citrus research international

CRI GROEP
ANNUAL RESEARCH REPORT 2013/2014

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

1 INTRODUCTION

Tim G Grout (Manager: Research & Technical)

In order to fulfil our mission statement, CRI endeavours to maximise grower competitiveness by developing, supporting, coordinating and providing Research and Technical services to the citrus industry. Although researchers may prefer to focus on their research alone, their expertise is required to support DAFF with market access issues, the maintenance of the Citrus Improvement Scheme and knowledge transfer to both exporting and emerging citrus growers. CRI continues to receive most of its funding from the levy administered by the Citrus Growers Association on fresh citrus exports, but opportunities for alternative funding are pursued if the requirements do not conflict with identified research priorities. CRI researchers seconded to universities within the CRI Group Alliance regularly benefit from THRIP funding, as do some university researchers that are funded by the citrus industry.

Once again, citrus black spot (CBS) was the dominant market access issue that consumed vast amounts of time normally assigned to other important tasks. However, despite the extra demands, most needs were met without compromise. The prospects of broadening future access to the USA market from production areas in SA where CBS occurs are promising. With the fruit fly *Bactrocera invadens* becoming more widespread in the northern areas, the demand for researcher support actually declined slightly, but this was more than taken up by planning for a systems approach to control false codling moth in EU citrus shipments in the near future. Nine years of engagement culminated in an agreement with the USA to revert to a 22-day cold treatment for citrus exports to USA in 2014 and further engagements took place between SA and Japan, China, S Korea, Thailand, Vietnam, India and the Philippines to gain, retain or optimise access for SA citrus to these markets.

Within the Integrated Pest Management (IPM) research portfolio, four times as much funding was spent on false codling moth (FCM) research as on any other programme, because it is likely to be the next major market access challenge after CBS. The research scope involved biorational and biological preharvest approaches, as well as postharvest detection and control methods. The entomologist vacancy in the Western Cape province was filled by Dr Martin Gilbert who is also working on FCM. Research requested by the Japanese authorities on the susceptibility of *Ceratitis capitata* larvae to temperatures around 1°C in different types of citrus was completed and other research to improve the level of control of all fruit flies was conducted.

Within the Disease Management research portfolio, CRI's CBS researchers were involved in South African and international CBS expert panels formulating responses to EFSA's CBS Pest Risk Assessment in addition to a collaborative project with USA, Brazilian and Argentinian researchers. The epidemiology of CBS and its global movement are also being investigated in addition to new fungicides. Dr Jan van Niekerk took over from MC Pretorius as programme coordinator for soilborne diseases so that MC could strengthen our extension services. Apart from research on Citrus Tristeza Virus and Greening disease, a project in the graft transmissible diseases programme was started to compare the horticultural performance of old-clone material with clean Citrus Improvement Scheme (CIS) material. Research on the control of postharvest diseases is critical and residue loading of various fungicides through different application techniques, including use of the JBT flooder, is under investigation.

The Horticultural research portfolio has been suffering from a lack of capacity but we were able to fill our preharvest horticulturist vacancy with Jakkie Stander who has practical experience in the citrus industry. Progress made during this last report period includes confirmation that thiabendazole does reduce chilling injury and that lycopene in the rind of red grapefruit also reduces chilling injury. The prevention of water loss between harvest and waxing reduces the likelihood of pitting in Valencias and mandarins. It is now also clear that humates and fulvates, when applied with N, P or K, can increase microbial activity in some soils and reduce leaching of N. Possible effects of climate change are receiving attention, one being sunburn that is being seen quite frequently in the evaluation of new mandarin hybrids in hot areas. Even the choice of rootstock can have an effect on susceptibility to sunburn by changing the shape of the tree canopy.

The CIS continues to be operated by CRI with advice from the CIS Advisory Committee. The number of buds supplied by the Citrus Foundation Block continues to increase and during 2013/4 demand for Nadorcott 1 mandarin exceeded that for Eureka lemon with mandarin hybrids in general comprising 42% of the citrus budwood requested. There was still a need for authorised cutting of budwood in nurseries to meet demand but this decreased by 9.7% to 30.9%.

Although there was no CRI Citrus Research Symposium in 2013, attendance at the five regional CRI Postharvest Workshops continued to grow and far exceeded that normally seen at the biennial Research Symposium. Attendance at the regional production workshops in autumn and spring has improved but this is

still a relatively new idea and the timing of the autumn workshop may not be ideal. Technology transfer to emerging growers and provincial government extension officers is starting to reap rewards, particularly in Limpopo where growers must manage citrus in the presence of *B. invadens.* Other means of technology transfer continue to be utilised by CRI researchers, such as the SA Fruit Journal, Cutting Edge and CRInet, and where possible researchers endeavour to publish their results in refereed scientific journals so that they can be cited in possible future market access challenges.

INLEIDING

Tim G Grout (Bestuurder: Navorsing & Tegnies)

Om ons missie na te streef, poog CRI daarna om produsente se mededingendheid te verbeter deur die ontwikkeling, ondersteuning, koördinering en die verskaffing van navorsing en tegniese dienste aan die sitrusbedryf. Alhoewel navorsers verkies om slegs op hul navorsing te fokus, word hul kundigheid vereis om DAFF te ondersteun met marktoegangskwessies, instandhouding van die Sitrusverbeteringskema en die oordrag van kennis aan beide die produsente wat uitvoer en die opkomende sitrusprodusente. CRI ontvang steeds die meeste van sy befondsing van die heffing wat deur die Citrus Growers Association geadministreer word op vars sitrus wat uitgevoer word. Geleenthede vir alternatiewe befondsing word egter ook ondersoek as die vereistes nie teenstrydig is met geïdentifiseerde navorsingsprioriteite nie. CRI navorsers wat aan universiteite binne die CRI Groep Alliansie gesekondeer is, trek gereeld voordeel uit THRIP-befondsing, asook sommige Universiteitsnavorsers wat deur die sitrusbedryf befonds word.

Sitrus swartvlek (SSV) was weer die belangrikste marktoegangskwessie wat baie van die tyd in beslag geneem het wat gewoonlik aan ander belangrike take spandeer word. Ten spyte van die addisionele eise, is egter aan die meeste behoeftes voldoen sonder kompromie. Die vooruitsigte om toekomstige toegang na die VSA-mark vanuit produksie gebiede in SA waar SSV voorkom uit te brei, lyk belowend. Met die vrugtevlieg *Bactrocera invadens* wat al meer wydverspreid in die noordelike gebiede voorkom, het die vraag na navorsersondersteuning eintlik effens afgeneem, maar dit is opgeneem deur die beplanning vir ŉ sisteembenadering vir die beheer van valskodlingmot in sitrusverskepings na die EU in die nabye toekoms. Nege jaar van onderhandelinge het gelei tot ŉ ooreenkoms met die VSA om terug te keer na ŉ 22-dae-koue behandeling vir sitrusuitvoere na die VSA in 2014. Verdere onderhandelinge het ook tussen SA en Japan, China, S-Korea, Thailand, Viëtnam, Indië en die Filippyne plaasgevind om toegang vir Suid-Afrikaanse sitrus na hierdie markte te verkry, te behou of te verbeter.

Binne die Geïntegreerde Plaagbeheer (IPM) navorsingsportefeulje is vier maal meer van die befondsing op valskodlingmot (VKM) navorsing spandeer as op enige ander program, omdat dit waarskynlik die volgende groot uitdaging vir marktoegang na SSV gaan wees. Die omvang van die navorsing het bio-rasionale en biologiese vooroes benaderings ingesluit, sowel as na-oes opsporing en beheermetodes. Die vakante entomologie pos in die Wes-Kaap is deur dr Martin Gilbert gevul wat ook op VKM werk. Navorsing wat deur die Japanese owerhede versoek is op die vatbaarheid van *Ceratitis capitata* larwes vir temperature rondom 1°C in verskillende sitrustipes is afgehandel en ander navorsing om die vlak van beheer van alle vrugtevlieë te verbeter, is uitgevoer.

Binne die Siektebestuur navorsingsportefeulje was CRI se SSV- navorsers betrokke by die Suid-Afrikaanse en internasionale SSV deskundige panele wat antwoorde op EFSA se SSV Pes Risiko Analise geformuleer het. Hul was ook betrokke by n gesamentlike projek met die VSA, Brasiliaanse en Argentynse navorsers. Die epidemiologie van SSV en sy wêreldwye verspreiding is ook bykomend tot nuwe swamdoders ondersoek. Dr Jan van Niekerkhet by MC Pretorius as programkoördineerder vir grondgedraagde siektes oorgeneem sodat MC ons voorligtingsdienste kan versterk. Afgesien van navorsing op sitrus Tristeza Virus en Vergroeningsiekte, is ŉ projek in die ent-oordraagbare siekteprogram begin om die hortologiese prestasie van die ou-kloon materiaal met skoon materiaal van die Sitrusverbeteringskema (SVS) te vergelyk. Navorsing op die beheer van na-oes siektes is van kritieke belang en residu-ladings van verskillende swamdoders deur verskillende toedieningstegnieke, insluitend die gebruik van die JBT Flooder, word tans ondersoek.

Die Hortologie navorsingsportefeulje het gebuk gegaan onder ŉ gebrek aan kapasiteit, maar ons was in staat om ons vooroes tuinboukundige vakature met Jakkie Stander, wat praktiese ervaring in die sitrusbedryf het, te vul. Vordering wat tydens die laaste verslagperiode gemaak is, sluit bevestiging in dat thiabendazole koueskade verminder en dat likopeen in die skil van rooi pomelo's ook koueskade kan verminder. Die voorkoming van waterverlies tussen oes en waksaanwending verminder die waarskynlikheid van gepokte skil in Valencias en mandaryne. Dit is nou ook duidelik dat "humates" en "fulvates", wanneer dit saam met N, P of K toegedien word, mikrobiese aktiwiteit in sommige gronde kan verhoog en die loging van N kan verminder. Die moontlike gevolge van klimaatsverandering geniet ook aandag, een daarvan is sonbrand wat heel dikwels in die evaluering van nuwe Mandarynkruisings in warmer gebiede waargeneem word. Selfs die

keuse van ŉ onderstok kan ŉ uitwerking hê op die vatbaarheid vir sonbrand deur die vorm van die boomdak te verander.

Die SVS word steeds deur CRI bedryf met advies van die SVS Advieskomitee. Die aantal ogies wat deur die Sitrus Grondvesblok voorsien word het steeds in 2013/4 vermeerder en die aanvraag vir Nadorcott 1 Mandaryn het die van Eureka suurlemoene oorskrei. Die Mandaryn kruisings het 42% uitgemaak van die sitrus entmateriaal wat aangevra is. Alhoewel daar steeds ŉ behoefte vir gemagtigde sny van enthout in kwekerye was om in die aanvraag te voldoen, het dit met 9,7% tot 30,9% afgeneem.

Alhoewel daar nie ŉ CRI Sitrusnavorsingsimposium in 2013 was nie, het die bywoning van die vyf plaaslike CRI Na-oes werkswinkels voortgegaan om te groei en het by verre die normale bywoniing van die tweejaarlikse Navorsingsimposium oortref. Bywoning van die plaaslike produksie werkswinkels in die herfs en lente het verbeter, maar dit is nog steeds ŉ relatiewe nuwe idee en die tydsberekening van die herfs werkswinkel mag dalk nie ideaal wees. Tegnologie-oordrag aan die opkomende boere en voorligters van die provinsiale regering begin om vrugte af te werp, veral in Limpopo waar produsente sitrus in die teenwoordigheid van *B. invadens* moet bestuur. Ander maniere van tegnologie-oordrag word steeds deur CRI navorsers benut, soos die SA Vrugte Joernaal, Snykant en CRInet, en waar moontlik poog navorsers om hul resultate in gekeurde wetenskaplike joernale te publiseer, sodat dit in die toekoms in moontlike marktoegangsuitdagings aangehaal kan word.

2 MARKET ACCESS TECHNICAL COORDINATION

Coordinator: Vaughan Hattingh and Elma Carstens (CRI)

2.1 Summary

The crisis with the EU CBS regulations dominated this report period. The pursuit of amendments to the EU regulations through the IPPC dispute settlement process was placed on hold when the EU indicated that it would revise its assessment of the risk posed by CBS. Despite extensive and detailed input by an international panel of CBS experts, the EU failed to align its risk assessment and regulations with the advice provided by this expert panel. It is apparent that the EU has chosen a path that is contrary to the reigning international scientific view on CBS and the intervention of international trade facilitation platforms, such as the International Plant Protection Convention and the SPS Committee of the World Trade Organisation remain options that offer prospects for resolution. Nine years of engagement culminated in an agreement with the USA to revert to a 22-day cold treatment for citrus exports to USA in 2014. The prospects of broadening future access to the USA market from production areas in SA where CBS occurs are promising. There were ongoing engagements between SA and Japan, China, S Korea, Thailand, Vietnam, India and the Philippines to gain, retain or optimise access for SA citrus to these markets. Continued incursions of the fruit fly *Bactrocera invadens* were detected and the status of SA was changed to one that recognises presence of the pest in certain areas, whereas others remain pest free with *B. invadens* under official control. Considerable attention was given to biosecurity concerns and amendments to regulations to better protect SA's biosecurity are expected in the near future.

Opsomming

Die krisis met die EU se regulasies vir CBS het hierdie verslagperiode oorheers. Die aksies om die EU se regulasies deur die "IPPC dispute settlement process" te wysig is op ys geplaas met die aanduiding van die EU dat die evaluering van die risiko wat CBS inhou, hersien gaan word. Ten spyte van uitgebreide en gedetailleerde insette deur ŉ internasionale paneel van CBS deskundiges, het die EU misluk om sy risikobepaling en regulasies in ooreenstemming met die advies wat deur hierdie paneel voorsien is, aan te pas. Dit is duidelik dat die EU ŉ pad gekies het wat teenstrydig is met die huidige internasionale siening oor CBS, en die ingryping van forums vir internasionale handelsfasilitering soos die Internasionale Plantbeskermingskonvensie en die SPS Komitee van die Wêreldhandelsorganisasie bly opsies wat vooruitsigte vir ŉ oplossing kan bied. Nege jaar van onderhandelinge het tot ŉ ooreenkoms met die VSA gelei, om na ŉ 22-dae koue behandeling vir sitrusuitvoere na die VSA in 2014 terug te keer. Die vooruitsigte om die toekomstige toegang na die VSA vanaf produksie-areas in SA waar CBS voorkom uit te brei, lyk belowend. Daar was voortdurende skakeling tussen SA en Japan, China, S-Korea, Thailand, Viëtnam, Indië en die Filippyne om toegang vir SA na die markte te verkry, te behou of te verbeter. Nuwe introduksies van die vrugtevlieg, *Bactrocera invadens* is voortdurend gevind en die status van SA is verander na een wat erken dat die pes in sekere areas teenwoordig is, terwyl ander steeds vry is van *B. invadens,* maar onder amptelike beheer. Baie tyd is aan bekommernisse oor biosekuriteit spandeer, en veranderinge aan regulasies om SA se biosekuriteit beter te beskerm word in die nabye toekoms verwag.

