meeting on packhouse management with Sundays River BEE farmers	Melton Mulaudzi
21 Apr 15	CRI Boardroom, Nelspruit	Attending the Citrus Emergence Export Excellence Training Workshops	Andrew Mbedzi
21 Apr 15	Patensie Pack house	Attending study group meeting on pack house management with Patensie BEE farmers	Melton Mulaudzi
22 Apr 15	CRI Boardroom, Nelspruit	Attending the Citrus Emergence Export Excellence Training Workshops	Andrew Mbedzi
22 Apr 15	Letas farm	Delivering bursaries application forms from Citrus Academy	Melton Mulaudzi
23 Apr 15	CRI Boardroom, Nelspruit	Attending the Citrus Emergence Export Excellence Training Workshops	Andrew Mbedzi
23 Apr 15	Eden Agric Blinkwater	Attending meeting with the farmers with the purpose of forming Alice Cooperative	Melton Mulaudzi
24 Apr 15	Musunda Citrus Farm, Vhembe	Attending Vhembe Citrus Study Group Session One at Musunda Citrus Farm	Andrew Mbedzi
04 May 15	Mkharo Farm, Ngezimane	Farm visit to assist with the scouting of pests in the orchards	Andrew Mbedzi
05 May 15	Lungane Farm, Makonde	To check the status of the citrus trees-Trees are not being irrigated now for the last 8 years	Andrew Mbedzi

07 May 15	ARC Offices, Stellenbosch	Attending Fruit South Transformation Workshop at the ARC Offices in Stellenbosch	Andrew Mbedzi
12 May 15	Zebediela Citrus Estate, Zebediela	Attending a meeting on the Development of the Zebediela Citrus Estate Business Plan	Andrew Mbedzi
13 May 15	Zebediela Citrus Estate, Zebediela	Attending a meeting on the Development of the Zebediela Citrus Estate Business Plan	Andrew Mbedzi
14 May 15	Agri-Village 1 Offices, Polokwane	Attending a meeting on the Renewal of the Memorandum of Understanding Between CGA and LDA	Andrew Mbedzi
5 May 15	Patensie Hontel	Attending Citrus Emergence Export Excellence Workshop	Melton Mulaudzi
6-8 May 15	ARC offices in Cape Town	Attending Western Cape fruit South Africa Transformation meeting	Melton Mulaudzi
15 May 15	Torties, Jerico and White Citrus Farms	Monitoring picking of nules, Satsumas and Palmer navels fruit	Melton Mulaudzi
18-21 May 15	Khangela, Luthando, Siyaphambile, SRV	Organizing the Citrus Emergence Export Excellence Workshop	Melton Mulaudzi
25 May 15	Oakdene farm	Assisting the farmer with the burst-up irrigation mainline from the river	Melton Mulaudzi
27 May 15	Peddie Ripplemead farm	Monitoring the picking of Clementines and attending to red scales challenges	Melton Mulaudzi
27-28 May 15	Peddie, Mthatha Bizana	Attending greening committee awareness meeting and B.I meeting	Melton Mulaudzi
28 May 15	Du Roi IMP, Nursery & LAB	Drop-off CRI Scouting Booklet and Finalizing Planned Visit to LAB and Nursery	Andrew Mbedzi
29 May 15	Dept. of Agric. Makwarela	Attending Vhembe Citrus Technical Committee meeting on the Planning of Hosting of the Citrus Field Day	Andrew Mbedzi
08 Jun 15	Gonzana, Oakdene, Lovers & Orange	Monitoring picking of Nova and navels and sanitations of the orchards	Melton Mulaudzi
09 Jun 15	Jerico, Eden and Konzi	Monitoring picking of clementines, navels and lemons	Melton Mulaudzi
10-11 Jun 15	Peddie Ripplemead and Naudeshoek	Citrus Harvest day function at Ripplemead farm with Govt. (MEC of Agriculture in the Province and his or top management)	Melton Mulaudzi
12 Jun 15	Lettas and Greenwood farms	Monitoring picking of Navel and sanitation was a major challenge	Melton Mulaudzi
18 Jun 15	White River, Mpumalanga	Meeting on the Progress of Newly Planted Lemon Trees at Sibonelo farm	Andrew Mbedzi
18 Jun 15	Lovers Retreat, Toties & Jordan	Monitoring picking of the fruit and the sanitations of orchards	Melton Mulaudzi
19 Jun 15	Sibonelo Lemon Farm, Eilandshoek	Assess the Progress of 5.5ha Newly Planted Lemon Trees	Andrew Mbedzi
19 Jun 15	Peddie Ripplemead farm	Attending Ripplemead Pack house meeting with DAFF, NAMC, Peddie Municipality Manager and Local Extension	Melton Mulaudzi
22 Jun 15	DFA Citrus Farm, Dennilton	Assess Dennilton Farmers Association 400 Burned Citrus Trees	Andrew Mbedzi
22-23 Jun 15	Western Cape Hortgro Office	Attending CPAC meeting and Presentation of Calaska trading on the funding of about R6.7M	Melton Mulaudzi
23 Jun 15	DRDLR Offices, Nelspruit	Attending the Mpumalanga Province District Land Reform Committee (DLRC) Stakeholder meeting	Andrew Mbedzi

26 Jun 15	Mpofu training centre	To arrange Jacomien visit to Mpofu training centre to introduce Mrs. Desiree Shonken	Melton Mulaudzi
27 Jun 15	Show Ground, Makhado	Meeting Soetdoring Farmer Mr. Mathidi giving him Citrus Academy Educational DVDs and Discussing Pruning of Citrus	Andrew Mbedzi
30 Jun 15	DRDLR Offices, Brits	Attending the North West Province District Land Reform Committee (DLRC) Stakeholder meeting	Andrew Mbedzi
30 Jun – 1 Jul 15	King William Agric. Training Division	Attending Mentorship with Mr Mbaleni with his Manager and Lukhanyo from CGA	Melton Mulaudzi
01 Jul 15	Chauke's Farm, Nghezimane	Arranging and Planning of Water Farmers Day	Andrew Mbedzi
02 Jul 15	Agric. Offices, Makwarela	Vhembe Citrus Technical Committee meeting on the Organization of Water farmers Day at Chauke's Farm	Andrew Mbedzi
03 Jul 15	Agric. Offices, Makhado	Briefing Makhado Extension Officers on a New Citrus Farmer (Mamusha Boerdery) in Dendron (formerly Soetdoring Farm)	Andrew Mbedzi
07 Jul 15	Mpofu Training Centre-Battlesden farm Alice	Attending meeting with Ms. Jacomien de Klerk , Desiree , Siyabula Katyana and Mpofu officials	Melton Mulaudzi
08 Jul 15	Luthando farm, Kirkwood, SRV	Attending citrus study group on Irrigation scheduling, Pruning and Fertilization with Andre Combrink from SRCC	Melton Mulaudzi
08 Jul 15	Rural Dev. & Land Reform Offices, Nelspruit	Attending District Land Reform Committee meeting on Land Reform Projects and Agri-Parks	Andrew Mbedzi
09 Jul 15	Patensie Boplaas	Attending citrus study group pertaining Irrigation scheduling, Pruning and Fertilization	Melton Mulaudzi
14 Jul 15	Peddie Reillyvale/ siyamila farm	Attending citrus study group pertaining Irrigation scheduling, pruning and Fertilization	Melton Mulaudzi
15 Jul 15	Thulwane, Inthathakuza and Umhlavawethu	Monitoring pruning and harvesting of early navels. Umhlavawethu was still having a major irrigation challenges	Melton Mulaudzi
15 Jul 15	Mabunda Citrus Farm, Xitlakati	Attending Mabunda Board Meeting on the Picking and Packing of Mabunda Citrus Fruit	Andrew Mbedzi
16 Jul 15	Nkwaleni Community Hall	Attending citrus study group pertaining Irrigation scheduling , pruning and Fertilization	Melton Mulaudzi
16 Jul 15	Agric. Offices, Makwarela	Vhembe Citrus Technical Committee meeting on the Arrangement and Planning of the Citrus Field Day	Andrew Mbedzi
19 Jul 15	Port Elizabeth, Eastern Cape	To Attend the CGAGDC Board of Directors Strategic Workshop with Mankhili	Andrew Mbedzi
20 Jul 15	Kronenhoff, Kirkwood, SRV	Attending CGAGDC Board of Directors Strategic Workshop	Andrew Mbedzi Melton Mulaudzi
21 Jul 15	Kronenhoff, Kirkwood, SRV.	Attending CGAGDC Board of Directors Strategic Workshop	Andrew Mbedzi Melton Mulaudzi
24 Jul 15	Gonzana, Torties, Orange	Monitoring picking of navels, fertilisers and chemicals delivered	Melton Mulaudzi
28 - 30 Jul 15	Post St Johns, Mthatha	Attending greening committee awareness meeting and B.I.	Melton Mulaudzi
29 Jul 15	Mariveni Citrus Farm, Letsitele	Dropping-off Mariveni Export Training Workshop Certificates	Andrew Mbedzi