2.2 EUROPE (EU)

The 2013 export season started with the threat that exports may be banned if the threshold of five interceptions for CBS is exceeded in the EU. To put the crisis to which the EU CBS situation developed in 2013 into perspective, a brief synopsis of developments to date is provided here. CBS was first regulated as a quarantine organism in the EU in 1992. South Africa and other countries objected to this as being in conflict with scientific information relevant to the phytosanitary risk of CBS. In 2000, on invitation from the EU, South African scientists conducted a CBS Pest Risk Assessment (PRA) and concluded that fruit is not a pathway for the spread of CBS and the climate in the EU is unsuitable for the establishment of CBS. The EU contested the assessment and there were several further technical exchanges and additional research conducted in the period 2001-2007. In 2008 the EU called upon the European Food Safety Authority (EFSA) to provide their scientific opinion on the CBS situation. EFSA expressed an opinion contrary to that of the 2000 CBS PRA conducted by SA scientists and indicated that EFSA was of the opinion that the EU CBS Regulations were appropriate. South Africa challenged this opinion and in 2010 called upon the International Plant Protection Convention (IPPC) for dispute settlement. The USA conducted an independent CBS PRA in 2010 and also concluded that fruit is not a pathway for the spread of CBS. An IPPC facilitated bilateral engagement was held between representatives from EU and SA in Rome in 2013. SA requested the IPPC to proceed to the step where an international panel of experts is convened to assess the dispute. EU declined to consent, indicating that they were requesting EFSA to conduct a revised CBS PRA. The IPPC process was accordingly placed on hold pending the outcome of the EFSA CBS PRA. In July 2013 EFSA published a draft PRA and called for public comment. An international panel of scientists with CBS expertise compiled a detailed commentary on the draft PRA and provided it to EFSA. The international panel indicated that it disagreed with the draft EFSA PRA. The international scientific CBS panel agreed with earlier PRAs which had concluded that fruit is not a pathway for CBS and the EU is not climatically suitable for establishment of CBS. EFSA published its finalised CBS PRA in February 2014. It was evident that EFSA had largely disregarded the input provided by the international scientific panel and EFSA reached conclusions that were strongly divergent from the conclusions of the international scientific panel and previous PRAs. EFSA maintained its opinion that citrus fruit imports could be a risk to parts of the EU and that the current regulations are necessary. DG SANCO indicated that it would move to strengthen its CBS regulations in 2014.

In December 2013, after 34 interceptions of CBS in the EU, citrus imports into the EU from SA production areas outside of CBS Pest Free Areas were banned. However, the ban only applied to fruit from the 2013 season. Considering that all 2013 citrus exports from SA had long since been concluded and the ban was not applicable to the 2014 season, it did not disrupt the flow of any export fruit.
Extensive engagements were undertaken with role players in South Africa and the EU. Several newsletters were issued through the CGA to ensure that all parties remain informed on an ongoing basis. The CGA increased capacity to deal with various aspects of the CBS strategy. After publication of the EFSA PRA in February 2014, the European Commission's DG SANCO commenced drafting regulations for additional 2014 CBS measures for consideration by the EU's Standing Committee on Plant Health (SCPH).

2.3 JAPAN

There were two ongoing issues pertaining to Japan market access. The first outstanding issue was the broadening of access for soft citrus cultivars to include all other SA mandarins in addition to Clementines. During this reporting period feedback was received from Japan-MAFF about the inclusion of other mandarins. Japan-MAFF indicated that they viewed all other mandarins as each being a different citrus type and according to their requirements would each need Mediterranean fruit fly cold treatment efficacy data. Considering that there are 32 mandarin cultivars in question, this is clearly not a reasonable or feasible requirement. CRI compiled a draft response, indicating why the Japanese requirements were unreasonable, and supplied it to SA-DAFF for compilation of a response to Japan. The second outstanding issue was the adoption of a revised cold treatment condition for the export of all citrus types. The experimental work requested by Japan to clarify aspects of the experimental results previously supplied by SA was completed. A report on these supportive findings will be supplied to SA-DAFF for communication with Japan in the next report period.

2.4 USA

At the end of the previous reporting period a submission was supplied to SA-DAFF, requesting a technical meeting with USDA-APHIS in South Africa to discuss the four long outstanding issues, namely (1) reversion of the FCM cold treatment period from 24 d to 22 d, (2) equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all SA production areas), (3) expansion of CBS pest free areas to include the whole of the W Cape in the work plan and (4) adoption of CBS pest free places of

production (N Limpopo region). Three new requests were included in the submission, namely (1) the division of the Western Cape province into two areas, each with its own running average, (2) SA citrus fruit to be allowed to enter the USA through additional ports in Texas, New Orleans and Miami and (3) the termination of the obligatory cutting of SA citrus fruit upon arrival in the USA.

A bilateral video conference was held between SA-DAFF and USDA-APHIS in November 2013 to further discuss the outstanding issues. It was agreed in principle to revert to the 22 d treatment in 2014 on a trial basis (pilot programme). A revised binomial sampling procedure for FCM was envisaged as an alternative to the former biometric sampling procedure. This entailed a 200 fruit sample per consignment with cutting of all the fruit and rejection on detection of the second FCM larva. The meeting was followed by a confirmatory letter from USDA-APHIS in December 2013.

Meetings were held with SA-DAFF and industry role players in January 2014 to discuss the proposals. It was decided to propose to the USA to run a Pilot Programme for 2014, but with the retention of the current biometric sampling and inspection procedure and rejection threshold of seven FCM larvae per consignment. SA-DAFF submitted this to USDA-APHIS in February 2014. A bilateral meeting was held between SA-DAFF and USDA-APHIS in March 2014 and the following pilot programme for exports in 2014 was agreed upon: A 22-day cold treatment with the current biometric sampling and inspection procedures and the current FCM threshold of 7 larvae per consignment. Additionally, 200 asymptomatic fruit must be cut per consignment to further determine the infestation level of FCM. This reversion to the 22-day cold treatment came after 9 years of discussions and exchange of information.

The following outstanding matters were also discussed in the bilateral meeting (1) Ports of entry – the inclusion of the port in Miami, Florida was not approved because there are no facilities for the certification of the cold treatment but the inclusion of the ports in Houston, Texas and New Orleans, Louisiana may be allowed in 2015 if the 2014 pilot program is successful. Importing through the port of Wilmington in Delaware was approved, (2) Termination of the obligatory cutting of fruit in the USA: USDA-APHIS rejected this proposal, (3) Importation of citrus from SA under the same CBS conditions as apply to US domestic trade – it was indicated that USDA-APHIS will proceed with the process to make this possible and (4) Recognition of the whole of Western Cape Province as a Citrus Black Spot (CBS) pest free area and the CBS Pest free places of production in areas of low pest prevalence – it was indicated that USDA-APHIS will reconsider these requests after finalisation of the importation of citrus fruit from CBS areas in South Africa**.**

2.5 CHINA

The two outstanding issues for this market were the acceptance of non-containerised bulk shipping and a systems approach for FCM. The Chinese Authorities raised the following concerns regarding the use of non-containerised shipping: (1) maintenance of phytosanitary risk mitigation and (2) the control of the cold treatment during shipping. Information addressing these concerns was submitted to SA-DAFF. However, by the end of this reporting period, the information had not yet been submitted to the Chinese authorities. CRI engaged with the Chinese Academy of Agricultural Science to investigate the prospect of revitalising a dormant research cooperation agreement as a means of facilitating progress with these matters.

2.6 SOUTH KOREA

The clarification/interpretation of point 5.1 of the amended export protocol remained problematic in that it has been interpreted as requiring packed consignments to be covered with plastic or mesh from packing to port. SA-DAFF was again provided with information for communication and discussion with the South Korean authorities before the start of the 2014 export season. This did not resolve the issue and SA-DAFF was again requested to take it up with the South Korean Authorities as part of a technical discussion. By the end of this reporting period this issue remained unresolved. SA's application for inclusion of mandarins in the S Korean export program remained pending further assessment by S Korea.

2.7 THAILAND

The protocol to export citrus to Thailand was signed in 2012. In the first export season no phytosanitary problems were encountered but in the 2013 export season consignments were rejected for the presence of Fullers Rose Weevil. Information on the distribution and occurrence of this pest in citrus orchards was provided to SA-DAFF in January 2014 prior to the approval of the orchards for the 2014 export season.

2.8 VIETNAM

In July 2013, the export of citrus fruit to Vietnam was suspended pending the submission of Pest Information Packages (PIPs). This information was provided to the Vietnamese Authorities in 2008 by SA-DAFF but it was submitted as a PIP for *Citrus* spp and not as individual PIPs for each citrus type. The Vietnamese Authorities, however, wanted a PIP for each citrus type. The PIP for *Citrus* spp was amended by CRI and in August 2013 SA-DAFF submitted the PIP for sweet oranges to the authorities and the PIPs for Lemons, Grapefruit and Soft Citrus in March 2014. No feedback was received from the Vietnamese Authorities during this reporting period despite follow-ups made by SA-DAFF.

2.9 INDIA

Exports of fresh citrus fruit are allowed to India under specific import conditions. One of the conditions is that the consignment must be inspected and found to be free from *Phyllosticta citricarpa*. However, scientific articles indicate that this pest occurs in India and SA-DAFF was requested to seek removal of this requirement. SA-DAFF submitted the request to India, but by the end of the reporting period no feedback had been received from the Indian Authorities.

2.10 NEW MARKETS

2.10.1 The Philippines

In 2009, SA-DAFF submitted a Pest Information Package to the Philippine Authorities to gain access for all fresh citrus fruit types from South Africa. In October 2012 the first draft PRA with proposed phytosanitary requirements for importation of fresh *Citrus* spp. was received from the Philippine Authorities. Additional information was supplied to SA-DAFF in January 2013 and SA-DAFF submitted the information to the Philippine authorities in June 2013. In January 2014 a proposed final PRA was received from The Philippines. Inputs were requested from CRI on the pests listed as being of phytosanitary concern. The requested information on the listed pests was provided to SA-DAFF in March 2014. In June 2013 a request was also received from the Philippines for a technical visit to South Africa but the visit was later postponed to June 2014.

2.10.2 Australia and Lebanon

No feedback was received from these countries during this reporting period, despite several follow up requests by SA-DAFF.

2.11 IMPORTS

2.11.1 Egypt

In March 2014, SA-DAFF requested input from CRI on information received from Egypt. The information pertained to the occurrence of *Brevipalpus lewisi* in Egypt as well as on the details of the USDA accepted cold treatment protocol for *Bactrocera zonata*. CRI recommended that the proposed procedures would provide appropriate phytosanitary risk mitigation.

2.11.2 Import conditions

By the end of this reporting period revision of the import conditions for Citrus vegetative propagation material was still pending. In 2013 there was a shortage of seed available to the industry and to avoid a shortage of trees, seed was imported under permit from reputable sources (Australia and USA).

2.12 BIOSECURITY AND REGULATIONS

An incursion of the exotic fruit fly *Bactrocera invadens* was detected in SA in 2010. After successful eradication of this pest in different areas in 2010, 2011 and 2012, South Africa notified the IPPC in March 2013 that the status of *B. invadens* in SA had changed from “transient, actionable and under eradication” to “present, only in some areas and under official control”. The pest was considered to be present in the Vhembe district of the Limpopo province.

In December 2013 a further report was sent to the IPPC to report on detection of incursions of *B. invadens* in South Africa in isolated areas in the Mopani municipal district of Limpopo province, in the Bojanala Platinum and Ngaka Modiri Molema municipal districts of the North-West province, in Tshwane in the Gauteng

province, in the Ehlanzeni municipal district in Mpumalanga province and in uThungulu and Umkhanyakude municipal districts in the KwaZulu-Natal province. The report further stated that in the affected areas of Bonjanala Platinum district of North-West Province and in Tshwane in the Gauteng province, no further detections of *B. invadens* were found for more than 12 weeks, or three life cycles, after the last fruit fly had been detected. The status in these districts is now Absent: Pest eradicated. By the end of the reporting period incursions of *B. invadens* had been detected in parts of all provinces except for the Eastern- and Western Cape. Quarantine and eradication measures were implemented in accordance with the relevant national action plan and accordingly the official *B. invadens* status of South Africa remained unchanged at the end of the report period.

A document was released in April 2014, reporting on the CBS monitoring survey that was conducted in February 2013 in the magisterial districts with commercial citrus plantings in the Northern Cape province. The survey confirmed the absence of CBS in the region. The survey was conducted in response to a request from the EU for more information in support of SA's request to the EU to recognise the N Cape as CBS free.

In January 2014 another CBS monitoring survey was conducted in some of the magisterial districts of the Northern Cape, North West and Free State provinces. This survey was conducted as a periodic confirmatory survey to maintain official recognition of the CBS pest free status of the region. At the end of the report period the laboratory report was still outstanding, but its subsequent release confirmed the continued CBS pest free status of the region.

The retention of the area of low CBS pest prevalence in the far northern region of the Limpopo province came into question after rejections of export citrus from this region in 2013. Meetings were held with SA-DAFF and the producers to discuss the maintenance of this area. The implementation of a control programme for CBS was agreed upon and it was decided that the status of the area will again be considered following a survey to be conducted in 2015.

The new Plant Health (Phytosanitary) Bill, that is a revision of the Agricultural Pests Act (Act No. 36 of 1983) and amendments to Regulations R110 and R1013 of the Agricultural Pests Act had not been published in the Government Gazette by the end of the report period. Amendments to Regulation R110 will include the compulsory notification of the occurrence of listed quarantine pests in pest free areas, the declaration of the whole Western Cape province as a CBS pest free area, the inclusion of the presence of *B. invadens* in the Vhembe district of the Limpopo province and the establishment of buffer zones to protect the Citrus Greening free status of the Eastern Cape province.

Detection surveys were conducted in magisterial districts of the Western Cape to monitor the spread of African citrus greening disease within the province. Positive trees were found in the Malmesbury district and the trees were removed. A delimiting survey will be conducted to confirm that there has been no spread of the disease in that specific area. Detection surveys were also conducted in the Kat River area of the Eastern Cape and no positive trees were found. Early warning detection surveys were conducted in KZN to safeguard against the risk of Asiatic greening and the official report confirmed absence of the disease in South Africa.

In 2012 South Africa was notified by the Chinese Authorities that *Xanthomonas anoxopodis* (Citrus canker) had been detected in seed consignments imported from South Africa. The seed was returned to South Africa. Laboratory tests were conducted on the seeds and the nursery from which the seed originated was inspected, sampled and tested. The results confirmed the continued absence of citrus canker in SA. CRI requested SA-DAFF to submit this information to China in the form of an official report to confirm the continued absence of the pest from SA. The communication from SA was still pending at the end of the report period.

3 PORTFOLIO: INTEGRATED PEST MANAGEMENT

3.1 PORTFOLIO SUMMARY

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

The mood in the citrus industry during the 2013/14 season has been dominated by the citrus blackspot (CBS) crisis in the European Union. Consequently so too has a tremendous amount of time, attention and money been spent, both politically and scientifically, on addressing the issue. Despite this, much of the southern African citrus industry experienced an exceptionally successful export season, highlighting the tenacity of the industry to deal with adversity. This same tenacity and resolve is being channelled towards dealing with the entomological issues which have the potential to create similar crises for the industry. Top

of this list is false codling moth (FCM), which was the subject of a recently completed pest risk analysis (PRA) conducted by the European Plant Protection Organisation (EPPO). The expectation is that this may lead to more stringent regulations for exporting of citrus from Africa to Europe, in a similar vein to the current case with CBS.

CRI's entomological research team expanded during the past year with the appointment of the extremely experienced entomologist, Dr Marin Gilbert, in the Western Cape. In line with the citrus industry's and CRI's research priorities, the bulk of Martin's time is being spent on FCM research. Additionally, CRI continues to build and benefit from research associations with a number of other entities, particularly universities (Rhodes, Stellenbosch, Nelson Mandela Metropolitan, Pretoria and Free State). With any university association comes students and consequently several of the projects reported within the IPM Portfolio, include post-graduate students, which bodes well for entomological research succession in our industry. Additionally, a number of research associations with a range of other private entities complete the landscape within the IPM Portfolio. Examples are e-nema (Germany), Du Roi IPM, Ibbenbüren and Xsit.