30 Jul 15	Lulekani Agric. Offices, Phalaborwa	Attending Mopani Citrus Study Group on Citrus Spray Programme, Invader Fruit Fly and Vhembe Citrus Field Day	Andrew Mbedzi
31 Jul 15	Chauke's Farm, Nghezimane	Check Progress on the Planning of Water Farmers Day	Andrew Mbedzi
31 Jul 15	White citrus Farm	Monitoring of picking of navels citrus fruit	Melton Mulaudzi
02-6 Aug 15	Western Cape Paarl	Attending Solidaridad workshop Western Cape partnership with SIZA and FRUITSA	Melton Mulaudzi
05 Aug 15	Chauke's Farm, Nghezimane	Delivering Banners, Chairs for the Water Farmers Day	Andrew Mbedzi
06 Aug 15	Chauke's Farm, Nghezimane	Attending Vhembe Citrus Study Group On Water Use and Water Stress on Citrus.	Andrew Mbedzi
07 Aug 15	Soetdoring Farm, Dendron	Assisting the New Citrus Farmer with the Pruning of the Citrus Trees.	Andrew Mbedzi
13-14 Aug 15	Northern Cape	Attending meeting with Northern Cape farmers on new company	Melton Mulaudzi
16-17 Aug 15	Western Cape Paarl	Attending CPAC meeting at Hortgro on Candlewood, Calaska trading presentation requesting R6, 7million for citrus project	Melton Mulaudzi
18 Aug 15	Western Cape Paar/ Stellenbosch and Chamomile farm	Visit to Chamomile farm by DAFF, Western Cape Provincial and marketing forum members	Melton Mulaudzi
19 Aug 15	WC Stellenbosch Agricultural Training institute	Attending presentation by Solidarida to the marketing Forum. Solidaridad is proposing to work with smallholders farm	Melton Mulaudzi
20 Aug 15	SASA Offices (Cane Growers) KZN	Attending KZN MoU Steering Committee Meeting on the Implementation of the MoU Between CGA and Department	Andrew Mbedzi Melton Mulaudzi
21 Aug 15	Dube Trade Port, King Shaka Airport	Attending the KZN Extension Forum Meeting at the Dube Trade Port	Andrew Mbedzi Melton Mulaudzi
25 Aug 15	Seloane Farm, Phalaborwa	Farm Visit to Check on the Development of the Planted Lemon Seedlings	Andrew Mbedzi
26 Aug 15	Agric. Offices, Makhado	Delivering Integrated Pest Management Booklets	Andrew Mbedzi
27 Aug 15	Gillimburg Citrus Farm, Mokopane	Attending Waterberg Citrus Study Group on Picking, Packing and Grading of the Citrus Fruit	Andrew Mbedzi
27-28 Aug 15	Kwazulu Natal Durban Garden	Attending National Female awards in Kwazulu Natal Province	Melton Mulaudzi
28 Aug 15	AGRIC. Office, Makwarela	Delivering the Invader Fruit Fly Kits (Lynfield Traps, M3, Dichlofos Tablets & Mat Blocks)	Andrew Mbedzi
02 Sep 15	Rural and Land Reform Offices, Brits	Meeting with Thabiso regarding the New Acquired Citrus Farms in Zand Drift and Mooinooi	Andrew Mbedzi
02 Sep 15	Port Elizabeth	Developing Agenda for Citrus Information Day with Mr. Hannes Bester and preparation of combined study group for SRV and Patensie growers at Boplaas farm	Melton Mulaudzi
03 Sep 15	Motsuenyane Foundation Off., Pretoria	Discussing the present state and future of Dennilton Citrus Farm with Pius Motsuenyane	Andrew Mbedzi
03 Sep 15	African Sand B&B PE-Patensie Boplaas and Fort Beaufort	Attending combined study group on spring pests and handing over of B.I trap, FCM, Fruit fly trap as a grant to the growers by DRDAR from Cacadu District	Melton Mulaudzi
04 Sep 15	Soetoring Farm, Dendron	Assisting the farmer with the Citrus spraying programme	Andrew Mbedzi

04 Sep 15	Greenwood	Monitoring fertilization	Melton Mulaudzi
05 Sep 15	Musunda Farm, Musunda	Assist the local Extension Officer with the irrigation challenges on the Farm	Andrew Mbedzi
07 Sep 15	Topkat farm	To discuss the new development of business plan of the farm which need to submitted to DRDRL East London	Melton Mulaudzi
09 Sep 15	Agric. Offices, Makwarela	Attending Vhembe Citrus Technical Committee Meeting on the organization and planning of the Citrus field day Event	Andrew Mbedzi
09-10 Sep 15	East London Escape Palace	Attending greening committee meeting with Dr Hennie Le Roux and Provincial Government from DRDAR	Melton Mulaudzi
11 Sep 15	Premier Regent ICC – Fort Beaufort	Attending DLRCS meeting with Eric Nohamba chairperson of Alice KAT Trust	Melton Mulaudzi
15 Sep 15	Western Cape Paarl Hortgro Office	The EO of Candlewood presented on Candlewood Empowerment Trust project funding	Melton Mulaudzi
15 Sep 15	Swadini, Hoedspruit	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
16 Sep 15	Swadini, Hoedspruit	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
16 Sep 15	Western Cape Paarl Hortgro Office	Solidaridad Rural Horizon meeting on EC awareness workshop	Melton Mulaudzi
17 Sep 15	Cape College	Attending developmental inputs meeting with growers, Amathole EOs and growers	Melton Mulaudzi
17 Sep 15	Loskop Dam, Groblersdal	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
18 Sep 15	Loskop Dam, Groblersdal	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
21 Sep 15	Luthando Farm, Willow tree Kronenhoff, SRV	Attending Eastern Cape Provincial working committee with Dr Sonandi, Lukhanyo, Kalawe on training of Extension officers	Melton Mulaudzi
22 Sep 15	Kronenhoff – Willow tree Masifunde	Attending Citrus Information Day with growers and other stakeholders	Melton Mulaudzi
22 Sep 15	CRI Boardroom, Nelspruit	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
23 Sep 15	CRI Boardroom, Nelspruit	Attending CRI Citrus Production, IPM and Diseases Management Workshop	Andrew Mbedzi
23 Sep 15	Oakdene, Jerico, Konzi and Eden	Monitoring delivering of grant pesticides by Provincial Department of Agriculture	Melton Mulaudzi
25 Sep 15	Easy Farm, Tshivhilwi	Preparation of venue for the Vhembe Citrus Field Day Event	Andrew Mbedzi
28 Sep 15	Kirkwood Kronenhoff	Preparation for Solidaridad workshop / meeting with growers	Melton Mulaudzi
28 Sep 15	Agric. Offices, Makwarela	Attending Vhembe Citrus Technical Committee Meeting on the finalization of the Agenda for the Citrus Field Day Event	Andrew Mbedzi
29 Sep 15	Patensie Tobacco	Attending Solidaridad workshop with Karin from Solidaridad, Mike from Siza Standard, Lukhanyo and Patensie farmers	Melton Mulaudzi
30 Sep 15	Willow tree Masifunde Sonke, SRV	Attending Solidaridad workshop with Karin from Solidaridad, Mike from Siza Standard, Lukhanyo and SRV farmers	Melton Mulaudzi
30 Sep 15	Tzaneen Country Lodge, Letsitele	Attending DAFF Annual Citrus coordinating Meeting at the Tzaneen Country Lodge in Tarentaalraand, Letstele	Andrew Mbedzi
01 Oct 15	Easy Farm, Tshivhilwi	Promoting the competitiveness of Citrus Farming in a Dynamic Economic Environment	Andrew Mbedzi

01-2 Oct 15	Mentorskraal, Jeffreys Bay	Attending CRI Production workshop with both commercial and BEE growers	Melton Mulaudzi
02 Oct 15	Agric. Offices, Makhado	Meeting to clear up equipment and furnisher that were used during the Citrus Field Day Event at Easy Farm	Andrew Mbedzi
05 Oct 15	Birchwood Hotel, Johannesburg	Attending the Chamber Executive Committee Meeting on the update of the CGA Grower Development Company	Andrew Mbedzi Melton Mulaurdzi
06 Oct 15	Birchwood Hotel, Johannesburg	Attending the Citrus Growers Development Chamber Meeting on the update of the CGA Grower Development Company	Andrew Mbedzi Melton Mulaudzi
07 Oct 15	Agric. Office, Makwarela	Preparation meeting for the Vhembe Citrus Study Group to be held at Mamusha Boerdery in Dendron on 9th of October 2015	Andrew Mbedzi
08 Oct 15	Levubu Citrus Farms, Levubu	To check on the extent of citrus orchard removal in the area	Andrew Mbedzi
08 Oct 15	Nkwaleni Community Hall	Attending citrus study group meeting on Citrus spring pest complex and CBS control	Melton Mulaudzi
09 Oct 15	Mamusha Boerdery, Dendron	Attending Vhembe Citrus Study group on Responsible Use of Agro-chemicals, Monitoring and Control of Citrus Pests and Diseases	Andrew Mbedzi
09 Oct 15	Torties, Gonzana, Jordan, Oakdene & White Citrus Farms	Monitoring bollworm sprays	Melton Mulaudzi
13 Oct 15	East London Road Lodge to Fish River	Provincial workshop on the Eastern Cape relation with other stakeholders and financial budgets for upcoming growers	Melton Mulaudzi
14 Oct 15	City Lodge Hotel, Port Elizabeth	Attending Pre-season meeting with DAFF and growers	Melton Mulaudzi
15 Oct 15	Sibonelo Lemon Farm, Eilandshoek	To check the Progress on 3.5ha of Newly Planted Citrus Trees and office building	Andrew Mbedzi
16 Oct 15	Thekwane Lemon Farm, Thekwane	To deliver Citrus Academy Export Training Workshop Certificate	Andrew Mbedzi
19 Oct 15	Du Roi IPM, Mariveni, Vhembe	Deliver Scouting Booklets on my way to Vhembe and Waterberg for Meetings	Andrew Mbedzi
20 Oct 15	Alicedale Farm, Tshipise	To arrange with Rabelani to assist Victor Masilo of Mooinooi in Brits with irrigation	Andrew Mbedzi
21 Oct 15	Radium Citrus farms, Waterberg	Farm visits with the district coordinator to check on planting of new citrus trees	Andrew Mbedzi
21-25 Oct 15	Paarl in the Western Cape	CPAC meeting at Hortgro on Candlewood, Calaska trading requesting R6,7million for citrus project at Swellendam	Melton Mulaudzi
22 Oct 15	Danstaat farm, Limpopo River	Assisting the farm to plan the new planting of the citrus orchards	Andrew Mbedzi
26 Oct 15	Fort Cox College	Training meeting with Mrs Qampi on training farm workers of Nwenwe Cooperatives funded by ARC through PPECB	Melton Mulaudzi
27 Oct 15	Mooivalei Farm, Thabazimbi	To assist them to find some other nearby farms who can use the packhouse	Andrew Mbedzi
27 Oct 15	Eden, Blinkwater	Discussion about the 2015 spraying programme for BEE Growers	Melton Mulaudzi
28 Oct 15	Ongerep Farm, Lephalale	To check on the allocation of new citrus trees given out by the Govt. Greening Division	Andrew Mbedzi