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

Unsurprisingly, the programme which has received the most funding and which has the highest number of registered projects and researchers involved is the FCM Programme. Twenty research projects are reported, a record number for the programme. Thirteen of these focussed on pre-harvest management issues. Three of the pre-harvest control projects focussed on aspects of mating disruption. It was concluded that a mating disruption "overkill" approach held promise as a means to reduce FCM risk to negligible levels. Also interesting was the investigation of a novel mating disruption – or rather, mating inhibition – compound. Seven of the pre-harvest control projects concentrated on aspects of biological control of FCM: three on virus, one on fungi, one on nematodes and two on parasitoids. These studies range from high-tech fundamental science, such as investigating genetic signals for susceptibility or resistance to virus, to highly applied studies, such as large scale field trials with entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN). Both of these pathogens showed tremendous potential by significantly reducing FCM infestation of fruit. A study on the biology and behaviour of pupating FCM is intended to enable a more targeted approach with EPNs and EPFs. A second study on FCM behaviour and biology examined occurrence and movement of the pest in multicrop systems in the Western Cape. Only one project examined the efficacy of spray treatments against FCM, revealing Runner, Delegate and Cryptogran as the most effective options, albeit not significantly.

Three projects focussed on post-harvest detection of FCM in fruit. The first demonstrated that Microfocus X-ray tomography could probably detect 100% of FCM infestations in fruit within a reasonably short scan. The second project investigated the use of behavioural responses of FCM larval parasitoids to indicate infested fruit. The third detection project was simply a probit 8.7 survey for FCM infestation of export lemons. A further three trials focussed on post-harvest treatments for disinfesting fruit of FCM. The first proved that a combination of cold and ionising radiation could provide probit 9 disinfestation of fruit. The second also examined cold, but as a risk mitigation, rather than quarantine, treatment. The final post-harvest treatment project demonstrated some potential with vapour heat. The final FCM project proposed to verify inspection standards within a systems approach which is being devised as a potential alternative to cold sterilisation for FCM.

The fruit fly programme attracted the second highest level of funding, after FCM. This was mainly due to the work conducted on the new invasive fruit fly pest in South Africa, *Bactrocera invadens*, which has now been declared present in the Vhembe district of Limpopo Province and has been detected in a number of other areas in six out of South Africa's nine provinces. For local fruit fly pests, the focus was mainly on finding new proteinaceous baits and bait application methods for more effective control. This was addressed in two projects. A new paper-based bait station was found to be promising in field tests. On the other hand, new autolysate attractants were found to be less attractive than the commercially available attractant HymLure. For *B. invadens*, a project on the dispersal capacity of the pest was initiated in April 2013. Information obtained in this project will provide a basis in determining the effective size of area to be treated and quarantined for the pest.

In the programme covering mealybug and other phytosanitary pests, issues pertaining to both mealybug and carob moth management were addressed, as well as a post-harvest treatment trial. Gamma irradiation at 150 Gy as a post-harvest treatment for citrus mealybug, confirmed its value as a phytosanitary treatment. EPFs showed good potential against both citrus mealybug and citrus thrips in laboratory bioassays. The first of the carob moth projects looked at the morphology and ecology of carob moth in citrus orchards. Another study conducted in the Vaalharts region, indicated that carob moth infestation of citrus was higher than for

FCM, as a result of probable migration from pecan nut orchards. Lastly, post-harvest fumigation with a GRAS fumigant and CO_2, targeted against a range of phytosanitary pests, provided total mortality of grain chinch bug, and indicated that fruit fly was slightly more susceptible than FCM.

The focus in the non-phytosanitary key pests programme is on pests such as citrus thrips, citrus psylla and red scale. Two EPF isolates gave disappointing results in the field against thrips and mealybug. EPFs were also evaluated against red scale in laboratory bioassays and although they caused some mortality to crawlers they had no effect on older life stages. Some new chemistry was evaluated in a field trial against the green citrus leafhopper but results were not very good. Several botanical and organic products demonstrated some promise against woolly whitefly in laboratory bioassays.

Only one project received attention in the minor pests and mites programme in this report period and that was on the development of an attractant for fruit-piercing moths. Banana was shown to be the most effective attractant.

During the research year in question CRI entomologists and many of the other entomologists working within this programme, 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 and Cutting Edge publications.

PORTEFEULJE OPSOMMING

Die gemoedsstemming in die sitrusbedryf gedurende die 2013/14 seisoen is deur die sitrus swartvlek (SSV) krisis in die Europese Unie gedomineer. Daarom is daar ook geweldige tyd, aandag en geld op die saak gespandeer – albei polities en wetenskaplik. Ten spyte daarvan het ŉ groot deel van die Suid-Afrikaanse sitrusbedryf ŉ besonderse suksesvolle jaar beleef, ŉ demonstrasie van die bedryf se volharding wanneer met teenspoed gekonfronteer. Dieselfde volharding en vasberadenheid word gefokus op die entomologiese uitdagings wat die vermoë het om dieselfde tipe krisisse vir die bedryf te veroorsaak. Bo op hierdie lys is valskodlingmot (VKM), wat die onderwerp van ŉ pas voltooide plaag risiko analise (PRA) is wat deur die Europese Plantbeskermings Organisasie (EPPO) uitgevoer is. Die verwagting is dat hierdie tot strenger regulasies vir die uitvoer van sitrus van Afrika Europa toe kan lei, soortgelyk aan die huidige geval met SSV.

CRI se entomologiese navorsings span het gedurende verlede jaar gegroei met die aanstelling van die baie ervare entomoloog, Dr Martin Gilbert, in die Wes-Kaap. In lyn met die sitrusbedryf en CRI se navorsings prioriteite word die meerderheid van Martin se tyd op VKM navorsing deurgebring. CRI bou aanhoudend verhoudings met ŉ verskeidenheid ander navorsingsinstansies waaruit daar voordeel getrek kan word. Dit sluit veral universiteite in (Rhodes, Stellenbosch, Nelson Mandela Metropolitaan, Pretoria, Vrystaat). Met enige universiteit samewerking kom daar studente. Gevolglik is daar heelwat na-graadse student betrokkenheid op verskeie van die projekte in die IPM Portefeulje, wat tot groot voordeel is vir entomologiese opvolging in ons bedryf. Derhalwe bestaan daar ŉ klomp navorsingsgenootskappe met ander private instansies, wat die landskap binne die IPM Portefeulje voltooi. Voorbeelde is e-nema (Duitsland), Du Roi IPM, Ibbenbüren en Xsit.

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

Soos verwag, die program wat die meeste bevondisng ontvang het en wat die meeste geregistreerde projekte en betrokke navorsers het, is die VKM program. Twintig navorsings projekte word gelys, ŉ rekord hoeveelheid vir die program. Dertien van hierdie het op vooroes bestuurs kwessies gefokus. Drie van die vooroes beheer projekte het op verskillende aspekte van paringsontwrigting gefokus. Die gevolgtrekking is gemaak dat ŉ paringsontwrigting “oordosis” benadering belofte getoon het as ŉ metode om VKM risko te verminder tot ŉ weglaatbare vlak. Ook interessant was die ondersoek aan ŉ nuwe paringsontwrigting molekule. Sewe van die vooroes beheer projekte het gefokus op verskillende aspekte van biologiese beheer van VKM: drie op virus, een op swamme, een op nematodes en twee op parasiete. Hierdie studies strek van hoë tegnologie basiese wetenskap, soos die ondersoek aan genetiese seine vir vatbaarheid of weerstandbiedendheid vir virus, tot baie toegepaste studies, soos grootskaalse veldproewe met entomopatogeniese swamme (EPS) en entomopatogeniese nematodes (EPN). Albei van hierdie patogene het geweldige potensiaal getoon deur hulle beduidende vermindering van VKM besmetting van vrugte. ŉ Studie op die biologie en gedrag van VKM papies sal hopelik tot meer suksesvolle toediening van EPNs en EPFs lei. ŉ Tweede studie op VKM gedrag en biologie en die voorkoms en beweging van die plaag in ŉ

veelvulgige-gewas stelsel in die Wes-Kaap uitgevoer. Net een projek het die werking van spuit behandelings teen VKM ondersoek en het onthul dat Runner, Delegate en Cryptogran die mees doeltreffende opsies was, al nie betekenisvol nie.

Drie projekte het gefokus op na-oes opsporing van VKM in vrugte. The die eerste het gewys dat Mikrofokus X-straal tomografie blykbaar 100% van VKM besmettings in vrugte opgetel het met ŉ kort skandering. Die tweede projek het die gebruik van gedrags reaksies van VKM larwe parasiete om VKM besmette vrugte aan te dui ondersoek. Die derde opsporings projek is eenvoudig ŉ probit 8.7 opname vir VKM besmetting van uitvoer suurlemoene. ŉ Verdere drie proewe het gefokus op na-oes behandelings vir disinfestasie van vrugte vir VKM. Die eerste het bewys dat ŉ kombinasie van koue en inoiserende bestraling, probit 9 disinfestasie van VKM in vrugte kan veroorsaak. Die tweede het ook koue ondersoek, maar as ŉ riskio vermindering eerder as ŉ kwarantyn behandeling. Die finale na-oes behandelings projek het gewys dat damp hitte ook dalk ŉ doeltreffende behandeling mag wees. Die finale VKM projek se doel was om inspeksie standaarde binne ŉ stelsels benadering, as ŉ moontlike alternatief vir koue sterilisaie vir VKM, te verifieer.

Die vrugtevlieg program het die tweede hoogste bevondsings vlak gelok, na VKM. Hierdie is hoofsaakliks as gevolg van die werk wat gedoen is op die nuwe indringer vrugtevlieg plaag in Suid-Afrika, *Bactrocera invadens*, wat nou as teenwoordig verklaar is in die Vhembe distrik van Limpopo Provinsie en is ook in verskeie ander streke in ses van Suid-Afrika se nege provinsies ontdek. Vir plaaslike vrugtevlieg plae is die fokus hoofsaakliks op die ontwikkeling van nuwe proteïen lokase en lokaas toedienings metodes vir meer doeltreffende beheer. Hierdie is in twee projekte aangespreek. ŉ Nuwe papier-gebaseerde lok-stasie het in veldproewe belowend opgetree. Aan die ander kant, is nuwe outolisaat lokmiddels minder aantreklik as die kommersieel beskikbare HymLure. Vir *B. invadens*, is ŉ projek op die verspreidings vermoë van die plaag in April 2013 geïnisieer. Inligting wat uit hierdie projek uitkom sal die basis skep vir ŉ bepaling van die korrekte grote van area om te behandel en onder kwarantyn te plaas vir die plaag.

In die program wat witluis en ander kwarantyn plae dek is kwessies oor albei witluis en karobmot toegespreek sowel as ŉ na-oes behandelings projek. Gammabestraling teen 150 Gy as ŉ na-oes behandeling vir sitruswitluis, het sy waarde as ŉ fitosanitêre behandeling bevestig. EPFs het goeie belofte getoon teen albei sitruswitluis en sitrusblaaspootjie in laboratorium biotoetse. Die eerste van die karobmot projekte het die morfologie en ekologie van karobmot in sitrus boorde ondersoek. Nog ŉ studie, wat in die Vaalharts streek uitgevoer is, het aangedui dat karobmot besmetting van sitrus hoër was as vir VKM, heel waarskynlik as gevolg van sy migrasie van pekanneut boorde. Laastens, het na-oes beroking met ŉ GRAS berokings middel en CO_2, gemik teen ŉ reeks fitosanitêre plae, totale mortaliteit van graanstinkbesie gegee en het aangedui dat vrugtevlieg effens meer gevoelig was as VKM.

Die fokus in die nie-fitosanitêre sleutel plae program is op plae soos sitrusblaaspootjie, sitrusbladvlooi and rooidopluis. Twee EPF isolate het teleurstellende resultate in veldproewe teen blaaspootjie en witluis gegee. EPFs is ook teen dopluis in laboartorium biotoetse geëvalueer en alhoewel hulle ŉ mate van kruiper mortaliteit veroorsaak het, het hulle geen effek op die ouer lewensstadiums gehad nie. Sommige nuwe chemie is in ŉ veldproef teen die groen sitrusblaarspringer getoets, maar resultate was nie baie goed nie. Verskeie botaniese en organiese produkte het belowend gelyk teen wollerige witluis in laboratorium biotoetse.

Net een projek het enige aandag gekry in die program oor minder belangrike plae en myte gedurende die verslag tydperk en dit was op die ontwikkeling van ŉ lokmiddel vir vrugtesteek motte. Dit is gewys dat piesang die mees doeltreffende lokmiddel was.

Gedurende die laaste navorsingsjaar het CRI entomoloë en verskeie ander entomoloë wat binne die program werk, aktief deelgeneem in plaaslike en internationale wetenskaplike kongresse. 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 en Snykant publikasies gedoen.

3.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

3.2.1 Programme summary

Despite the citrus black spot (CBS) European Union (EU) crisis currently overshadowing all other aspects of pest and disease control in the citrus industry, there is an underlying realisation that a similar situation with false codling moth (FCM) could occur in the near future. A pest risk assessment (PRA) on FCM, conducted by the European Plant Protection Organisation (EPPO) was completed in September 2013. It is possible that member state governments could respond by implementing stricter import regulations for FCM-susceptible produce from Africa. Top of the list would be citrus. Consequently, research on FCM is being conducted with even greater urgency than before. Research focus has swung away from purely conventional pre-harvest control, towards technologies and systems that could ensure the export of FCM-free consignments of fruit. In addition, research has become more innovative in trying to remedy the FCM problem effectively.

During the 2013-14 research cycle, 20 research projects were conducted within the FCM programme. Thirteen of these focussed on pre-harvest management issues, six focussed on post-harvest issues and the remaining project aimed to assemble all practices (pre- and post-harvest) into a systems approach.

Three of the projects focussed on aspects of mating disruption. The first aimed to develop a novel, effective and more affordable dispenser (3.2.2). Although something was devised, its efficacy was marginally inferior to Isomate and thus the focus of the study changed slightly. It was demonstrated that a mating disruption "overkill" approach, regardless of affordability, could be more effective than conventional mating disruption. The further investigation of this approach, along with that of some novel mating disruption products and approaches, was conducted within a new project (3.2.17) executed in the Western Cape. Unfortunately FCM presence was extremely low at the trial site, precluding the acquisition of any meaningful results. The third mating disruption project, which was actually termed as mating inhibition, was conducted with a novel compound, rather than the conventional FCM pheromone blend normally used (3.2.12). Caged field trials were conducted to determine whether mating and consequently fruit infestation could be reduced. Unfortunately, problems were experienced with the dispensing technology and the remainder of the time on the project was spent on better understanding and improving this in the laboratory.

Seven of the projects concentrated on aspects of biological control of FCM: three on virus, one on fungi, one on nematodes and two on parasitoids. After many years of research, our understanding of the virus (CrleGV), how it works and hence how to use it optimally has become far more sophisticated. For example, one of the projects looks at the differences in FCM response to CrleGV at genomic level to determine the degree of genetic differences elicited by different time and temperature conditions. Thirty-one genes were evaluated under different conditions using RT-qPCR in order to identify the best genes for evaluating interactions. The ultimate objective would be to identify those genes in FCM responsible for susceptibility to virus infection or alternately to resistance. Thus the best virus-host combinations could be structured. The second CrleGV project (funded by Rhodes University) looked at the effect of molasses, as a virus adjuvant, on neonate FCM larvae (3.2.15). Molasses resulted in decreased larval movement (by distance) on the fruit surface, increased time spent on the fruit surface (due to increased feeding) and reduced penetration of fruit. The final CrleGV project is investigating the UV-susceptibility of both Cryptogran and Cryptex in the laboratory and the field and determining the rate (and mode) of breakdown (3.2.13). Light microscopy, electron microscopy, qPCR and bioassays are being employed to ultimately determine the frequency of reapplication required.

In the project devoted to studying entomopathogenic fungi (EPF) for FCM control, results have been compelling (3.2.6). The three most promising local isolates outperformed two commercial isolates in laboratory bioassays and in the field, persisted in the soil whilst still remaining infective over a six month period. Results of a large scale field trial suggested the better performance of a *Beauveria bassiana* isolate than two *Metharizium anisopliae* isolates with a reduction in FCM infestation of approximately 80%. Results with entomopathogenic nematodes (EPN) were also promising (3.2.8). Due to variability in results in trials with once-off application of EPNs in spring, monthly applications were assessed. Survival and persistence of EPNs at three sites was better with the monthly applications than the once-off treatment. At one trial site in the Eastern Cape, FCM infestation of fruit was reduced by 72% where *Heterorhabditis bacteriophora* was applied monthly, whereas the once-off application only reduced infestation by 28%. Another project, closely related to the previous two, examined the pupating behaviour and biology of FCM (3.2.10). The role of soil type, soil moisture, soil compaction, air temperature, groundcover and shade on pupation is being examined. It is hoped that this knowledge will lead to more effective application of EPFs and EPNs.

The two projects on biological control with parasitoids, looked at late season augmentation with egg parasitoids (3.2.4) and classical biological control with a larval parasitoid (3.2.16). In 2012/13, *Trichogrammatoidea cryptophlebiae* egg parasitoids were released monthly in four citrus orchards from January to April. At most sites, FCM levels were too low to obtain meaningful results. At one site, FCM levels were very high. However, so too was natural parasitism, thus obscuring any impact which the released parasitoids might have had. The study was repeated during the 2013/14 season, but again, results were not convincing. The other project aims to introduce the effective larval parasitoid, *Agathis bishopi*, from the Eastern Cape into the Western Cape. However, this is being preceded by a survey, which has thus far revealed the occurrence of no parasitism of FCM larvae in the Citrusdal and Stellenbosch citrus growing areas.

A second study on FCM behaviour and biology examined occurrence and movement of the pest in multicrop systems throughout the year (3.2.18). This was conducted in citrus, grapes, plums, nectarines, apples and pears in the Western Cape, monitoring both FCM and fruit fly using traps. Generally, FCM levels were highest in nectarines, even weeks after fruit had been harvested. The study will be continued for at least three seasons.

There was only one project which examined the efficacy of spray treatments against FCM, including chemical and biological sprays (3.2.21). Runner and Delegate were the most effective treatments, followed by Cryptogran. However, there were no statistically significant differences between the efficacy of any of the products.

Three projects focussed on post-harvest detection of FCM in fruit. The first mainly examined the use of X-ray, but also tested the capability of some of the commercially available optical equipment for pack houses (3.2.3). In a study conducted on Satsuma mandarins, the time of a tomography X-ray scan was ultimately reduced from 35 minutes to 34 seconds, still appearing to detect 100% of FCM penetrations in fruit. The next detection project was equally ambitious and innovative – examining the potential of the larval parasitoid, *Agathis bishopi*, to indicate infested fruit by interpreting its behaviour. It is not yet possible to draw conclusions on the potential of this technology. However, the option of training the wasps exists. The final FCM detection project involves lemons (3.2.20). Despite many attempts, it has not been possible to prove that lemons cannot host FCM larvae. This project therefore aims to test – and hopefully prove – at the probit 8.7 level (thus a total of 29956 fruit) whether lemons packed for export can be infested with FCM.

A further three trials focussed on post-harvest treatments for disinfesting fruit of FCM. The first tested a combination of cold and ionising radiation as a probit 9 quarantine treatment for FCM larvae. A combination of 60 Gy ionizing radiation followed by cold for 16 days at 2.5°C killed more than 99.7% of treated larvae. Of those that survived, none succeeded in mating and producing offspring. A second post-harvest treatment trial also examined cold. However, this was an incomplete cold treatment for risk mitigation rather than a quarantine treatment (3.2.7). The most effective treatment tested was 2°C for 18 days, as no 1st and 2nd instar larvae survived. Survival in fruit was slightly higher than in diet, with 4.35% and 0.04% of 5th instars surviving in each medium respectively. The final post-harvest treatment trial examined the effect of heat (vapour heat) as opposed to cold (3.2.11). A temperature of 46°C for 6 h at 53% RH resulted in 100% mortality of larvae but this may be a risky treatment for fruit quality. A shorter or lower heat treatment, followed by a short cold treatment, which would be safer for fruit, is now being investigated.

Finally, a project was conducted to verify proposed inspection standards within a systems approach (3.2.19). Indications to date are that a) pre-harvest levels of FCM are a good indication of post-harvest risk, and b) current arbitrarily selected standards are likely to be quite accurate. However, more data are required for greater certainty.

Programopsomming

Ten spyte van die stitrusswartvlek (SSV) Europese Unie (EU) krisis wat tans alle ander aspekte van plaag- en siekte-bestryding in die sitrusbedryf oorskadu, is daar ŉ onderlêende bewustheid dat ŉ soortgelyke situasie met valskodlingmot (VKM) in die nabye toekoms kan plaasvind. ŉ Plaagrisiko analise (PRA) oor VKM, uitegevoer deur die Europese Planbesekrmings Organisasie (EPPO) is in September 2013 voltooi. Dit is moontlik dat ledestaat regerings kan reageer met die implementering van strenger invoer regulasies vir VKM-vatbare produkte van Afrika. Bo op die lys sal sitrus wees. Gevolglik word navorsing op VKM met selfs groter dringendheid as voorheen uitgevoer. Navorsings fokus het weggeswaai van suiwer konvensionele vooroes-bestryding, na tegnologië en stelsels wat die uitvoer van VKM-vrye vrug besendings kan verseker. Benewens het navorsing meer inoverend geword in ŉ poging om die VKM probleem doeltreffend op te los.

Gedurende die 2013-14 navorsingssiklus is 20 projekte binne die VKM program uitgevoer. Dertien van hierdie het gefokus op vooroes bestuurs kwessies, ses op na-oes kwessies en die oorblywende projek se doel was om alle praktyke (voor- en na-oes) bymekaar te bring in a stelsels benadering.

Drie van die projekte het gefokus op verskillende aspekte van paringsontwrigting. Die doel van die eerste was om ŉ oorspronklike, doeltreffende en meer bekostigbare vrysteller te ontwikkel (3.2.2). Al was iets ontwikkel, was sy werking effens swakker as dié van Isomate en dus het die klem van die studie ietwat verander. Dit is gedemonstreer dat ŉ paringsontwrigting "oordosis" benadering, ongeag die bekostigbaarheid, meer doeltreffend as gewone paringsontwrigting kan wees. Die verdere ondersoek van hierdie benadering, saam met die ondersoke van nuwe paringsontwrigtings produkte en benaderings, is binne ŉ nuwe projek (3.2.17) in die Wes-Kaap uitgevoer. Ongelukkig is VKM druk by die proefperseel besonders laag, wat die verkryging van enige betekenisvolle resultate verhoed het. Die derde paringsontwrigting projek is eintlik as paringsvoorkoming voorgestel en is met ŉ oorspronklike molekule uitgevoer, eerder as die gewone VKM feromoon mengsel wat gewoonlik gebruik word (3.2.12). Hok-veldproewe is uitgevoer om te bepaal of dit moontlik was om paring en gevolglik vrug besmetting te verhoed. Ongelukkig is probleme met die vrystellings tegnologie ondervind en die res van die projek is in die laboratorium deaurgebring om hierdie beter te verstaan en te verbeter.

Sewe van die projekte het op verskillende aspekte van biologiese beheer van VKM gefokus: drie op virus, een op swamme, een op nematodes en twee op parasiete. Na baie jare se navorsing het ons begrip van die virus (CrleGV), hoe dit werk en hoe om dit optimaal te gebruik, baie meer gesofistikeerd geword. Byvoorbeeld het een van die projekte verskille in VKM se reaksie teen CrleGV besmetting op genomiese vlak ondersoek om die mate van genetiese verskille teen verskillende tydsduur en temperatuur omstandighede te bepaal. Een-en-dertig genes is onder verskillende omstandighede evalueer met gebruik van RT-qPCR om die beste genes te identifiseer om interaksie te evalueer. Die uiteindelike doel is om die genes in VKM te identifiseer wat verantwoordelik is vir vatbaarheid vir virus infeksie of alternatief vir weerstandbiedendheid. Dus sal dit moontlik wees om die beste virus-gasheer kombinasies te struktureer. Die tweede CrleGV projek (deur Rhodes Universiteit bevonds) het gekyk na die effek van molasse, as ŉ virus byvoegsel, op pasuitgebroeide VKM larwes (3.2.15). Molasse het ŉ vermindering in beweging van larwes op die vrug oppervlak veroorsaak (gemeet in afstand), ŉ verlenging in tydsduur deurgebring op die vrug oppervlak (as gevolg van verhoogde voeding) en verminderde penetrasie van vrugte. Die finale CrleGV projek ondersoek die UV-vatbaarheid van albei Cryptogran en Cryptex in die laboratorium en in die veld en bepaal die koers (en manier) van afbraak (3.2.13). Lig-mikroskopie, elektron-mikroskopie, qPCR en biotoetse word gebruik om uiteindelik die benodigde intervalle vir hertoediening te bepaal.

In die projek op entomopatogeniese swamme (EPS) vir VKM beheer, is resultate oortuigend (3.2.6). Die drie mees belowende plaaslike isolate het beter gevaar as twee kommersiele isolate in laboratorium biotoetse en in die veld het hulle oor ŉ ses-maande tydperk voortgeduur sonder om hulle infektiwiteit te verloor. Resultate van ŉ grootskaalse veldproef het aangedui dat ŉ *Beauveria bassiana* isolaat beter gevaar het as twee *Metharizium anisopliae* isolate, met ŉ vermindering in VKM besmetting van om en by 80%. Resultate met emntomopatogeniese nematodes (EPN) is ook belowend (3.2.8). As gevolg van wisselvalligheid in resultate met eenmalige toediening van EPNs in die lente, is maandelikse toedieinings ondersoek. Oorlewing en langwerkendheid van EPNs by drie persele is beter met die maandelikse toedieinings as die eenmalige toediening. By een proefperseel in die Oos-Kaap, is VKM besmetting van vrugte met 72% verminder waar *Heterorhabditis bacteriophora* maandeliks toegedien is, waar die eenmalige toediening besmetting met net 28% verminder het. Nog ŉ projek, verwant aan die vorige twee, het die gedrag en biologie van VKM papies ondersoek (3.2.10). Die rol van grondtipe, grondvog, grondkompaksie, lug temperatuur, grondbedekking en skaduwee op die papie stadium is ondersoek. Dit word gehoop dat hierdie kennis tot meer doeltreffende toediening van EPFs en EPNs sal lei.

Die twee projekte op biologiese beheer met parasiete, het gekyk na laat seisoen loslatings van eier parasiete (3.2.4) en klassieke biologiese beheer met larwe parasiete (3.2.16). In 2012/13 is *Trichogrammatoidea cryptophlebiae* eier parasiete maandeliks losgelaat in vier sitrusboorde van Januarie tot April. By meeste persele is VKM vlakke te laag om betekensivolle resultate te kry. By een perseel is VKM vlakke baie hoog maar so ook was natuurlike parasitisme wat enige impak wat die losgelate parasiete kon gehad het geduister het. Die studie is gedurende die 2013/14 herhaal maar weereens is resultate nie oortuigend nie. Die doel van die ander projek is om die doeltreffende larwe parasiet, *Agathis bishopi*, wat van die Oos-Kaap kom, in die Wes-Kaap los te laat. Hierdie word egter eers deur ŉ opname voorafgegaan, wat tot dusver aangedui het dat geen VKM larwes in die Citrusdal of Stellenbosch streke geparasiteer is nie.

ŉ Tweede studie op VKM gedrag en biologie het die voorkoms en beweging van die plaag in ŉ multi-gewas stelsel deur die jaar ondersoek (3.2.18). Hierdie is in sitrus, druiwe, pruime, nektariens, appels en pere in die Wes-Kaap uitgevoer. Albei VKM en vrugtevlieg is met lokvalle gemoniteer. Oor die algemeen is VKM

vlakke hoër in nektariens, selfs weke nadat vrugte geoes is. Die studie sal vir minstens drie seisoene uitgevoer word.

Daar is net een projek wat die doeltreffendheid van spuit behandelings teen VKM ondersoek het, insluitend chemiese en biologiese bespuitings (3.2.21). Runner en Delegate is die mees doeltreffende behandelings gevolg deur Cryptogran, maar daar is geen statisties betekenisvolle verskil tussen enige van die behandelings nie.

Drie projekte het gefokus op na-oes opsporing van VKM in vrugte. Die eerste het hoofsaakliks die gebruik van X-straal ondersoek maar het ook die vermoë van sommige van die kommersieel beskikbare optiese toerusting vir pakhuise getoets (3.2.3). In ŉ studie uitgevoer op Satsuma mandaryne is die tydsduur van ŉ tomografie X-straal skandering uiteindlik van 35 mnute tot 34 sekondes afgebring. Dit het geblyk dat dit nogsteeds 100% van VKM besmettings in vrugte opgespoor het. Die volgende opsporings projek was ewe ambiseus en inoverend. Dit het die vermoë van die larwe parasiet, *Agathis bishopi*, ondersoek om besmette vrugte aan te dui deur interpretasie van sy gedrag. Dit is nog nie moontlik om gevolgtrekkings te maak omtrent die potensiaal van hierdie tegnologie nie, maar daar bestaan ŉ moontlikheid om die wesper op te lei. Die finale VKM opsporings projek behels suurlemoene (3.2.20). Ten spyte van baie pogings, was dit nog nie moontlik om te bewys dat suurlemoene nie ŉ gasheer vir VKM larwes kan wees nie. Daarom is die doel van hierdie projek om te toets – en hopelik om te bewys – teen die probit 9.7 vlak (dus ŉ totaal van 29956 vrugte) of suurlemoen, gepak vir uitvoer, met VKM besmet kan wees.

ŉ Verdere drie proewe het gefokus op na-oes behandelings vir VKM disinfestasie van vrugte. Die eerste het ŉ kombinasie van koue en ioniserende bestraling as ŉ probit 9 kwarantyn behandeling vir VKM larwes ondersoek. ŉ Kombinasie van 60 Gy ioniserende bestraling gevolg deur koue vir 16 dae teen 2.5°C het meer as 99.7% van behandelde larwes dood gemaak. Van die wat oorleef het kon nie een van hulle paar en dus nageslag produseer nie. ŉ Twede na-oes behandelings proef het ook die effek van koue ondersoek, maar hierdie was onvoldoende koue behandeling vir risiko vermindering eerder as ŉ kwarantyn behandeling (3.2.7). Die mees doeltreffende behandeling wat getoets is is 2°C vir 18 dae, want geen 1de en 2de instar larwes het oorleef nie. Oorlewing in vrugte was effens hoër as in die dieet, met oorlewing van 4.35% en 0.04% 5de instars onderskeidelik. Die finale na-oes behandelings proef het die effek van hitte (damp hitte) in teenstelling met koue, ondersoek (3.2.11). ŉ Temperatuur van 46°C vir 6 h teen 53% RH het 100% mortaliteit van larwes veroorsaak maar mag dalk ŉ riskio vir vrug kwaliteit inhou. ŉ Korter of laer hitte behandeling, gevolg deur ŉ kort koue behandeling wat veileger vir vrugte sal wees, word nou ondersoek.

Laastens is ŉ projek uitgevoer om inspeksie standaarde binne ŉ stelsels benadering te verifieer (3.2.19). Tans is aanduidings dat a) vooroes vlakke van VKM ŉ goeie aanduiding van na-oes risiko is, en b) huidige eiemagtige standaarde lyk redelik akuraat. Nietemin word meer data benodig vir groter sekerheid.