28-29 Oct 15	Road Lodge Hotel in East London	Meeting on the re-building of Ripplemead packhouse with Land Bank, DRDAR and Peddie Eos in Bisho Govt. Offices	Melton Mulaudzi
29 Oct 15	Bathokoa Farm, Mokopane	Meeting with the Farmer and his Mentor on the Replanting of 6ha of Citrus	Andrew Mbedzi
30 Oct 15	DRDLR Offices in East London	Recap status meeting with Mr. Ncedisa from DRDLR, Ms. Susan, Lawrence Mgadle and Lukhanyo from CGA	Melton Mulaudzi
30 Oct 15	Agric. Offices, Makhado	Assisting the Makhado citrus coordinators with their citrus presentations for the citrus study groups	Andrew Mbedzi
02 Nov 15	Eden, Blinkwater	Handing over the new planting DVD from Citrus Academy	Melton Mulaudzi
03 Nov 15	Torties, Jerico & Cape College	Handing over the new planting DVD from Citrus Academy	Melton Mulaudzi
04 Nov 15	Ripplemead & Naudeshoek	Handing over the new planting DVD from Citrus Academy	Melton Mulaudzi
04 Nov 15	Seloane Farm, Phalaborwa	To check progress on the panted 30 ha of lemon trees	Andrew Mbedzi
05 Nov 15	Sundays River Valley Citrus Farms	Handing over the new planting DVD from Citrus Academy	Melton Mulaudzi
06 Nov 15	Multi-spray, Central Park in Nelspruit	Organizing the Multi-spray day for the Vhembe Citrus Farmers	Andrew Mbedzi
06 Nov 15	Hocksberg	Meeting Mr. & Mrs. Dick to discuss the purpose of requesting the database of KAT river growers	Melton Mulaudzi
09 Nov 15	White River	Meeting Wally on the re-use of old oil to be collected from small farmers who are using diesel pumps	Andrew Mbedzi
12-13 Nov 15	Crown Hotel in King Williams Town	Attending Ripplemead Pack house meeting with NAMC, DRDAR Provincial officers and Lukhanyo	Melton Mulaudzi
17 Nov 15	Peddie Ripplemead Farm and PE Town Lodge	Developing 2016 calendar activities for Provincial working committee with Dr. Sonandi, Ms. Gayiya, Mfundo, Mpangeva, Lawrence and Lukhanyo	Melton Mulaudzi
18 Nov 15	PE North End DAFF offices	Attending greening committee meeting with Provincial Government from DRDAR. Mr. Preggy chaired the meeting	Melton Mulaudzi
19 Nov 15	SASA office Durban	Attending KZN MoU working committee meeting regarding Extension Services, Farmers support, identification of Extension officers to be trained and marketing	Melton Mulaudzi
19 Nov 15	Ushukela Offices, Mt. Edgecombe in KZN	Steering Committee meeting on the Implementation of the MoU between CGA and the KZN Department of Agriculture	Andrew Mbedzi
24 Nov 15	Rust de Venter, Waterberg	Attending Waterberg Citrus Study group on Planting Management and Care of Young Citrus Trees	Andrew Mbedzi
25 Nov 15	Mariveni Farm, Letsitele	Attending Mopani Citrus Study group on the effect of marketing Citrus Fruit from areas with Oriental Fruit Fly	Andrew Mbedzi
26 Nov 15	Sibonelo Lemon Farm, Eilandshoek	Meeting with the Farm Manager regarding picking and water issues and also giving him planting DVD from Citrus Academy	Andrew Mbedzi
30 Nov 15	City Lodge Hotel, OR Tambo in JHB.	Attending Chamber Executive Committee meeting on the development of CGA Grower Development Company	Andrew Mbedzi Melton Mulaudzi
03 Des 15	Lidell, Letas & Greewood farms	Handing over the new planting DVD from Citrus Academy	Melton Mulaudzi

04 Des 15	Agric. Offices, Makwarela	Attending a meeting on the planning and arrangements of the Multi-spray day in the Vhembe district	Andrew Mbedzi
04 Des 15	Eden, Orange Grange Jordan Farms	Monitoring the spraying programme of CBS control and FCM control	Melton Mulaudzi
07 Des 15	Jerico, Konzi, Eden & Greenwood Farms	Monitoring CBS control and placement of delta traps for FCM control but no traps have been applied during my visit	Melton Mulaudzi
08 Des 15	ETC–Port Elizabeth	Attending provincial public consultation workshop on the draft amended AgriBEE sector code (60 days public comments)	Melton Mulaudzi
09 Des 15	Empangisweni Trust, Gluckstad in Vryheid	Check progress of planted 60ha of Eureka lemons and planting of 40ha of Oranges	Andrew Mbedzi Melton Mulaudzi
10 Des 15	Sun Valley Farms and A & D Farm KZN	Witness the empowerment progress being Implemented in the farm	Andrew Mbedzi Melton Mulaudzi
11 Des 15	Madzikane, Sokhela and Donovale Farms	Check progress on the recommendations given during the Previous Farm visits	Andrew Mbedzi Melton Mulaudzi
15 Des 15	King Shaka Airport, KZN	Meeting with Justin the CGA CEO on the staffing negotiations of the CGA Grower Development Company	Andrew Mbedzi Melton Mulaudzi
13 Jan 16	Cape College	MoA Between Alice KAT Growers and Dr. Phehane of ARC and housing all cultivars allocated to small growers at CGAGDC	Melton Mulaudzi
14 Jan 16	Mooinooi in Brits, NW	Due diligence on Citrus farms Grootfontein 346 JQ, Komrivier 347 JQ and Groenkloof 464 JQ	Andrew Mbedzi
14 Jan 16	Alice KAT Jerico Farm	Attending fixing of irrigation and monitoring spraying programme of CBS and Thrips	Melton Mulaudzi
15 Jan 16	Winterveldt Farm, Pretoria	Check progress on the water rights and installation of pipeline from the river	Andrew Mbedzi
18 Jan 16	Komatipoort, Sebonelo and Nelspruit Riverside	Farmers and Citrus academy students interviews for the CGA Transformation publication	Andrew Mbedzi
18 Jan 16	Okdene, Lovers Retreat	Monitoring delta traps for FCM, and fruit fly traps but there were nothing in place	Melton Mulaudzi
19 Jan 16	Bu/buckridge, Letsitele and Hoedspruit	Farmers and Citrus academy students interviews for the CGA Transformation Publication with Louise Brodie	Andrew Mbedzi
19 Jan 16	Gonzana farm	Monitoring irrigation systems and advising the grower to open the lateral pipes to flush dirt out	Melton Mulaudzi
20 Jan 16	Battesden/ Huduza farms	Assisting the farmer to fixed the lateral pipes	Melton Mulaudzi
20 Jan 16	Easy Farm & Chauke's (Mkharo) Farm	Farmers and Citrus academy students interviews for the CGA Transformation publication with Louise Brodie	Andrew Mbedzi
21 Jan 16	Hammaskraal in Pretoria	Farmers and Citrus academy students interviews for the CGA Transformation publication with Louise Brodie	Andrew Mbedzi
21 Jan 16	Kirkwood Luthando farm	Citrus study group on soil and leaf analysis, Oriental Fruit fly and Fruit fly control and developing 2016 activities plan	Melton Mulaudzi
22 Jan 16	Patensie Boplaas farm	Citrus study group on Soil and leaf analysis, Oriental Fruit fly and Fruit fly control and developing 2016 activities plan	Melton Mulaudzi

22 Jan 16	Fruit SA Offices Lynwood in Pretoria	Farmers and Citrus academy students interviews for the CGA Transformation publication with Louise Brodie	Andrew Mbedzi
25 Jan 16	Drive to Tzaneen in Limpopo	To attend the Citrus Research International (CRI) Citrus Post-Harvest Workshops	Andrew Mbedzi
25-26 Jan 16	Nkwaleni Community Hall	Citrus study group on Soil and leaf analysis, Oriental Fruit fly and Fruit fly control and developing 2016 activities plan	Melton Mulaudzi
26 Jan 16	Fairview Hotel in Tzaneen	Attending the Citrus Research International (CRI) Citrus Post-Harvest Workshops	Andrew Mbedzi
27 Jan 16	Fairview Hotel in Tzaneen	Attending the Citrus Research International (CRI) Citrus Post-Harvest Workshops	Andrew Mbedzi
27 Jan 16	Torties farm	Monitoring the planting of Satsuma at Torties and also monitoring receiving of the 50000 Nules seedling from Northern Cape Nursery	Melton Mulaudzi
28 Jan 16	City Lodge Hotel, OR Tambo International	Attending the Chamber Executive Committee Meeting on CGA Grower Development Company	Andrew Mbedzi Melton Mulaudzi
29 Jan 16	Blinkwater	Delivering the notes concerning pesticides from Government regarding Global GAP preparation	Melton Mulaudzi
01 Feb 16	Temo-Towers in Polokwane	Attending Meeting with the Provincial Coordinator on the 2016/17 Citrus Activity Plan	Andrew Mbedzi
03 Feb 16	Greenwood Farm	Monitoring planting of Nadorcott with Carrizo rootstock from Joubert Nursery at Kirkwood	Melton Mulaudzi
05 Feb 16	Mpofu training Centre	Attending information day on beef production and food processing with MEC of DRDAR	Melton Mulaudzi
08 Feb 16	Central Park in Nelspruit	Attending a Meeting with the Multispray Company on holding the information Day in Vhembe District	Andrew Mbedzi
09 Feb 16	LVCC in Nelspruit	Attending the Citrus Research International (CRI) Citrus Post-Harvest Workshops	Andrew Mbedzi
09 Feb 16	Sundays River Valley Valentine Hall	Attending EU CBS meeting with DAFF and growers	Melton Mulaudzi
10 Feb 16	LVCC in Nelspruit	Attending the Citrus Research International (CRI) Citrus Post-Harvest Workshops	Andrew Mbedzi
10 Feb 16	Torties Farm	Monitoring planting of Satsuma and 50000 Nules seedlings from Northern Cape Nursery	Melton Mulaudzi
11 Feb 16	Den Staat Farm, Weipe in Musina.	Attending Vhembe Citrus Study group on Scouting of Citrus Pests and Control of the Oriental Fruit Fly	Andrew Mbedzi
15 Feb 16	Rust de Venter Citrus Farms,	Follow-up on Citrus Seedlings that were given to farmers by the Department	Andrew Mbedzi
15 Feb 16	Kwadesi-Sundays River Valley-Willow Tree	Attending Eastern Cape Working Committee with Western District Extension officers and beneficiaries	Melton Mulaudzi
16 Feb 16	Mmabatho Palms Hotel, Mahikeng, NW	Attending Provincial and District Land Reform Committees orientation and Training Workshop	Andrew Mbedzi
16-17 Feb 16	African Sand to Jefferys Bay Mentors	Attending CRI Post-Harvest workshop with farmers and CRI Staff	Melton Mulaudzi

17 Feb 16	Mmabatho Palms Hotel, Mahikeng, NW	Attending Provincial and District Land Reform Committees Orientation and Training workshop	Andrew Mbedzi
18 Feb 16	Ratanang Citrus Project, Thabazimbi	Attending Waterberg Citrus Study group on Irrigation, Citrus Pests and Diseases Control	Andrew Mbedzi
23 Feb 16	Lovers retreat	Monitoring Satsuma coloring break and fruit maturity testing and the testing Satsuma has MRL of Runner	Melton Mulaudzi
24 Feb 16	CRI Boardroom, Nelspruit	Attending The Citrus Growers Association Roadshows on Renewal of the Levy	Andrew Mbedzi
24 Feb 16	Sihlangule Coop Blue waters	Received pesticides from Govt. Grants. Was advised to prune trees and also apply Erase herbicides	Melton Mulaudzi
25 Feb 16	DFA Citrus Farm in Dennilton	Checking progress on the revival of 400 burned Citrus Trees	Andrew Mbedzi
25 Feb 16	Peddie Ripplemead farm	Attending packhouse meeting with farmers and Government Extension officers and Agricultural Economics from Bisho	Melton Mulaudzi
29 Mrt 16	Tshipise Avetura, Tshipise	Attending The Citrus Growers Association Roadshows on Renewal of the Levy	Andrew Mbedzi
01 Mrt 16	Agric. Offices in Makwarela	Attending Vhembe Citrus Technical Committee Meeting on the Planning of Citrus Activity Plan for the 2016/17	Andrew Mbedzi
02 Mrt 16	DRDLR Offices in Rustenburg	Business Plan Evaluation Committee Meeting of Elandskraal 469 JQ, and Zoutpansdrift 415 JQ BPs	Andrew Mbedzi
02 Mrt 16	Lidell farm	Monitoring hail damage. At least 20% of the fruit have dropped down	Melton Mulaudzi
04 Mrt 16	Jordan farm	Monitoring the Nadorcott block against thrips and orange dog caterpillar	Melton Mulaudzi
09 Mrt 16	East London to KZN Nkwaleni	Preparation of Citrus information day at Nkwaleni Processing plant with farmers, Andrew and other Stakeholders	Melton Mulaudzi Andrew Mbedzi
10 Mrt 16	Nkwaleni Valley Processing, KZN	Attending Kwazulu Natal Citrus Information Day at the Nkwaleni Valley Processing Plant	Andrew Mbedzi Melton Mulaudzi
11 Mrt 16	PE CRI office	Attending Performance appraisal with Mr Hannes Bester Manager Extension	Melton Mulaudzi
13-14 Mrt 16	Western Cape Paarl	Attending and preparation of CPAC meeting at Hortgro offices	Melton Mulaudzi
14 Mrt 16	DRDLR Offices	Attending the District Land Reform Committee on the Establishment Beneficiary Selection Committee (BSC)	Andrew Mbedzi
15 Mrt 16	Sundays River Addo African Inn	Attending PPECB citrus information day with Exporters and pack houses managers	Melton Mulaudzi
17 Mrt 16	DRDLR Offices	Attending the District Land Reform Committee on the Establishment Beneficiary Selection Committee (BSC)	Andrew Mbedzi
18 Mrt 16	Diep in Die Berg, Pretoria	Attending Citrus Growers Development Chamber Meeting and the Launch of CGA Grower Development Company	Andrew Mbedzi Melton Mulaudzi
22 Mrt 16	Easy Farm, Tshivhlwi	Drop-off Agro-chems for Testing Oranges and Farm visit	Andrew Mbedzi
22 Mrt 16	East London Airport to KZN Cedar	Attending KZN Extension Summit at Cedar College	Melton Mulaudzi
24 Mrt 16	Bathokoa Farm, Mokopane	Check progress on the newly planted 6ha of Navel citrus Trees	Andrew Mbedzi

8.3 RESEARCH PRIORITIES / NAVORSINGSPRIORITEITE 2015-16

8.3.1 INTEGRATED PEST MANAGEMENT

AREA	*INTEGRATED PEST MANAGEMENT*	
	Topic	**Priority description**
Baviaans		
Citrusdal Swellendam	Slakke	Groter wordende problem veral op laat-Mandaryne (hoë inkomste kultivars) – word langer "blootgestel" weens plukvenster. Beheer maatreëls onvoldoende.
	Karobmot	Produsente voel verwarring heers t.o.v. VKM en beheermaatreëls.
	Woolly White Fly	Raak toenemende plaagprobleem en beheermaatreëls onvoldoende – sommige produsente het 3-4x gespruit met o.a. Buprofezin/Nemesis. Berg areas in Citrusdal 'n groot problem, maar raak ook nou in die Vallei 'n problem. Biologiese beheeropsies?
	Onder net	Toenemende netoprigting in beide areas: Plaag toename: myte, witluise, wollerige witvlieg, residuele afbreek – langer?
	Australiese wolluis	Raak toenemend meer
Beitbridge Tshipise Weipe	False Codling Moth	Bly steeds nommer 1 prioriteit
Breederivier		
Burgersfort		
Constantia		
Hoedspruit		
Katrivier	Thrips	Find softer alternative option to Abamectin
	Mealybug	Investigate softer control options & evaluate navel susceptibility for different selections
	Lemon moths	Develop monitoring system
	Netting	Determine effect of nets on OPM & DM
Malelane/Komatipoort		
Marble Hall		
Midknight	No further details	
Nelspruit	Rind pitting	Urgently require treatments which are superior to TBZ and which will contain pitting once fruit is discharged from cold storage in overseas markets
Nkwaleni		
Ohrigstad		
Oranjerivier	False Codling Moth	Meer werkswinkels moet aangebied word
	Vrugtevlieg	Watter produk kan die papies doodmaak?
	Dopluis	Organiese beheer?
	Grysmyt	Suurlemoene en nawels
	Bladspringer	Daar is geen geregistreerde produk nie – soek produk vir 1 maand voor oes
	Bolwurm	Toets Diapell – toets vir konstantheid
Patensie		
Pongola		
Rustenburg		
Sondagsrivier	Thrips	A: Remains a major problem resulting in fairly significant cull, particularly on navels and late set lemons B: Due to a limited choice of chemical options, producers are forced to use harsh (non-IPM friendly) products, resulting in other pest repercussions that require further chemical intervention

		C: Investigate alternative options to traditional chemical control of nymphs/adults (similar to the approach taken on FCM control), including biological control options and soil treatments
Stellenbosch Paarl Swartland		
Southern Natal		
Swaziland		
Swellendam		
Vaalharts		
Waterberg	Psylla	Beheer strategië op draende bome vir Psylla op S/L

8.3.2 DISEASE MANAGEMENT

AREA	*DISEASE MANAGEMENT*	
	Topic	**Priority description**
Baviaans		
Citrusdal Swellendam	Root rot	Huidige beheerprogramme onvoldoende
	Botrytis	Waarskynlik groter wordende problem weens klimaatsverandering = natter lente
	Copper stippling	Alternaria-bruinvlek groter wordende problem op verskeie vatbare kultivars. M45 kan nie gebruik word nie weens market (VSA & Kanada)
Beitbridge Tshipise Weipe	Citrus Black Spot	Bly die nommer 1 prioriteit. Alles moet gedoen word om hierdie problem op te los. Omrede dit meer ‘n politieke en finansiële geskil is as wat dit ‘n Fito probleem is sal CRI en CGA hierdie problem by die WHO moet oplos
Breederivier		
Burgersfort		
Constantia		
Hoedspruit		
Katrivier	Nematodes	Investigate cheaper fumigation options
	Postharvest	Find an alternative to Quazatine
Malelane/Komatipoort		
Marble Hall		
Midknight	No further details	
Nelspruit	Blight/rootstock	Identification of additional Blight-tolerant rootstocks, i.e. in addition to Swingle, Cleopatra and possibly X639
Nkwaleni		
Ohrigstad		
Oranjerivier	Alternaria	Organiese beheer op nawels en Manderyne
	Afrika vergroening	
	Wortelvrot	Organiese vrugte (organiese produk?)
	Biosekuriteit	Asiatiese ontgroening
Patensie		
Pongola		
Rustenburg		
Sondagsrivier	Root borne	A: Tree death/decline due to Armillaria-like symptoms is widespread and causing significant losses in orchards planted in virgin soil and on trifoliate rootstocks

		Continue with investigating the cause of the problem (identification of the causal agent) and develop a control strategy/recommendation B: Phytophthora root rot remains a serious problem on replant soils. Current recommendations are outdated and do not appear to be highly effective. Investigate new strategies, including commercial recommendations for fumigation and the reintroduction of beneficial microbes thereafter.
	Additional comment	There is a concern that trees originating from certified nurseries could partly be to blame for tree losses shortly after planting. Hence, a request to ensure that the CIS certification protocol is being properly implemented and adhered to.
	Post-Harvest	A: Continue with research on all aspects of cold sterilization including understanding its implications (negative) on fruit quality, shelf life, flavour, etc. B: Practical method to improve application of packhouse chemicals. C: Need an effective replacement for guazatine and continue search for softer alternatives to existing chemicals.
	Botrytis	A: Fairly common disease on lemons in seasons characterized by wet blossom periods (September). B: Benomyl is effective but there are concerns regarding frequent use and the likelihood of resistance development, as it still forms part of SRV CBS programme. Also, once Benomyl has been applied to protect the new season's blossom, no further picking is possible. C: Research to focus on: i) Finding new products (alternatives to Benomyl) including evaluation of biological products with little/no residue implications. ii) Understanding the dynamics of the disease to determine reasons for specific orchards being more susceptible than others.
Stellenbosch Paarl Swartland		
Southern Natal		
Swaziland		
Swellendam		
Vaalharts		
Waterberg		