3.2.2 FINAL REPORT: Investigation of the potential for the development of a locally produced mating disruption system

Project 955 (April 2009 – July 2013) by Sean Moore, Wayne Kirkman (CRI), Tanya Fullard & Martin Hill (RU)

Summary

Pheromone release rate from several different designs of dispenser was examined in an incubator at a relatively constant 29ºC. One dispenser type (ExpMD), with a semi-permeable membrane lid, was identified as having potential for further testing. This was due to a relatively constant rate of pheromone release. However, release rate from Isomate dispensers was 10 to 15 times higher. In field trapping trials conducted over 20 weeks, ExpMD caught almost five times the number of male FCM that were caught in Isomate loaded delta traps. In a large field trial, the efficacy of a number of treatments was compared. Isomate, Isomate Twintube, Checkmate, ExpMD, Cryptogran (two separate blocks) and a combination of Cryptogran and Isomate, were compared, where each treatment was applied to a 3-6 ha block. Isomate was significantly more effective than Isomate Twintube, Checkmate and one block of Cryptogran. However, infestation throughout the trial site did not appear to be even, placing doubt over the accuracy of the comparison. FCM infestation of fruit reached an unacceptable level in February throughout the trial site, leading to spraying out of the trial with Cryptogran. A second field trial was conducted, comparing various mating disruption and attract and kill products – both those commercially available and experimental products. Mating disruption “overkill”, a combination of Isomate and Checkmate (the latter applied fortnightly), was the most effective treatment in reducing both moth catches and fruit infestation. Finally, laboratory trials were conducted to compare the efficacy of different pheromone isomer blends for disrupting the ability of adult male FCM to locate virgin female FCM and thus mate. Unfortunately after numerous

attempts, an appropriate technique and protocol for conducting these trials could not be developed and the study was thus aborted. Evaluation of this trial is not yet complete.

Opsomming

Feromoon vrylatingstempo van verskeie verskillende vrylater ontwikkelings is in ŉ inkubator teen ŉ relatiewe konstante temperatuur van 29ºC vergelyk. Een van die ontwikkelings (ExpMD) met ŉ semi-deurdringbare membraan deksel, is uitgeken as belowend vir verdere proewe. Hierdie is as gevolg van ŉ relatief konstante vrylatingstempo. Nietemin was die vrylatingstempo van Isomate vrystellers nogsteeds 10 tot 15 maal hoër. In lokval proewe wat oor 20 weke in ŉ sitrus boord uitgevoer is, het ExpMD vyf maal soveel mannetjie VKM gevang as was Isomate in delta lokvalle gevang het. In ŉ groot boorproef is die werking van ŉ paar verskillende behandelings verglyk. Isomate, Isomate Twintube, Checkmate, ExpMD, Cryptogran (twee aparte blokke) en ŉ kombinasie van Cryptogran en Isomate is vergelyk waar elke behandeling in ŉ 3-6 ha blok toegedien is. Isomate is beduidend meer doeltreffend as Isomate Twintube, Checkmate en een van die Cryptogran blokke. Altans het dit voorgekom dat VKM besmetting nie deur die proefperseel eenvormig was nie, wat die akkuraatheid van die vergelyking laat betwyfel het. Vrugbesmetting het in Februarie deur die proefperseel ŉ onaanvaarbare vlak bereik. As gevolg hiervan is die hele perseel met Cryptogran uitgespuit. ŉ Tweede veldproef is uitgevoer om verskeie paringsontwrigting en lok-en-vrek produkte te vergelyk – albei dié wat kommersieel beskikbaar is en eksperimentele produkte. Paringsontwrigting “oordosis”, ŉ kombinasie van Isomate en Checkmate (ten laaste elke tweede week toegedien), was die mees doeltreffende behandeling, albei wat afname in mot vangstes en vrugbesmetting betref. Laastens is laboratoriumproewe uitgevoer om verskillende feromoon isomeer mengsels te vergelyk in hulle effektiwiteit om ŉ volwasse mannetjie VKM se vermoë om ŉ ongepaarde wyfie VKM op te spoor te versteur en dus om te paar. Na verskeie pogings, was dit ongelukkig nie moontlik om ŉ geskikte tegniek en protokol te ontwikkel om hierdie proewe uit te voer nie en dus is dié studie gestaak.

Introduction

The pest status of false codling moth (FCM) continues to grow, particularly with the imminent increase in stringency towards FCM interceptions by the European Union market. As of the 2010 export season, a zero tolerance standard for FCM interceptions was implemented for fruit destined for this most traditional of export markets. Citrus growers are therefore required to employ far more comprehensive control programmes for FCM than has been the case in the past. Such a requirement accentuates the benefit of an area-wide means of controlling FCM. The sterile insect technique (SIT) is generally recognised as potentially the most effective area-wide means of controlling a pest such as FCM. However, this technique is not available to all citrus growers in South Africa. Neither is it likely to be so in the near future, not only due to the immense cost and development time required in setting up such an initiative, but due to the unsuitability of certain regions. This could be due to the topography, size, cultivar distribution or orchard fragmentation of the region. However, mating disruption also has great potential for area-wide control of FCM (Shorey, 1973; Foster & Harris, 1997; Witzgall et al, 2008). Currently two mating disruption products are available in South Africa for control of FCM i.e. Isomate and Checkmate. Isomate is effective (Hanekom, 2006) but is manufactured in Japan and is expensive. Whenever the Rand weakens, it is inevitable that the product will become even more expensive. Additionally, due to the impending critical phytosanitary status of FCM, particularly in the EU, an “overkill” mating disruption programme should be tested in order to ascertain the full potential of the technology to address the problem.

Objectives

The objectives of this study were to:

- Develop an effective and affordable novel dispenser for use in mating disruption of FCM,
- Determine the most effective pheromone blend for mating disruption of FCM,
- Compare the efficacy of commercial and experimental mating disruption products in the field,
- Determine the full potential of mating disruption to address the FCM problem.

Materials and methods

Rate of release

Based on literature studies (eg Foster & Harris, 1997; McBrien et al, 1997; Tomaszewska et al, 2005) and some novel ideas, five fairly rudimentary pheromone dispensers were designed and assembled for a laboratory trial, which was initiated in January 2010. For proprietary purposes, only general descriptions will be provided for the dispensers. Twelve samples of each dispenser type (Table 3.2.2.1) were made, six each

being loaded with 100 µl of FCM pheromone and six without pheromone. The pheromone was made up as a three-component mixture of (E)-7-Dodecen-l-yl acetate, (E)-8-Dodecen-l-yl acetate and (Z)-8-Dodecen-l-yl acetate in the ratio 6:5:1 (Hofmeyr & Calitz, 1991; Hofmeyr et al, 1991). A second trial was initiated in May 2010, again using five dispensers, one of which was a repetition of that used in the previous trial and one of which was pure pheromone without formulation.

Table 3.2.2.1 Dispensers tested with and without FCM pheromone in laboratory trials.

Trial initiated January 2010	**Trial initiated May 2010**
Vial with semi-permeable membrane lid	Vial with no formulation
Safety gel in vial	Vial with semi-permeable membrane lid
Paraffinic wax and petroleum jelly mix in vial	Vial with polymer gel
Hard wax in vial	Hard wax discs
Paraffinic wax, emulsifier, vitamin E, soya oil and water mixture in vial	Checkmate in vial

A laboratory incubator was set at 27ºC. Dispensers were weighed before placing in the incubator and then again approximately weekly thereafter. For the first trial the scale which was used was only accurate to two decimal places. However, a new scale which was accurate to four decimal places was acquired for the second trial. The first trial ran from 23 January to 7 April 2010. The second trial ran from 3 May to 24 August 2010. From the regular measurements of weight, it was possible to calculate the rate and consistency of pheromone release.

A third trial was conducted to compare the rate of pheromone release from our novel membrane dispenser (Exp MD) with that of Isomate and Isomate-Twintube. A laboratory incubator was set at 27ºC. Dispensers were weighed before placing in the incubator and then again approximately weekly thereafter. The trial ran from 18 February to 1 July 2011. Seventeen measurements were therefore taken. Six replicates of each dispenser type were used.

Attractiveness

Six yellow delta traps with sticky floors were loaded with membrane dispensers, which each contained 100 µl of pheromone. A further six traps were loaded with Isomate dispensers and six traps were unloaded as control traps. These traps were hung in a three year old (planted 2007) orchard (no 54) of Newhall Navel orange trees on Far Away Farm in the Sundays River Valley on 23 November 2010. Traps were spaced evenly throughout the orchard, which measured 2 ha in size (1118 trees spaced 6 m x 3 m apart). Each week on the same day, male FCM caught in each trap were counted and sticky floors were replaced.

Efficacy

2010/11 field trial:

A block of around 24 ha on Vleiview Farm, Dunbrody Estates, in the Sundays River Valley, was selected for a field trial. The block consisted predominantly of a variety of late navel orange varieties, with one orchard being an early navel orange variety i.e. Newhall navels. All trees were planted in 2003 and were of almost identical size. Each orchard (of which there were eight) was around 3 ha in size.

Six different treatments were applied: Isomate, Isomate Twintube (not yet registered), Checkmate, experimental dispensers (ExpMD), Cryptogran and a combination of Isomate and Cryptogran. Isomate and Isomate Twintube were each applied to 6 ha blocks (two orchards each). Half of the Isomate block was also sprayed with Cryptogran. Checkmate, ExpMD and Cryptogran were each applied to 3 ha blocks (single orchards). However, two 3 ha blocks, separated from one another, were sprayed with Cryptogran. The difference in treatment size (3 ha vs 6 ha) should have had no negative influence on the smaller treatments, as they all formed part of a greater FCM control block, of which most was under mating disruption. Application rates and dates for all treatments are given in Table 3.2.2.2.

Table 3.2.2.2. Mating disruption and Cryptogran treatments applied in a field trial at Dunbrody Estates in the 2010/11 season.

Treatments	Orchards	Date and rate (per ha) of application							
Isomate	15	6 Oct	11 Jan						
		500	300						
Isomate Twintube	23 & 24	6 Oct	11 Jan						
		250	150						
Checkmate[1]	22	7 Oct	5 Nov	30 Nov	21 Dec	11 Jan	2 Feb	23 Feb	15 Mar
		102 ml in 65 L	98 ml in 62 L	135 ml in 80 L	117 ml in 75 L	107 ml in 72 L	111 ml in 75 L	111 ml in 75 L	118 ml in 80 L
ExpMD	21	12 Oct							
		555							
Cryptogran[2]	16 & 20	1 Dec	11 Feb						
		8221 L	8890 L						
Cryptogran + Isomate	14	Application rates and dates are as for Isomate and Cryptogran above							

[1]Application rate given in volume of product (in ml) per volume of water (in L) per ha.
[2]Cryptogran mixed at the registered rate of 10 ml + 250 ml molasses + 5 ml BreakThur per 100 L water.

One yellow delta trap, loaded with Lorelei pheromone dispenser, was hung in each treatment block on 12 October 2010. Traps were monitored weekly on the same day until 12 April 2011. Twelve data trees were marked in the centre of each treatment block. Each week from 4 January to 12 April 2011, all fruit which had fallen underneath each data tree was collected and taken back to the laboratory. There fruit were dissected and assessed for cause of fruit drop, with particular attention being paid to FCM or signs of FCM infestation.

2013/14 field trial:

A block of around 15 ha on Vleiview Farm, Dunbrody Estates, in the Sundays River Valley, was selected for a field trial. The block consisted of a variety of late navel orange varieties (Table 3.2.2.3). All trees were planted in 2003 at a spacing of 6 m x 3 m (rows x trees) and were of almost identical size. Each orchard was around 3 ha in size, consisting of two equal halves (A and B – 1.5 ha each) separated by a windbreak.

Six different treatments were applied and an untreated control was also used (Table 3.2.2.3). Half of the Last Call FCM block (17B) was also sprayed with Cryptogran. The Checkmate applied in the Isomate block (19) was not applied according to registration. Checkmate is registered to be applied every 21-28 days, not earlier than November and not more than 6 sprays per season. This mating disruption treatment can therefore be considered as an “overkill” and was included in order to determine whether FCM infestation could be eliminated, regardless of the affordability or practicality of the treatments.

Table. 3.2.2.3. Mating disruption, Attract and Kill and Cryptogran treatments applied in a field trial on Vleiview Farm (Dunbrody Estates) in the 2012/13 season.

Treatment	Orchard	Navel orange variety	Application details	Application date/s
Untreated	15A & B	Lane Late	-	-
Last Call	17A	Autumn Gold	3000 droplets/ha/application	6 applications, 5-weekly, between 11 Oct 2012 and 27 Mar 2013
Last call	17B	Autumn Gold	As for Last Call above	
+Cryptogran			[1]Full cover spray applied by farmer	19 Dec 2102 and 21 Feb 2013
Isomate	19A & B	Glenora	800 dispensers/ha	Isomate 11 Oct 2012 (500/ha) and 16 Jan 2013 (300/ha)
+ Checkmate			110 ml in 43 L/ha/	13 applications, fortnightly

			application	from 12 Oct to 25 Mar
Isomate	21A & B	Powell	As for Isomate above	
ExpMD	24A	Powell		14 Nov 2012
ExpAK	24B	Powell		14 Nov 2012

[1]Cryptogran mixed at the registered rate of 10 ml + 250 ml molasses + 5 ml BreakThur per 100 L water.

One yellow delta trap, loaded with Lorelei pheromone dispenser, was hung in each treatment block on 31 October 2012. Traps were monitored weekly on the same day and will continue to be monitored until harvest in 2013. Twelve data trees were marked in the centre of each treatment block. Where the block consisted of two equal halves of 1.5 ha each, 6 data trees were used in the middle of each of these. Each week from 2 January 2013, all fruit which had fallen underneath each data tree was collected and taken back to the laboratory. There fruit were dissected and assessed for cause of fruit drop, with particular attention being paid to FCM or signs of FCM infestation.

Comparison of different pheromone blends

Three and possibly four different isomers have been identified as components in the FCM female sex pheromone. Different combinations of these are used in various commercial pheromone products used for mating disruption or monitoring (Table 3.2.2.4). These different blends were prepared and separate groups of 24 h old male FCM adults exposed to each of them. Five male moths were placed into a curry tub with 1.5 ml of pheromone blend (in an open top Eppindorff vial) for 5 min, with the Curry tub lid sealed. Thereafter, 15 males (3 replicates of 5 males) were used from each treatment for each olfactometer trial.

Table 3.2.2.4. Proportions of isomers (in mg) used in pheromone blends for testing FCM mating disruption in the laboratory.

	Commercial product on which pheromone blend is based			
Pheromone Isomer	**Lorelei**	**Isomate 1**	**Isomate 2**	**Checkmate**
E7 – 12 Ac (dodecenyl acetate)	50.00	69.31	69.31	-
E8 - 12 Ac (dodecenyl acetate)	41.67	29.70	29.70	77.78
Z8 – 12 Ac (dodecenol)	8.33	0.99	-	22.22
E8 – 12 Ac (dodecenol)			0.99	-

Firstly, Y-tube (10 mm diameter) experiments were run in order to determine if the males' ability to locate a virgin female, when given a choice between a female and an empty vial, was disrupted and whether there was any difference in this disruption between males which had been exposed to different pheromone blends (Figure 3.2.2.1). Control males (not exposed to the mating disruptors) were included. The Y-tubes containing the insects were then placed under a laminar flow unit such that the air flow through the tube was approximately 0.44m/s. This allowed the female pheromones to disperse and reach the male. The experiment was run in the dark and moths were observed using a red light. Humidity and temperature were not controlled.

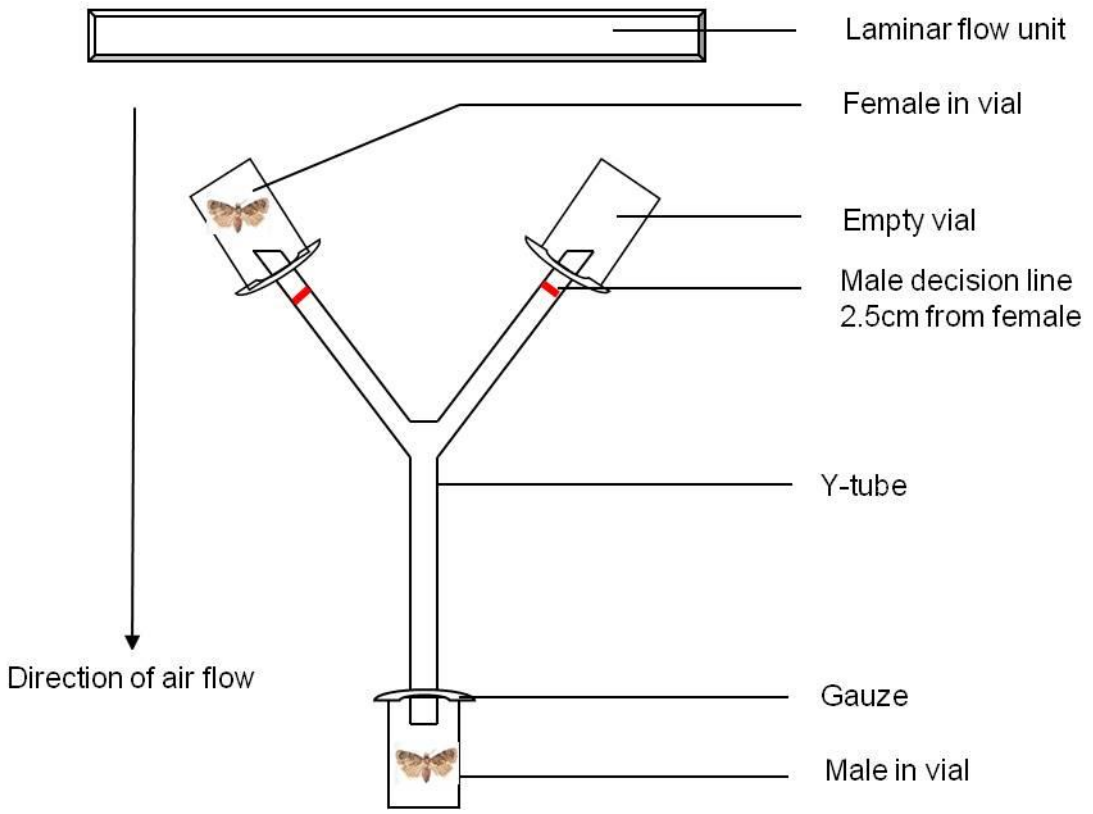

Figure 3.2.2.1. Y-tube with the position of male and female.

The first trial did not produce results, as moths did not appear to make decisions. A second experiment was therefore run, this time placing the male moth inside the Y-tube, rather than in a vial, and preventing escape by taping gauze onto the end of the tube (Figure. 3.2.2.2). Constant temperature and humidity were also maintained. This was only done with untreated moths, as it was necessary to first develop a suitable technique before treating moths.

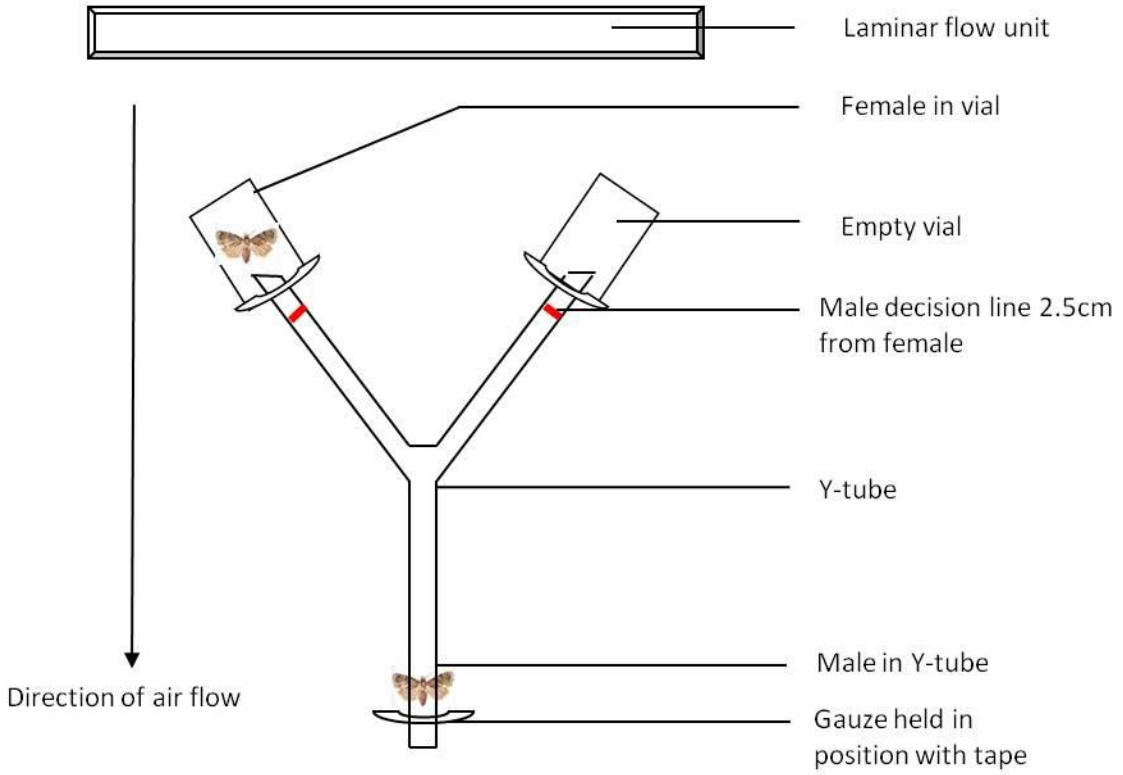

Figure 3.2.2.2. Y-tube with the position of male and female.

The second trial was also not successful. As these first two experiments did not work, a slightly different design was employed. A plastic pipe (12mm in diameter) was used instead of the Y-tube (Figure 3.2.2.3). Here the male simply had to move to the female, as choice was removed as a factor. The decision line was also moved much closer to the male (10cm away). Again only untreated moths (n=15) were used.

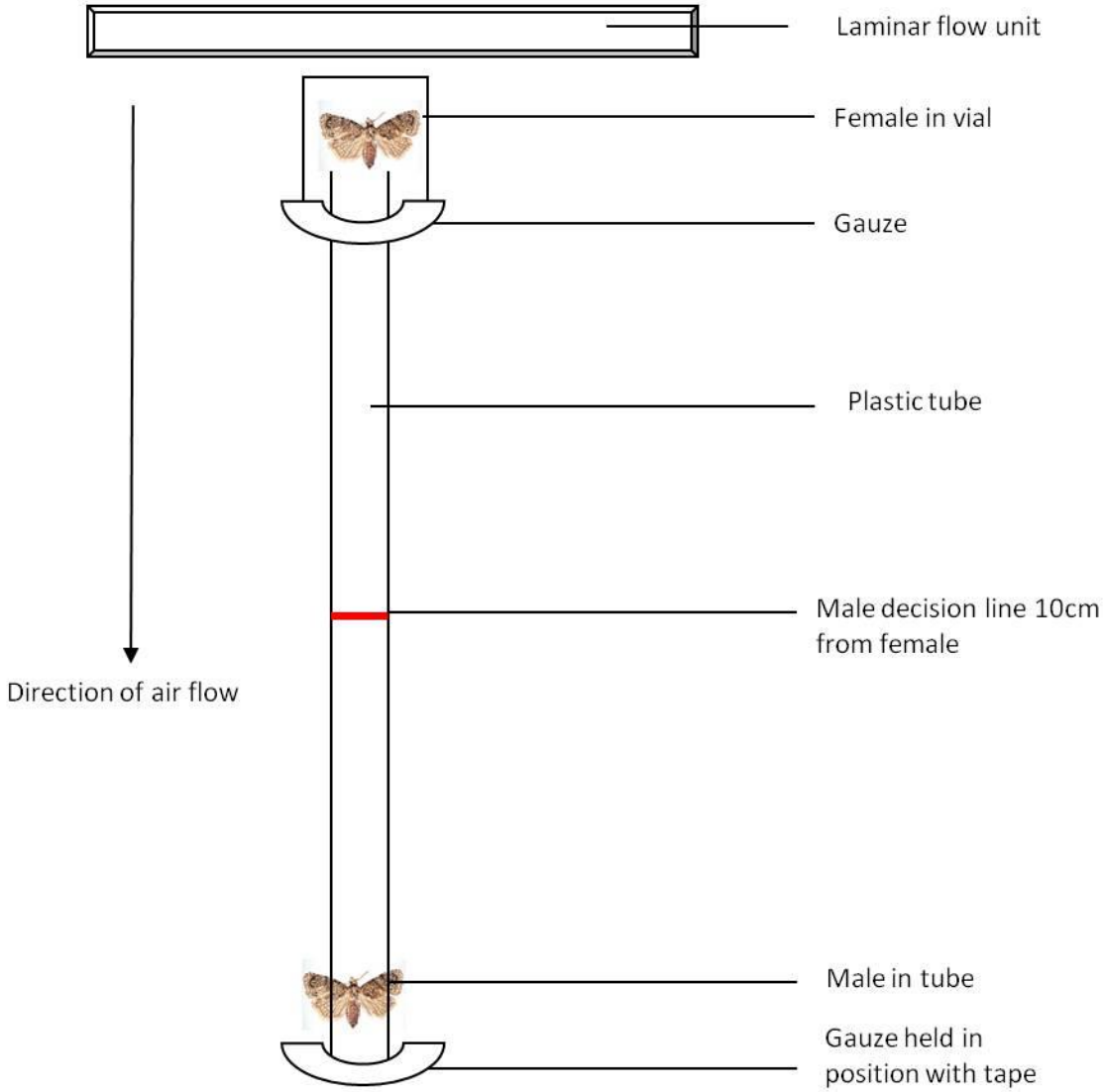

Figure 3.2.2.3. Tube with the position of male and female.

The same variable results were observed and a fourth design was employed. (Fig. 3.2.2.4).

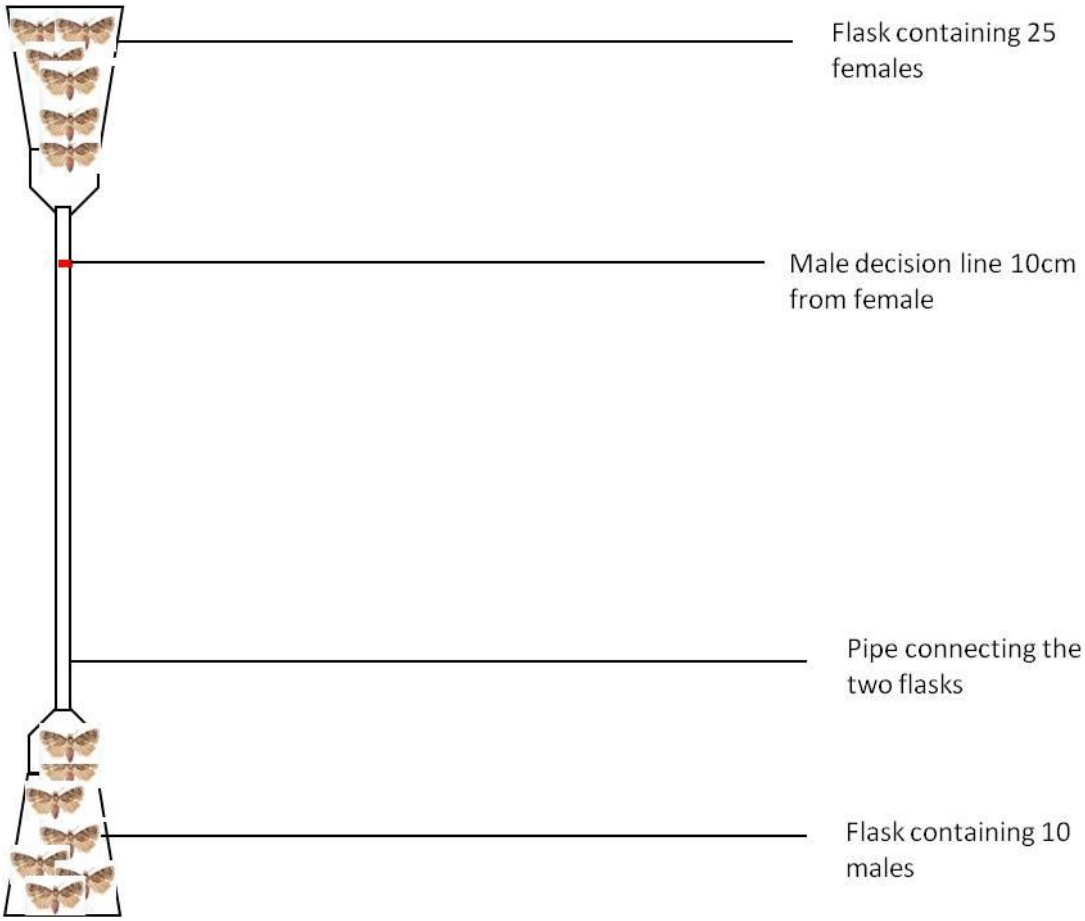

Figure 3.2.2.4. Flasks used to house males and females.

Results and discussion

Rate of release

In the first trial, temperature and humidity in the incubator were measured half-hourly, using a Hobo data logger. Unfortunately, the computer on which the data was downloaded crashed. Consequently, the data were lost.

The formulation of paraffinic wax, emulsifier, vitamin E, soya oil and water was unsuccessful. Release rate tests were therefore not conducted with such a dispenser. From 23 January to 7 April 2010, the safety gel dispenser appeared not to release any pheromone, as the pheromone-loaded dispensers gained an average of 0.272 g relative to the unloaded dispensers. During the same period, the wax and gel dispensers lost an average of 0.012 g and the wax dispensers lost an average of 0.008 g. The membrane dispensers lost an average of 0.047 g (Fig. 3.2.2.5). Release of pheromone from the two dispensers containing wax was considered to be too low to be useful in the field. Therefore the only one of these dispensers to be tested again in their exact form in a second trial was the semi-permeable membrane dispenser.

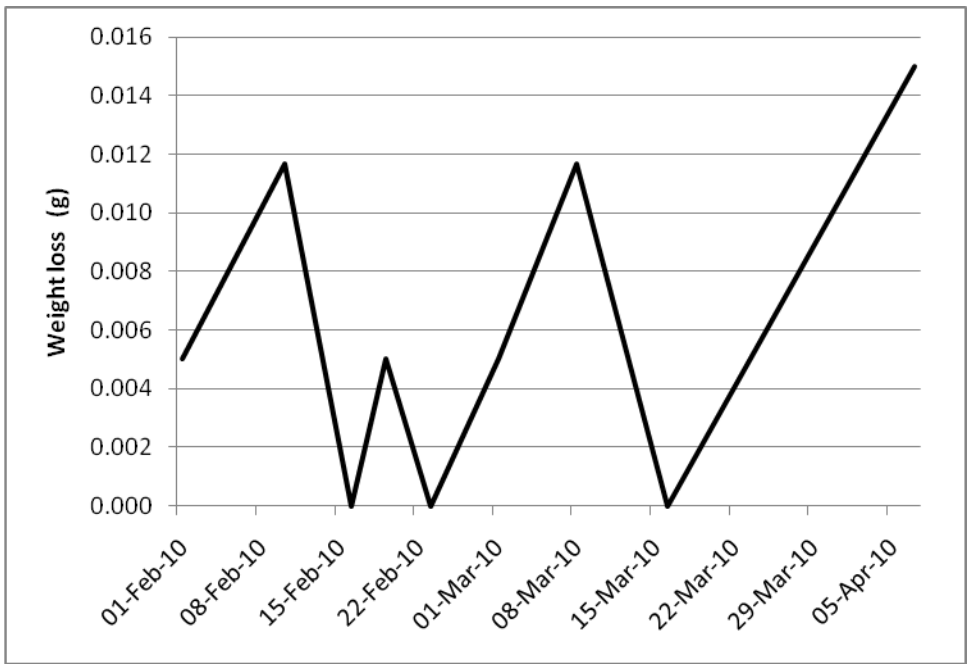

Figure 3.2.8.5 Weight loss of pheromone-filled semi-permeable membrane dispenser relative to empty dispenser over time, representative of rate of pheromone release (in a laboratory incubator).