8.3.3 HORTICULTURE

AREA	***FRUIT PRODUCTION & QUALITY MANAGEMENT***	
	Topic	**Priority Description**
Baviaans		
Citrusdal Swellendam	Onder net	Toenemende oprigting van nette in beide areas – invloed op produksie, kwaliteit (vruggrootte, interne & kleur)
	Girdling [Citrusdal]	Minder belangrik
	Alternate bearing	Bly 'n uitdaging veral op laat-Mandaryne – hoë inkomste kultivars
	Biostimulante	Raak belangriker, meer produkte beskikbaar, effektiwiteits-onsekerheid
	Sonbrand [Citrusdal]	Bly 'n uitdaging, Screen duo nie effektief, alternatief?
	Soil Health	Raak toenemend belangrik vir produsente – praktyke vir bevordering en bio-produkte

Beitbridge Tshipise Weipe	TBZ	Bly steeds nommer 1 prioriteit – die vervanging van TBZ met ‘n sort gelyke produk
Breederivier		
Burgersfort		
Constantia		
Hoedspruit		
Katrivier	Fruit set	Improve fruit set on all Mandarins and seedless lemons
	Internal Quality	Alternative for calcium arsenate
	Colour	Improve colour development on early varieties
	Pruning	Do economic study on benefits of pruning
	Netting	How to improve production and fruit colour under nets
	Alternate bearing	Prediction of alternate bearing for soft citrus
	Carbohydrates	As tool to predict alternate bearing on soft citrus
	Weed control	Need product to control “motvanger”
Malelane/Komatipoort		
Marble Hall		
Midknight		
Nelspruit	New varieties	Require new varieties which mature in June. It can be navels which colour well or Valencia-types which do not require manipulation to reduce acids
		A good quality Valencia-type maturing in July to August (must not require arsenate sprays to reduce acids). This variety is required to supplement Midknights and replace old clone Valencias.
	Rind Pitting	Urgently require treatments which are superior to TBZ and which will contain pitting once fruit is discharged from cold storage in overseas markets.
Nkwaleni		
Ohrigstad		
Oranjerivier	Sonbrand	
	Vrugset	Dit bly altyd ‘n problem op alle kultivars
	Vruggrootte	
	Kleur	Kleurontwikkeling op vroeë kultivars
	Raklewe	Bestuur van koueketting, hantering van vrugte, kyk na protokokke, boord tot pak
	Smaak van vrugte	Waarom die Nardorkot se smaak verander
	Bemesting	Watter invloed of voordeel het silica in die bemestingsprogram?
	Besproeiing	Bestuur van besproeiingstelsels
	Kultivars - Onderstamme	
	Koue skade	Koue skade / ryp in die boord. Voorligting asb.
	Vrugkwaliteit	Kleur van vrugte onder nette
	Grondgesondheid	
	Biostimulante	
Patensie		
Pongola		
Rustenburg		
Sondagsrivier	Creasing	Remains a major cull factor on navels in most seasons. Current management practices are not always sufficient to achieve commercial control
Stellenbosch Paarl Swartland		
Southern Natal		
Swaziland		
Swellendam		
Vaalharts		

Waterberg	Spuit	Doen proewe met elektrostatiese spuite en na doses aanpassings op etikette

8.3.4 CULTIVAR EVALUATION

RESEARCH PRIORITIES - NORTHERN & SOUTHERN AREAS - 2015-16		
	CULTIVAR EVALUATION	
CITRUS AREAS	**2013-14**	**2015-16**
Baviaans	Kry onderstam wat meer weerstandheid teen Armillaris. Kyk voordurend na verbeterde nawelseleksies vir die area om bestaande seleksies te vervang, ook later nawels. Enige niskultivars vir die area belangrik. Soek vroeë sagtesitrus in Satsuma se plek. Alternatiewe nawel in Palmer se plek.	Kultivars: Kyk na Cambria seleksie omtrent 6 weke vroeer as die huidige Cambrias. FCM word gevind in meer as 80% van ons nawelkultivars, seleksie minder vatbaar (Nawels). Soek meer vermer nawelseleksies. Onderstamme: Hier wissel dit van grond tot interne gehalte tot drag. Dink dit moet bespreek word vir die omstandighede/omgewing waarbinne geproduseer word. Produsente moet kennis neem van die beperkings van 'n onderstok binne sy omgewing en vereistes.
Beitbridge		Kultivars: Droë Valencia om Turkey (Juvalle) te vervang. Sagte sitrus proewe vorder goed. Onderstamme: Onderstamme vir Noordelike areas bestand teen Phytophthora.
Breederivier	Onderstamme: Soek onderstam wat fisiologiese skildefekte verbeter sonder om kwaliteite in te boet. Kultivars: Laat manderyne steeds groot behoefte. Meer deeglike evaluasie van nuwe kultivars voor vrystelling benodig. Soek vroeë sagtesiturs / Clementine in Nules tydgleuf met goeie vruggrootte en weerstand teen *Citrophthora*.	Cutivars & Rootstocks = High priority
Burgersfort		
Citrusdal	Nuwe kultivars alryd belangrik. Evalueer alle nuwe kultivars in area. Kry uitsluitsel oor onverenigbaarheid met Fukumoto en Mor t.o.v. Trifoliaat-onderstamme (Troyer, Carrizo, C35 en Swingle) - definitiewe onverenigbaarheid.	Cutivars & Rootstocks = High priority
Constantia		Cutivars & Rootstocks = High priority
Hoedspruit		Cutivars & Rootstocks = High priority
Katrivier	Find alternative rootstocks for replant soils. Update and disseminate information on old and new rootstocks in combination with cultivars. Find niche varieties for area, especially Mandarin/Soft citrus types. Draw up table for cross pollination between different varieites.	Cutivars & Rootstocks = High priority
Malelane/Komati.		Cultivars & Rootstocks = High priority
Marble Hall		Cutivars & Rootstocks = High priority
Midknight	C35 en ander verdwergde onderstamme	

Nelspruit	Seleksie van stabiele voortplantingsmateriaal.	Rootstock/Blight: Identification of additional Blight-tolerant rootstocks, i.e. in addition to Swingle, Cleopatra and possibly X639. Varieties: (1) Require new varieties which mature in June. It can be navels which colour well or Valencia-types which do not require manipulation to reduce acids. (2) A good quality Valencia-type maturing in July to August (must not require arsenate sprays to reduce acids). This variety is required to supplement Midknights and replace old clone Valencias.
Nkwaleni	This is ongoing research to find better varieties for the area. Need early grapefruit and other varieties to extend the season. Include CRI cultivars in Farmsecure trial sites (Graham Barry).	Cultivars & Rootstocks = High priority
Ohrigstad		
Oranjerivier	Evalueer verskillende kultivars per sitrustipe en onderstam om mees geskikte opsies vir area te vind.	Cutivars & Rootstocks = High priority
Patensie	Kry onderstam wat meer weerstand het teen Armillaria. Kyk voortdurend na verbeterde nawelseleksies vir die area om bestaande seleksies te vervang, ook later nawels. Enige niskultivars vir die area belangrik. Soek vroeë sagtesitrus in Satsuma se plek. Alternatiewe nawel in Palmer se plek.	Cutivars & Rootstocks = High priority
Pongola		Cutivars & Rootstocks = High priority
Rustenburg		Cutivars & Rootstocks = High priority
Sondagsrivier	Kry RL onderstam vir suurlemoene wat verdraagsaam is teen Phytophthora. Vind uit of Esselen Kwekery een het. Kyk ook na ander onderstamme vir suurlemoene wat minder groeikrag is as RL, maar meer as X639. Kry niskultivars vir area. Stel bestuurder vir nuwe kultivarmaatskappy aan. Kry fermer en beter nawelseleksies. Kry goeie vroeë nawel. Kry alternatiewe onderstamme vir herplant-situasies.	Rough lemon and X639 remain the only commercially compatible rootstock for Eureka lemons. However both rootstocks have their limitations. Rough lemon, in particular, has limited potential in replant situations, where it is highly susceptible to Phytophthora root/collar rot and nematodes. Find alternative rootstock options for Eureka lemons, with specific reference to replant soils.
Stellenbosch Paarl Swartland	Kry vroeë Valencia met beter interne gehalte vir VSA – laer suur en effens beter suiker. Benodig kruisbestuiwingstabel, veral vir laat manderyne.	Cultivars & Rootstocks = High priority
Southern Natal	Need early and late navels to spread packing season. Need replacement for Rustenburg and something later. Also need soft citrus varieties for this area.	Cultivars & Rootstocks = High priority
Swaziland		Cultivars & Rootstocks = High priority
Swellendam	Onderstamme: Soek onderstam wat fisiologiese skildefekte verbeter sonder om kwaliteite in te boet. Kultivars: Laat manderyne steeds groot behoefte. Meer deeglike	Cutivars & Rootstocks = High priority

	evaluasie van nuwe kultivars voor vrystelling benodig. Soek vroeë sagtesitrus/Clementine in Nules tydgleuf met goeie vruggrootte en weerstand teen *citrophthora*.	
Tshipise		Kultivars: Droë Valencia om Turkey (Juvalle) te vervang. Sagte sitrus proewe vorder goed. Onderstamme: Onderstamme vir Noordelike areas bestand teen Phytophthora.
Vaalharts	Benodig alternatiewe nawelseleksies vroeg, met beter kleur.	
Waterberg	Sagte sitrus vir hierdie spesifieke area. Vroeë Valencias.	Kultivars: 3 Onderstamme: 3
Weipe	Soek kultivars vir warmer klimate.	Kultivars: Droë Valencia om Turkey (Juvalle) te vervang. Sagte sitrus proewe vorder goed. Onderstamme: Onderstamme vir Noordelike areas bestand teen Phytophthora.