The second trial was run from 3 May to 24 August 2010. Temperature and humidity were again recorded half-hourly, using a Hobo data logger. Unfortunately, the data logger battery ran flat on 25 July, a month before termination of the trial. Up to this point, mean temperature recorded was 27.3ºC and mean humidity (RH) was 24.0% (Fig. 3.2.2.6).

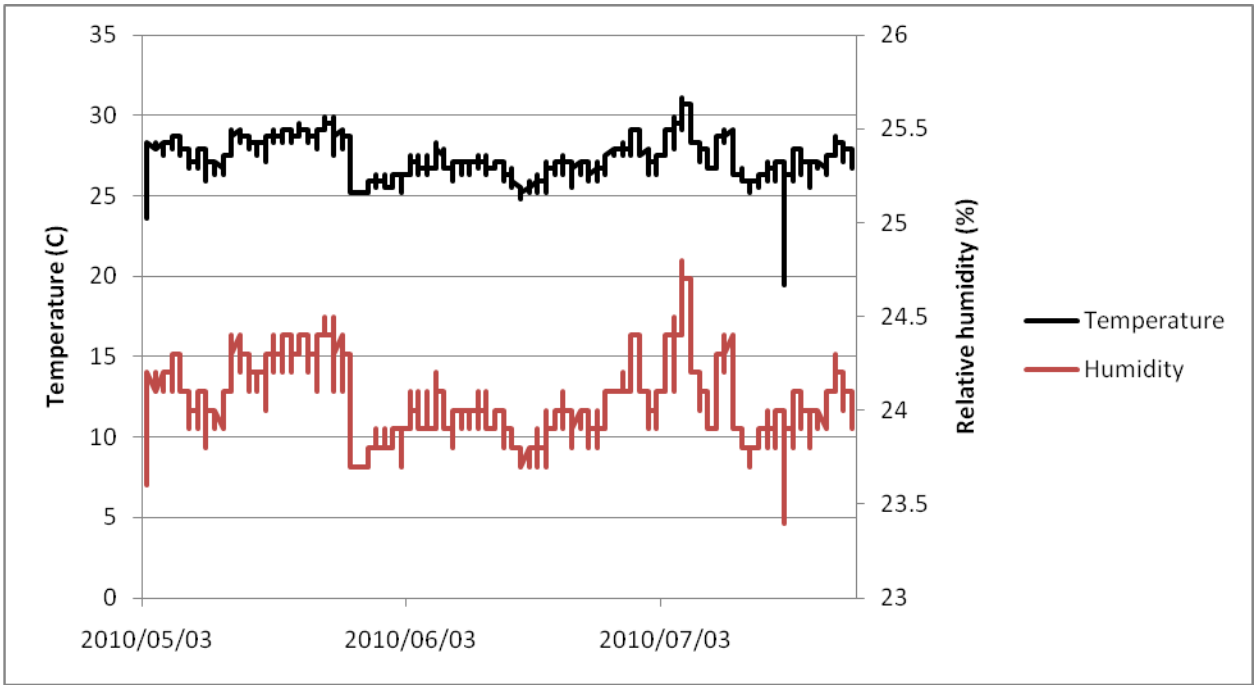

Figure 3.2.8.6 Temperature and humidity readings (taken half-hourly) in an incubator in a pheromone dispenser release trial, measured from 3 May to 25 July 2010.

During the first five weeks of evaluation, weight loss of (or from) a number of the dispensers was erratic. The first five weeks' data has therefore been excluded from the graphic display depicting pheromone release rate (Fig. 3.2.2.7). In this trial, weight loss of wax discs was very slow. Weight loss from the water-holding polymer dispenser was slightly faster and there was little difference in release rate from the membrane dispenser, Checkmate and unformulated pheromone. Although relative weight loss by the polymer dispensers appeared fairly consistent, the polymer desiccated rapidly and appeared to have little longevity as a formulation ingredient. Despite there being little difference in weight loss rate between unformulated pheromone and the membrane dispenser, it was decided to test the latter in the field, as release rate was

relatively constant. Although the membrane did not seem to slow the release rate, it would still be able to protect the pheromone from direct contact with the elements eg rain and wind. As the temperature in the incubator was relatively constant, it was also not clear whether the membrane could mitigate the rate of pheromone release at higher temperatures. Consistency of pheromone release rate is also probably not as important for mating disruption as it is for a monitoring device.

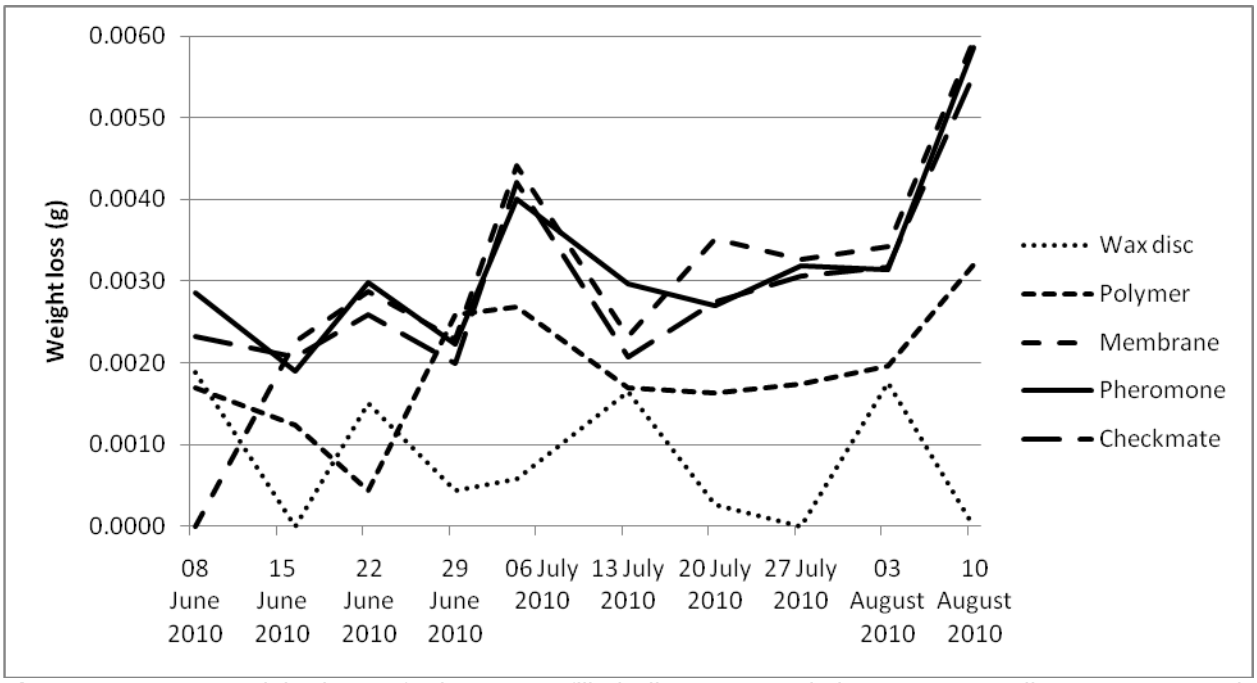

Figure 3.2.2.7. Weight loss of pheromone-filled dispensers relative to empty dispensers over time, representative of rate of pheromone release (29ºC in a laboratory incubator).

In the third release rate trial, the incubator temperature was relatively constant, with outer extremes of 23.63ºC and 29.90ºC a mean temperature of 27.27ºC ± 0.021 (mean ± SE) (Fig. 3.2.2.8). There was a slight rise in temperature early in April and a slight dip in temperature shortly after mid-April. The two Isomate dispenser types appeared to respond to this but Exp-MD did not (Fig. 3.2.2.9). Although a constant rate of release is important for the accuracy of a pheromone dispenser used for monitoring purposes, this is probably not the case for a mating disruption dispenser, as long as release rate remains above a critical minimum level.

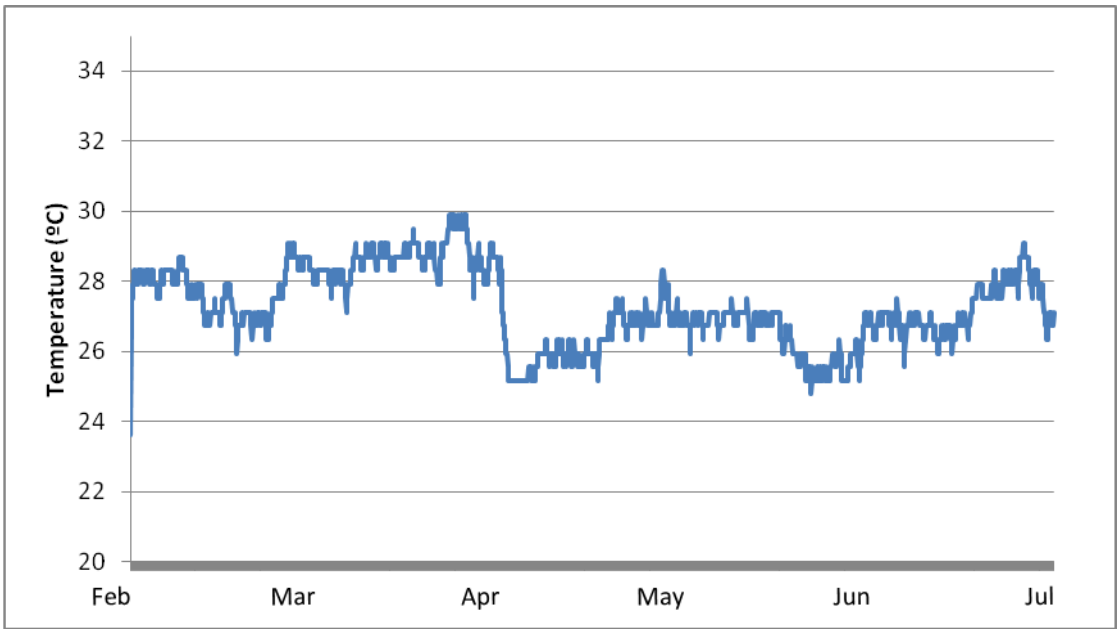

Figure 3.2.2.8. Incubator temperature recorded half-hourly for the full duration of the trial to measure rate of pheromone release from three different mating disruption dispensers.

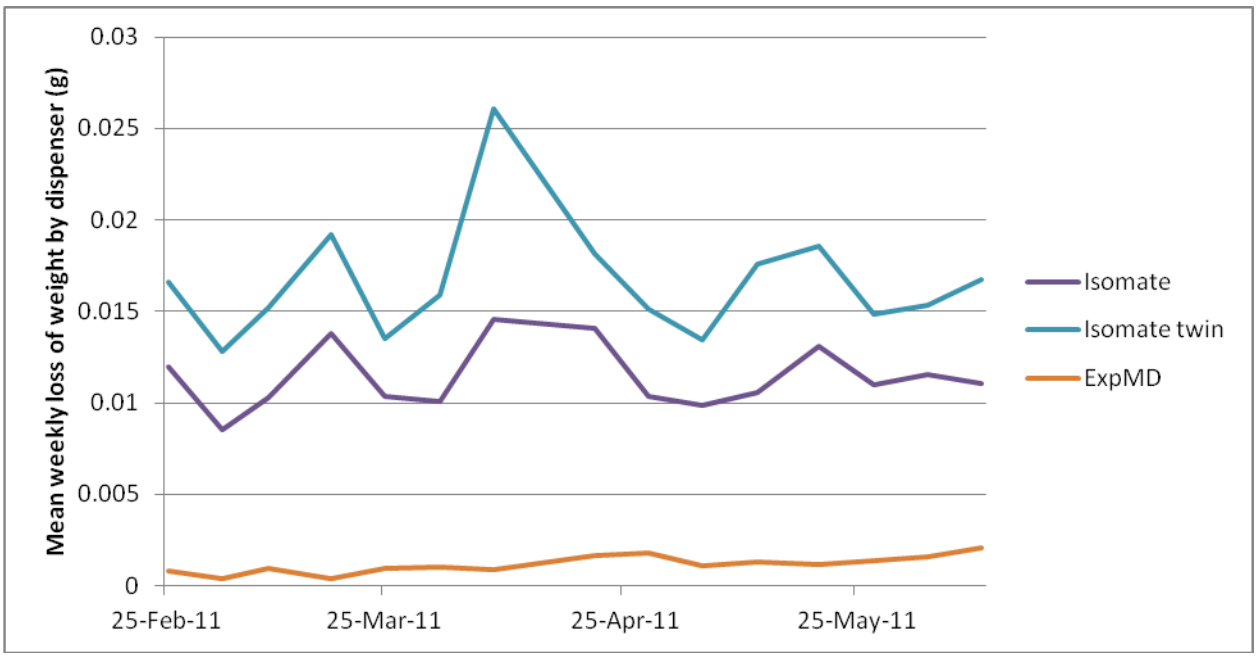

Figure 3.2.2.9. Rate of pheromone release from three different mating disruption dispensers.

Release rate from Exp MD appeared to be more consistent than that from the two Isomate dispenser types (Fig. 3.2.2.9). There was a peak in rate of release between March and April, which can be related to a peak in the temperature recorded during this time (Fig. 3.2.2.8). However, although consistency in rate of release is important for a dispenser used for monitoring purposes, it is not as important for a dispenser used for mating disruption, as long as release rate is above a certain threshold required to achieve the purpose of disrupting mating. Release rate from the Isomate and Isomate Twintube dispensers were respectively 10 and 15 times that of the release rate from the experimental dispenser (Fig. 3.2.2.9 and Table 3.2.2.5). This would explain why five times as many moths were caught in traps loaded with Exp MD compared to traps loaded with Isomate (Moore & Kirkman, 2011). Even from one dispenser, loaded in a delta trap, there must have been some disruptive effect, counter-acting the attractiveness of the dispenser, and thus allowing the ExpMD dispenser to attract more moths, despite the far lower release rate. However, despite this dramatic difference in release rate, a field trial showed only a negligible difference in catches of male moths (10 versus 8 over a 6 month period for ExpMD and Isomate, respectively) and a statistically insignificant difference in fruit infestation (0.81 versus 0.56 fruit infested per tree per week for ExpMD and Isomate, respectively) in blocks treated with the two dispenser types (Moore & Kirkman, 2011).

Table 3.2.2.5. Pheromone release rate from different mating disruption dispensers.

MD dispenser type	Total mass or pheromone released over 17 weeks (g)	Mean rate of pheromone released per week (g)
Isomate	0.1958	0.0115
Isomate Twin-tube	0.2910	0.0171
Exp MD	0.0195	0.0011

The total mass of pheromone released by the two Isomate dispensers over a 6 month period was almost 0.2 and 0.3 g each (Table 3.2.2.5). The ExpMD dispensers were only loaded with a total of 0.1 g each. It is possible that to achieve efficacy equivalent to that of Isomate, it will be necessary to load a greater mass (volume) of pheromone per dispenser. Another important factor is the greater surface area of the Isomate twist-tie compared to the vial-shape of ExpMD. A greater porous surface area will facilitate a higher rate of release.