8.4 STUDY GROUP CHAIRMEN FOR 2016

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8.5 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PORTFOLIOS AND PROGRAMMES IN 2015-16

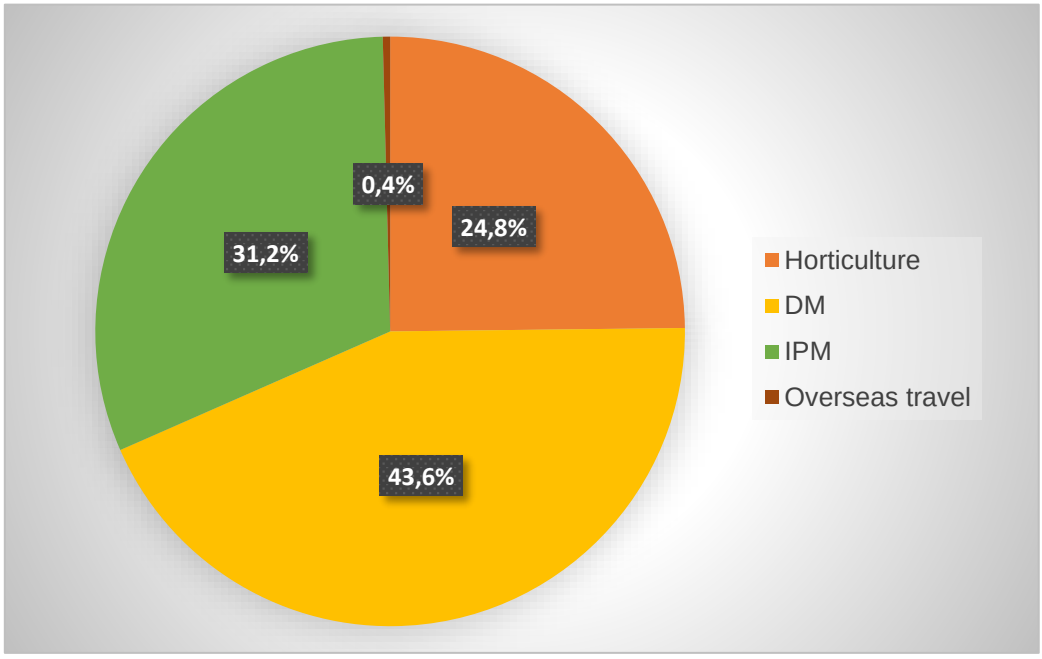

Fig. 8.5.1. Percentage funding in each CRI Portfolio and overseas travel for 2015-16.

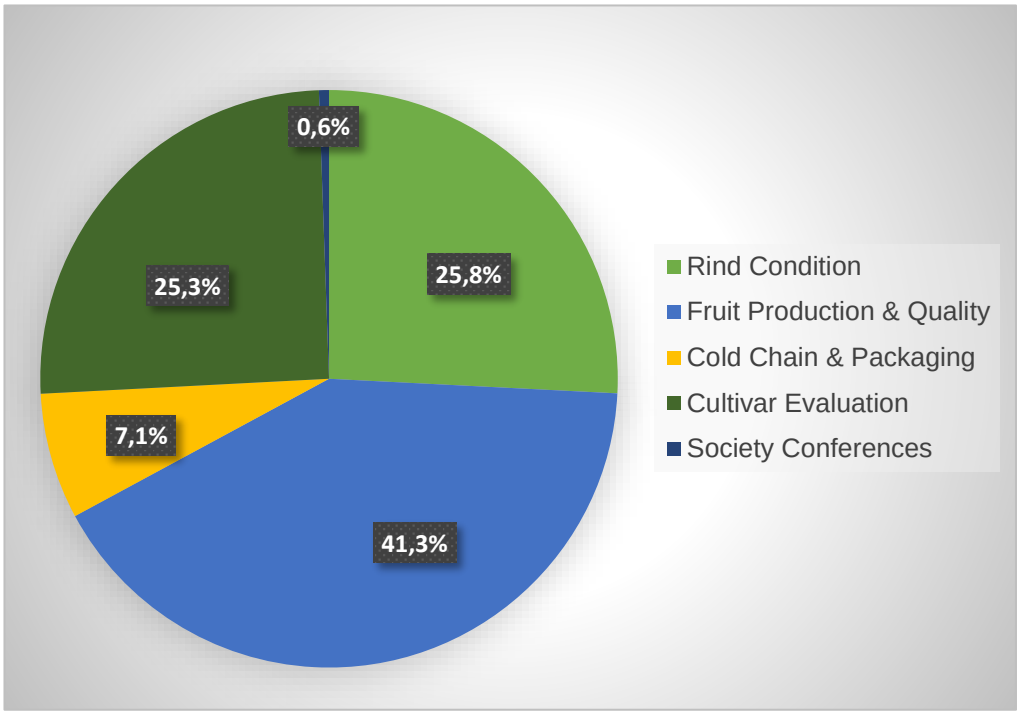

Fig. 8.5.2. Percentage funding to programmes in the CRI Research Portfolio: Horticulture for 2015-16.

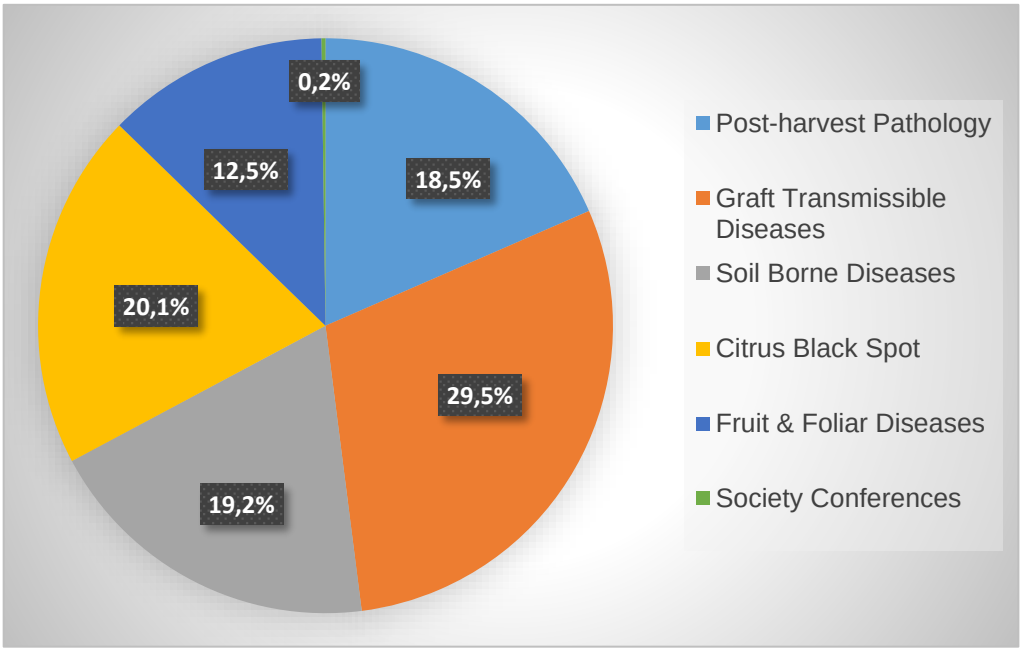

Fig. 8.5.3. Percentage funding to programmes in the CRI Research Portfolio: Disease Management for 2015-16.

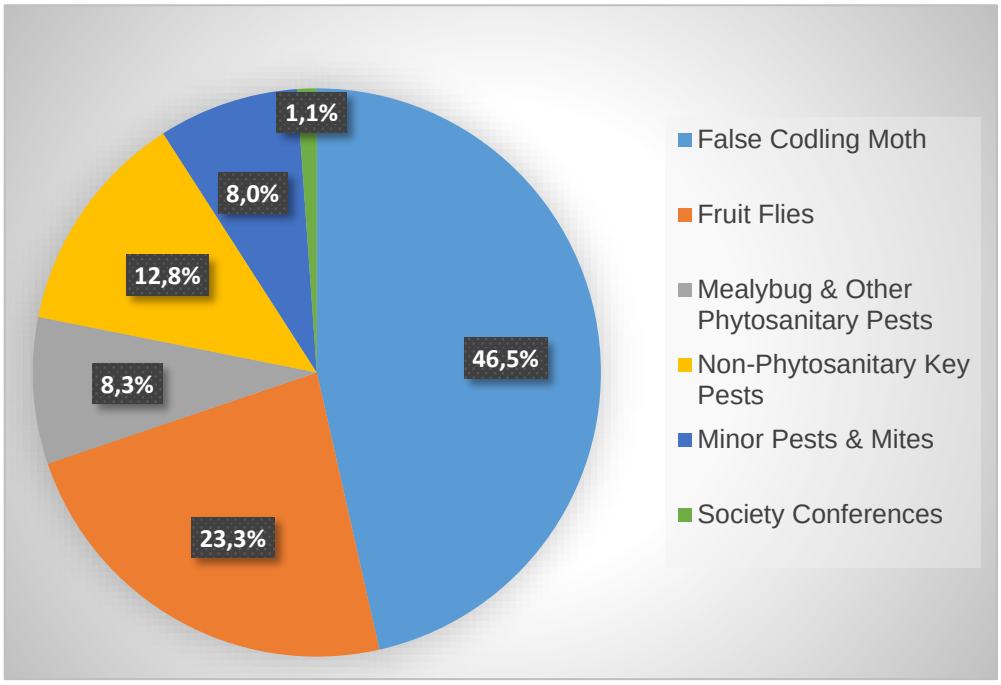

Fig. 8.5.4. Percentage funding to programmes in the CRI Research Portfolio: Integrated Pest Management for 2015-16.

8.6 OTHER MEANS OF TECHNOLOGY TRANSFER

8.6.1 **SA Fruit Journal** by Tim G Grout (CRI)

The SA Fruit Journal is received by every PUC holder and provides the best opportunity for detailed knowledge transfer because it allows for colour photographs and illustrations. During the report period there was a wide range of articles on horticulture, postharvest plant pathology and entomology, as well as some shorter news articles (Table 8.6.1.1). Low resolution downloads of the latest version can be obtained from http://www.safj.co.za/.

Table 8.6.1.1. S A Fruit Journal articles in 2015-16 besides Extension Briefs.

Issue	Pages	Title	Author/s
April/May 14(2)	21-25	Clementine selections evaluated in the cool inland area of Burgersfort	Joubert, J. and G. Barry
	26-28	The Israeli citrus industry: A South African perspective	Stander, O.P.J.
	31-34	Optimisation of fungicide application in citrus packhouses, a journey to precision Part 1	Erasmus, A., C. Lennox, C. Savage, & P. Fourie
	41-45	Progress in research on the control of peteca spot of lemon fruit: could ethylene metabolism influence susceptibility?	Cronje, P.J.R.
Jun/Jul 14(3)	78-79	Citrus Technical Field Day held in Western Cape	Bruwer, I.
	80	SAPPI continues to support the citrus industry	Groenewald, D.
	82-85, 87	The reproductive phenology of Citrus I: Introduction to the physiology of citrus flowering	Stander, O.P.J.
	88-91	Water research in citrus	Vahrmeijer, J.T., T.G. Grout, and N.J. Taylor
	93-94	The Last Word: Hennie le Roux	Le Roux, H.
Aug/Sep 14(4)	57-58, 60	Optimisation of fungicide application in citrus packhouses, a journey to precision Part 2	Erasmus, A., C. Lennox, C. Savage and P. Fourie
	61	A new wave of qualified agricultural entomologists.	Anonymous
	61	NMMU contributes to citrus entomology	Anonymous

	65-69	The reproductive phenology of Citrus II: Citrus floral ontogeny	Stander, O.P.J.
Oct/Nov 14(5)	70	CRI's Cutting Edge Milestone	Grout, T.G.
	73-74	Optimisation of fungicide application in citrus packhouses, a journey to precision Part 3: Imazalil residue loading in dip applications.	Erasmus, A., C. Lennox, C. Savage and P. Fourie
	77-83	The reproductive phenology of Citrus III: Morphogenesis from Flower to Fruit	Stander, O.P.J.
	83	Die Ballie Wahl-meriete toekenning vir die beste Hortologie student in Sitrusproduksie	Stander, O.P.J.
Dec/Jan 14(6)	31-35	Changing the direction of airflow inside refrigerated shipping containers improves fruit pulp temperatures and storage quality	Dodd, M.
	45	Huldeblyk: Johann-Chris Grobler	Anonymous
	47-48	Plant citrus, but what to plant…?	Fourie, P., M. le Roux, T. du Toit, H. Bester and H. Nel
Feb/Mar 15(1)	26-28	Phytotoxicity of GF-120 NF fruit fly bait on 'Nadorcott' mandarin fruit at the green and colour-break stages	Manrakhan, A., P.R. Stephen and P.J. R. Cronje
	30-34, 36	Some Oranges are Sweeter than Others…	Love, C.N., M.P. Hill and S.D. Moore
	86	The Last Word: Dr Jock Dankwerts	Brodie, L.

8.6.2 **CRI website** by Tim G Grout (CRI)

Bandwidth usage decreased significantly from 20.11 GB to 10.72GB during the report period which may be due to less new publicly-available material being uploaded. The number of unique visitors to the site also decreased from 39 338 in 2014/5 to 33 023 in 2015/6 (Table 8.6.2.1). Most bandwidth was used by unknown domains followed by domains from South Africa, then dot-net domains and dot-com. Of countries that could be identified, the most bandwidth was used in decreasing order by Turkey, Ukraine, Russia, Europe and China. Previously Brazil was accessing our website more than Turkey.

Table 8.6.2.1. Visits and page requests on www.cri.co.za since April 2015.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2014/5	39338	71519	383960	733795	20.11 GB
Apr 2015	3451	6758	19292	46730	727.31 MB
May 2015	3382	6606	22170	51686	826.06 MB
Jun 2015	2792	4540	19841	50553	912.64 MB
Jul 2015	2830	5027	18265	54355	993.39 MB
Aug 2015	2590	5002	18413	53842	1003.28 MB
Sep 2015	2465	4026	23415	53195	1.14 GB
Oct 2015	3179	6173	19139	53230	949.60 MB
Nov 2015	2577	5727	14289	44119	745.39 MB
Dec 2015	1926	3047	8622	28443	563.86 MB
Jan 2016	2340	3443	12949	45259	959.78 MB
Feb 2016	2613	3955	12723	47622	1.03 GB
Mar 2016	2878	4468	13437	43833	864.84 MB
Total 2015/6	33 023	58 772	202 555	572 867	10.72 GB

8.6.3 **CRInet** by Tim G Grout (CRI)

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 2015 calendar year are fewer than the average of 51 per annum for the last 9 years but this deviation is probably no cause for concern (Table 8.6.3.1). There are currently 468 CRInet members.

Table 8.6.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2016	6	3	0										
2015	5	2	3	3	2	3	12	4	4	1	3	1	43
2014	4	3	4	1	12	6	13	1	0	1	3	1	49
2013	1	15	0	7	3	0	2	4	6	13	1	6	58
2012	5	1	19	4	5	2	4	3	1	0	2	0	46
2011	14	3	5	2	8	24	2	3	3	2	2	2	70
2010	0	1	5	3	2	0	6	12	9	4	9	3	54
2009	1	7	3	6	11	0	6	8	4	2	1	2	51
2008	3	6	1	8	5	2	7	3	3	5	3	4	50
2007	5	2	7	1	1	2	4	2	5	4	3	3	39

8.6.4. **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 2015/6 are given in Table 8.6.4.1.

Table 8.6.4.1. Cutting Edge issues during 2015-16.

No.	Title	Issue	Author
191	National Road Traffic Act	Jan	M. Brooke
192	FCM Monitoring 2015	Jan	S.D. Moore & V. Hattingh
192	FCM Monitoring – of critical importance: Addendum	Feb	S.D. Moore & V. Hattingh
193	Name change of the invasive fruit fly and update on its pest status in SA	Feb	*Bactrocera invadens* Steering Committee
194	Update on the Guazatine EU MRL	Mar	P. Hardman
195	Food Safety Update	Mar	P. Hardman
196	Sampling procedure for *Phytophthora* and citrus nematode analysis and the latest price list for services rendered by the Diagnostic Centre	Apr	J. van Niekerk & E. Basson
197	Diligent postharvest disease management required at the beginning of the citrus harvest season	Apr	A. Erasmus, K. Lesar, MC Pretorius & P. Fourie
198	Food Safety Update	May	P. Hardman
199	Sour rot management	Jun	A. Erasmus, K. Lesar & P. Fourie
200	Clarification on the declaration of post-harvest treatments of citrus fruit for the UK	Jun	P. Hardman
201	CRI-CIS notice regarding Carnika navel and Midknight F17 Valencia	Jul	P. Fourie
202	Export Citrus Post-harvest Treatment Labelling Considerations	Aug	P. Hardman
203	Avoiding mealybug repercussions	Sep	S.D. Moore & T.G. Grout
204	The control of sour rot requires the diligent implementation and management of orchard practices to assist in reducing the incidence of this postharvest disease	Sep	A. Erasmus, K. Lesar, P. Hardman & P. Fourie
205	Amendment of SOLAS Chapter VI, Part A, Regulation 2: Shippers Mandatory Verification of the Gross Mass of a Packed Container	Nov	M. Brooke

206	Consumer Assurance Update November 2015	Nov	P. Hardman
207	2016 Export Citrus Post-harvest Treatment Labelling Considerations	Jan	P. Hardman
208	Management of citrus orchards during times of drought	Jan	MC Pretorius, T.Vahrmeijer, J. van Niekerk & H. le Roux
209	Further Update on Citrus Waxes and Declarations	Jan	P. Hardman
210	Southern African Citrus Improvement Scheme: Certified Nurseries (Feb 2016)	Feb	CIS
211	SOP for Dithiocarbamate Testing on citrus fruit for Canada - Suspended	Feb	P. Hardman
212	Japan Carton Declarations in 2016	Apr	P. Hardman
213	CRI-Diagnostic Centre – Services and Price List	Apr	J. van Niekerk & E. Basson
214	Fruit Export Industry Guidelines on Implementation of IMO, SOLAS Chapter VI, Part A, Regulation 2: Shippers Mandatory Verification of the Gross Mass of a Packed Container (VGM Guideline Part 1 of 3: 21st April 2016)	Apr	M. Brooke
215	Consumer Assurance and MRL Update	May	P. Hardman
216	Fruit Export Industry Guidelines on Implementation of IMO, SOLAS Chapter VI, Part A, Regulation 2: Shippers Mandatory Verification of the Gross Mass of a Packed Container (VGM Guideline Part 2 of 3: 27th May 2016 ; Revision 1: 14th June 2016 – inclusion of section 4 and update to section 3, 8 & 10)	Jun	M. Brooke
217	Control recommendations in areas with high fruit fly population pressure	Jun	A. Manrakhan, M. Gilbert & S.D. Moore
218	Warning of possible phosphonate phytotoxicity on late mandarin fruit	Jul	J. van Niekerk, P. Cronje, C. Kotze & MC Pretorius
219	Fosetyl-Al, Phosphorous acid and Phosphonates MRLs	Jul	P. Hardman
220	Postharvest recommendations for 2016 – Guidelines according to the factsheets issued by CRI	Jul	W. du Plooy, H. Bester & K. Lesar

9 PUBLICATIONS IN 2015-16

9.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

Chrysantus M.T., A. Manrakhan, J.H. Daneel, S.A. Mohamed, F. Khamis, S. Ekesi. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis rosa* Karsch (Diptera Tephritidae) populations from Kenya and South Africa. ZooKeys 540: 467-487.

Defraeye, T., P. Cronje, T. Berry, U.L. Opara, A. East, M. Hertog, P. Verboven and B. Nicolai. 2015.Towards integrated performance evaluation of future packaging for fresh produce in the cold chain. Trends in Food Science & Technology 44: 201-225.

Defraeye, T., P. Cronje, P. Verboven, U.L. Opara and B. Nicolai. 2015. Exploring ambient loading of citrus fruit into reefer containers for cooling during marine transport using computational fluid dynamics. Postharvest Biology & Technology 108: 91-101.

Defraeye, T., P. Verboven, U.L. Opara, B. Nicolai, P. Cronje. 2015. Feasibility of ambient loading of citrus fruit into refrigerated containers for cooling during marine transport. Biosystems Engineering 134: 20-30.

Defraeye, T., B. Nicolai, W. Kirkman, S. Moore, S. van Niekerk, P. Verbovena, P. Cronjé. 2016. Integral performance evaluation of the fresh-produce cold chain: A case study for ambient loading of citrus in refrigerated containers. Postharvest Biology and Technology 112: 1-13.

De Meyer, M., H. Delatte, S. Ekesi, K. Jordaens, B. Kalinová, A. Manrakhan, M. Mwatawala, G. Steck, J. Van Cann, L. Vaníčková, R. Břízová, M. Virgilio. 2015. An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. ZooKeys 540: 405-427.

De Villiers, M., V. Hattingh, D.J. Kriticos, S. Brunel, J.-F. Vayssières, A. Sinzogan, M.K. Billah, S.A. Mohamed, M. Mwatawala, H. Abdelgader, F.E.E. Salah, M. De Meyer. 2016. The potential distribution of *Bactrocera dorsalis*: considering phenology and irrigation patterns. Bull. Entomol. Res. 106: 19-33.

Erasmus, A., C.L. Lennox, L. Korsten, K. Lesar, P.H. Fourie. 2015. Imazalil resistance in *Penicillium digitatum* and *P. italicum* causing citrus postharvest green and blue mould: Impact and options. Postharvest Biology and Technology 107: 66-76.

Hofmeyr, J.H., V. Hattingh, M. Hofmeyr and J.P. Slabbert. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: validation of an ionising radiation and cold combination treatment. Afr. Entomol. 24(1): 217-224.

Hofmeyr, J.H., M. Hofmeyr, V. Hattingh and J.P. Slabbert. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: determination of ionising radiation and cold treatment conditions for inclusion in a combination treatment. Afr. Entomol. 24(1): 208-216.

Lado, J., P. Cronje, B. Alquézar, A. Page, M. Manzi, A. Gómez-Cadenas, A. D. Stead, L. Zacarías and M. J. Rodrigo. 2015. Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. Physiologia Plantarum 154: 469–484.

Magarey, R.D., S.C. Hong, P.H. Fourie, D.N. Christie, A.K. Miles, G.C. Schutte, T.R. Gottwald. 2015. Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia. Crop Protection 75: 104-114.

Manrakhan, A., Stephen, P.R.and Cronje, P.J.R. 2015. Phytotoxic effect of GF-120 NF fruit fly bait on fruit of mandarin (*Citrus reticulata* Blanco cv. Nadorcott): Influence of bait characteristics and fruit maturity stage. Crop Protection 78: 48-53.

Manrakhan, A., J.H. Venter, V. Hattingh. 2015. The progressive invasion of *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. Biol. Invasions 17: 2803-2809.

Moore, S., W. Kirkman and V. Hattingh. 2015. The host status of lemons for the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) with particular reference to export protocols. African Entomology 23(2): 519-525.

Moore, S.D., W. Kirkman, G.I. Richards and P.R. Stephen. 2015. The Cryptophlebia leucotreta granulovirus—10 Years of Commercial field use. Viruses 7: 1284-1312.

Mupambi, G., J.S. Verreynne, O.P.J. Stander and P.J.R. Cronjé. 2015. Optimal timing of application of 2,4-D on 'Navel' sweet orange [*Citrus sinensis* (Osbeck)] reduces the size of the navel-end. Journal of Horticultural Science & Biotechnology 90 (6): 619–625.

Nepgen, E. S., M.P. Hill, S.D. Moore. 2015. The effect of long-distance transportation on the fitness of irradiated false codling moth (Lepidoptera: Tortricidae) for use in a sterile insect release program. Journal of Economic Entomology 108(6): 2610-2619.

Ridgeway J.A. and A.E. Timm. 2015. Reference gene selection for quantitative real-time PCR normalization in larvae of three species of Grapholitini (Lepidoptera: Tortricidae). PLoS ONE 10(6): e0129026. doi:10.1371/journal.pone.0129026

Stander, O.P.J. and P.J.R. Cronje. 2016. Reviewing the commercial potential of hand thinning in citrus with a cost-benefit analysis of summer hand thinning of 'Nadorcott' mandarin. Hort Technology 26(2): 206-212.

Taylor, N.J. W. Mahohoma, J.T. Vahrmeijer, M.B. Gush, R.G. Allen, J.G. Annandale. 2015. Crop coefficient approaches based on fixed estimates of leaf resistance are not appropriate for estimating water use of citrus. Irrig Sci 33: 153-166.

Thackeray, S.R., S.D. Moore, M. Parkinson, M.P. Hill. 2015. Citrus thrips, *Scirtothrips aurantii* (Thysanoptera: Thripidae), damage and infestation in the presence of molasses. Crop Protection 78: 72-77.

van Dael, M., S. Lebotsa, E. Herremans, P. Verboven, J. Sijbers, U.L. Opara, P.J. Cronje, B.M. Nicolaï. 2016. A segmentation and classification algorithm for online detection of internal disorders in citrus using X-ray radiographs. Postharvest Biology and Technology 112: 205-214.

Zimba, K., M.P. Hill, S.D. Moore & U. Heshula. 2015. *Agathis bishopi* (Hymenoptera: Braconidae) as a potential tool for detecting oranges infested with *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). J. Insect Behav. 28: 618-633.

Zimba, K., S.D. Moore, U. Heshula & M.P. Hill. 2016. *Agathis bishopi*, a larval parasitoid of false codling moth *Thaumatotibia leucotreta*: laboratory rearing and effect of adult food on parasitism and longevity. Afr. Entomol. 24(1): 153-161.

9.2 SEMI-SCIENTIFIC PUBLICATIONS (Other than SA Fruit Journal)

Grout, T.G., Moore, S.D., 2015., Citrus, in: Prinsloo, G.L., Uys, G.M. (Eds.), Insects of cultivated plants and natural pastures in Southern Africa. Entomological Society of Southern Africa, Pretoria, South Africa, pp. 447-501.

10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Banda M., N.J. Taylor, J.T. Vahrmeijer. Validating sap flux density measurement methods in potted *citrus sinensis*. Combined Congress: SASHS, 18 – 21 January 2016, University of the Free State, Bloemfontein.

Coombes, Candice A, Martin P Hill, Sean D Moore, Jo F Dames. Entomopathogenic fungi as biological control agents of false codling moth. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Daneel, John-Henry, Aruna Manrakhan, Marc De Meyer. The efficacy of different attractants for monitoring Afrotropical fruit fly pests. Speed presentation. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Daniel, Claire A, Martin P Hill, Sean D Moore. Improving the cold tolerance of false codling moth through diet manipulation for improved performance in a sterile insect release programme. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Ehlers J, F Alférez, KI Theron and PJR Cronje. Non-chilling post-harvest pitting of Valencia orange. Combined Congress: SASHS, 18 – 21 January 2016, University of the Free State, Bloemfontein.

Gilbert, M. Trapping of fruit flies and false codling moth on multi-crop farms in the Western Cape. Speed presentation. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Goddard, Mathew, Martin Hill and Sean Moore. Developing an attractant for monitoring fruit-feeding moths in citrus orchards. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Grout, Tim G, Kim C Stoltz. Killing hitchhikers without affecting IPM. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Joubert, Francois, Martin Hill and Sean Moore. An audit of the efficacy of the sterile insect technique programme for false codling moth in the Sundays River Valley. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Kirkman, Wayne, Sean D Moore, Aruna Manrakhan, Martin Gilbert, John-Henry Daneel, Jeanne de Waal and Ralf-U Ehlers. Conservation and augmentation of entomopathogenic nematodes on citrus for control of false codling moth. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Love, Claire N, Martin P Hill and Sean D Moore. Know your enemy: investigating the pupation of false codling moth. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Makumbe, Louisa D M, Christopher W Weldon, Aruna Manrakhan. Optimisation of fluorescent pigment marking for the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), under African climatic conditions. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Manrakhan, Aruna, John-Henry Daneel and Pia Addison. Area-wide management of fruit fly pests in South Africa – the underlying basis and possible control tools. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Moore, Sean D. The future of microbial pesticides: fantasy or reality? XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Mwanza, Patrick, Mike Lee, Sean Moore, Gill Dealtry. Reapplication frequency of *Cryptophlebia leucotreta* granulovirus to protect against FCM infestation of citrus. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Nepgen, E S, M P Hill, S D Moore. Analysis of critical factors for the successful application of the Sterile Insect Technique against false codling moth. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

North JJ, PJR Cronje. Evaluation of a portable device for the efficient measurement of acidity in citrus. Combined Congress: SASHS, 18 – 21 January 2016, University of the Free State, Bloemfontein.

Peyper, Mellissa, Martin P Hill, Sean D Moore. *Anagyrus sp. nr. pseudococci* as a control agent of the key citrus pests, *Planococcus citri* and *Paracoccus burnerae.* XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Stander, O.P.J., Barry, G.H., Cronje, P.J.R. Influence of sink-source alterations on citrus (*Citrus* spp.) flowering. Combined Congress: SASHS, 18 – 21 January 2016, University of the Free State, Bloemfontein.

Taylor, N.J., Vahrmeijer J.T., van der Merwe S., Ibraimo N.A., Annandale, J.G. Modelling transpiration of citrus orchards. Combined Congress: SASHS, 18 – 21 January 2016, University of the Free State, Bloemfontein.

Thackeray, Sean, Martin Hill, Sean Moore, Matthew Parkinson. Citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) damage and infestation in the presence of molasses. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

Theron, Charmaine D, Aruna Manrakhan, Christopher W Weldon. Host utilisation by the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), in South Africa. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

van Niekerk, Sonnica, Martin P Hill, Sean D Moore. False codling moth population ecology in citrus orchards: the influence of orchard age. Speed presentation. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.

von Diest, Janina, Sean D Moore, Martin P Hill, Pia Addison and Antoinette P Malan. Interaction between entomopathogenic nematodes and entomopathogenic fungi for control of false codling moth. XIX Congress of the Entomological Society of Southern Africa, 12-15 July 2015, Rhodes University, Grahamstown.