Another means to try and improve efficacy would be to investigate different pheromone blends. If the best blend can be identified, this may be equally or more efficacious, even if used at a lower volume per dispenser. Currently different blends (ratios of different isomers) are used in different commercial products (Table 3.2.2.6).

Table 3.2.2.6. Different pheromone blends in different FCM monitoring and mating disruption products.

Isomer	Ratio of pheromone isomers in product*				
	Lorelei	Isomate1	Isomate2	Checkmate	Pherolure
E7 – 12 Ac (dodecenyl acetate)	6	65	65		Undisclosed
E8 - 12 Ac (dodecenyl acetate)	5	25	25	7	
Z8 – 12 Ac (dodecenol)	1	1		2	
E8 – 12 Ac (dodecenol)	0		1		

*Both mating disruption and monitoring products.

Attractiveness

A total of 20 moths were caught in six control traps (not loaded with any pheromone dispenser), in orchard 54 (Newhall Navels) on Far Away Farm in Sundays River Valley, over a 20-week period (Fig. 3.2.2.10). Comparatively, 187 moths were caught in Isomate loaded traps and 933 moths were caught in traps loaded with ExpMD dispensers. Despite ExpMD catching dramatically more moths than Isomate, this does not indicate that ExpMD is a more effective dispenser. Although attraction of moths is the purpose of a pheromone dispenser designed for monitoring, this is not the case with mating disruption. A mating disruption dispenser may certainly attract moths. However, mating disruption may be achieved through means other than attraction, such as some form of confusion. Nevertheless, it was good to see (through moth catches), that ExpMD continued to release pheromone at a detectable rate for at least 20 weeks. It was also good to see that there was a similar trend in catches between Isomate and ExpMD loaded traps.

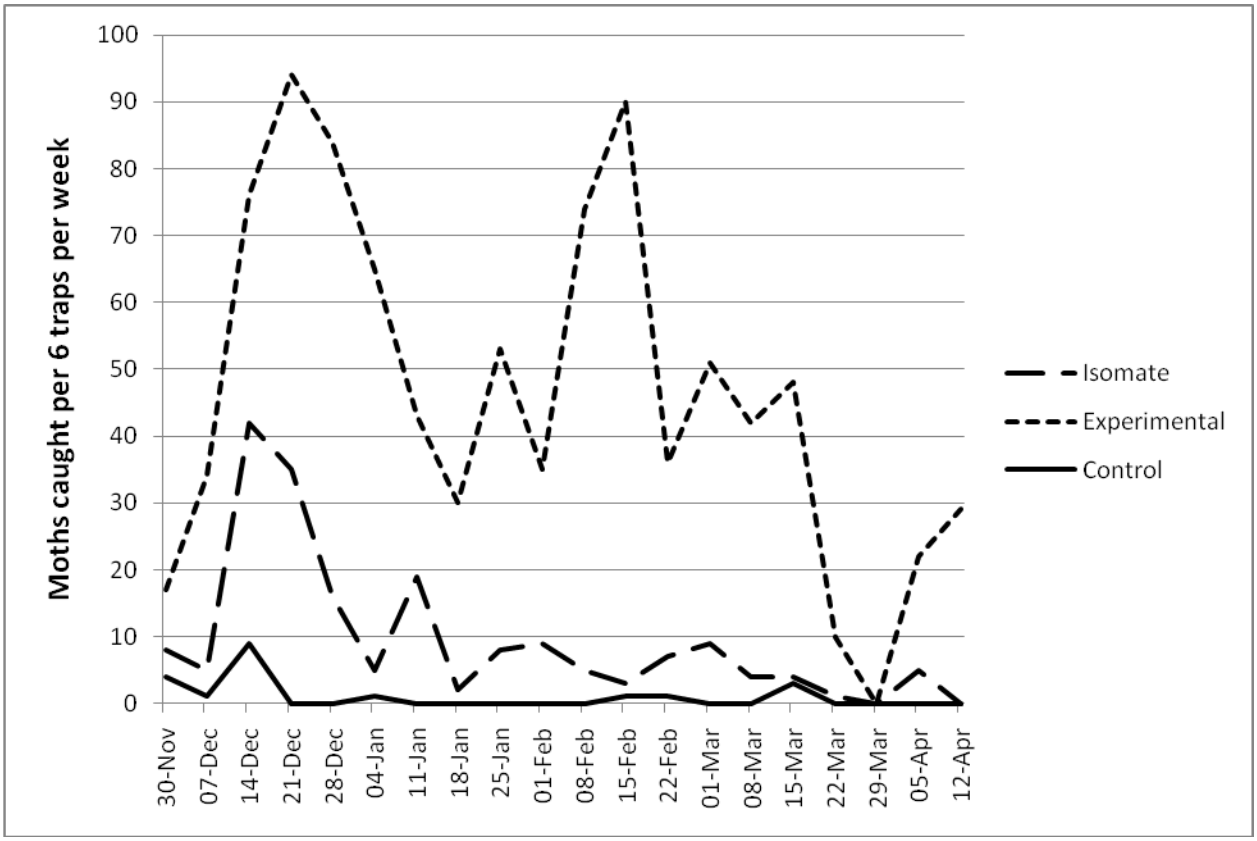

Figure 3.2.2.10 Male FCM caught per set of six evenly spaced yellow delta traps per pheromone dispenser type, in orchard 54 (Newhall Navel orange trees) on Far Away Farm, Sundays River Valley, 2010/11.

Efficacy

2010/11 field trial:

If effective, mating disruption should cause trap shutdown. One would therefore expect to catch very few moths in pheromone traps placed within areas under mating disruption. Conversely, virus (or any other spray) has no such effect. The comparison of moth numbers between orchards under mating disruption and

those sprayed with Cryptogran therefore has no bearing on the comparative efficacy of the treatments. However, a comparison of moths caught in the different mating disruption treatments is meaningful. Very few moths were caught in traps in the Isomate, Isomate Twintube and ExpMD blocks during the full duration of the trial (Fig 3.2.2.11). Moths were caught noticeably more frequently in the Checkmate block.

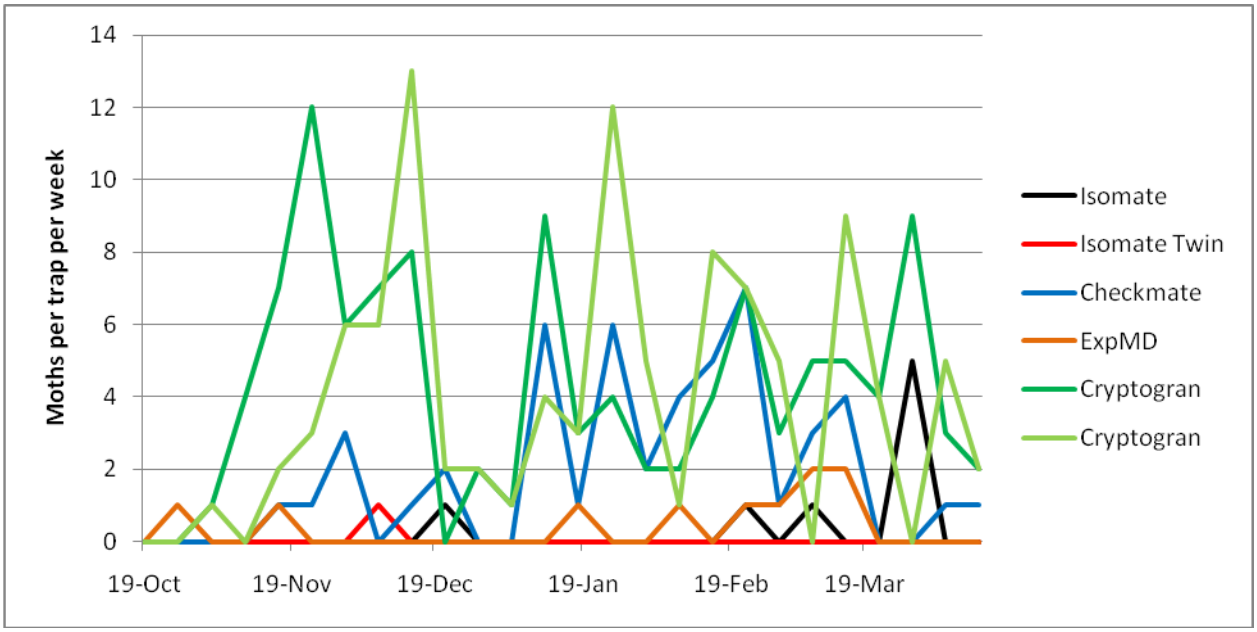

Figure 3.2.2.11. Adult male FCM caught per pheromone trap per week in the different treatment blocks.

Overall, fewest moths were caught in the Isomate Twintube block, followed by the Isomate block, the ExpMD block, the Checkmate block and the two Cryptogran blocks (Table 3.2.2.7). It is surprising that although only one moth was caught in the Isomate Twintube block throughout the entire duration of the trial, more infested fruit were recorded in this block than in any of the other treatments. It is therefore clear that trap catches alone are not an adequate measurement of the efficacy of a mating disruption treatment.

Only 10 moths were caught in the ExpMD treatment (Table 3.2.2.7). This was encouraging, as this was similarly low in comparison with Isomate. Of concern was the relatively high number of moths caught in the Checkmate treatment, despite the product being reapplied every 3-4 weeks.

Table 3.2.2.7. Total adult male FCM caught per pheromone trap for each treatment over the full duration of the trial (19 October 2010 to 12 April 2011).

Treatment	Orchard	Total moths caught per trap
Isomate	15	8
Isomate Twintube	23&24	1
Checkmate	22	49
ExpMD	21	10
Cryptogran	16	101
Cryptogran	20	110

Initially, fruit infestation was at a relatively low level, dropping to its lowest level during the month of January (Fig. 3.2.8.12). At this stage there was little difference in fruit infestation between treatments, with infestation slightly higher for the ExpMD and Isomate Twintube treatments. Infestation was initially lowest in the Isomate-Cryptogran block. FCM infestation peaked on 15 February, most conspicuously for Isomate Twintube, and remained relatively high for the remainder of the trial period (Fig. 3.2.8.12). As a result of this, the Twintube orchards (23 and 24) were sprayed out with Cryptogran on 21 February 2011. During that week, it was clear that infestation had increased to an disconcertingly high level throughout the entire trial site. Consequently, the remainder of the trial area was sprayed out with Cryptogran on 7 & 8 March 2010.

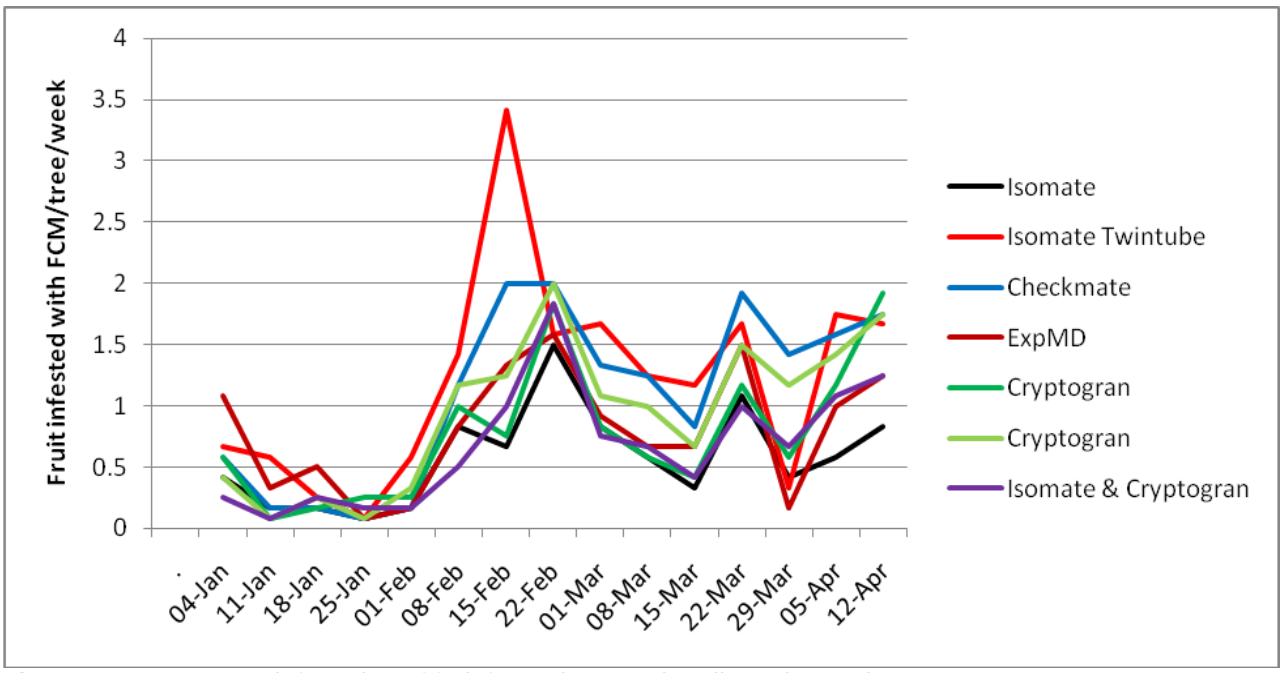

Figure 3.2.8.12. FCM infestation of fruit for various mating disruption and Cryptogran treatments.

The most effective treatment was Isomate, followed by Isomate and Cryptogran and then by Cryptogran alone (Table 3.2.8.8). However, there was no significant difference between most of the treatments. The only meaningful differences were that Isomate was significantly better than Isomate Twintube, Checkmate and one of the Cryptogran blocks. The initial superiority of the Isomate-Cryptogran treatment (Fig. 3.2.8.12) was not maintained. This must have been because this treatment was applied in the only orchard which consisted of early (Newhall) navels. Newhall navels are generally recognised as the most FCM-susceptible navel orange variety. As the Newhall is an early maturing navel variety, it will certainly increase in susceptibility to FCM before the late navels do i.e. all of the other trial blocks. This was therefore not entirely a fair comparison.

Table 3.2.8.8. Mean FCM infestation of fruit for each treatment over the full duration of the trial (4 January to 12 April 2011).

Treatment	Orchard	Mean fruit infested with FCM/tree/week*
Isomate	15	0.56a ± 0.06
Isomate Twintube	23&24	1.20d ± 0.0.06
Checkmate	22	1.08cd ± 0.10
ExpMD	21	0.81abc ± 0.09
Cryptogran	16	0.97cd ± 0.07
Cryptogran	20	0.78abc ± 0.08
Isomate & Cryptogran	14	0.66ab ± 0.09

*Bonferroni LSD multiple range test (α=0.05).

It was also surprising that Isomate Twintube performed so much worse than did Isomate or any of the other treatments for that matter (Table 3.2.8.8). This led to us questioning the uniformity of FCM pressure throughout the trial block. This question was supported by the difference in FCM infestation levels in the two Cryptogran blocks. The more heavily infested Cryptogran orchard (orchard 16) was on the northern extremity of the trial site, along with the Isomate Twintube orchards. In order to test this theory, infestation in fruit from six data trees in orchard 19 was examined from 1 March to 12 April. Orchard 19 did not fall within the trial, but was on the northernmost tip of the trial block. This orchard had been treated with Isomate by the farmer, according to the same criteria as were applied with the Isomate application in the trial. Mean FCM infestation in orchard 19 over this period was 1.30 infested fruit per tree per week. Comparatively, FCM infestation in orchard 15 (the trial Isomate treatment) was 0.67 i.e. almost half that in orchard 19, indicating that there was indeed uneven FCM activity through the trial site. Mean FCM infestation in orchard 23 and 24 (Isomate Twintube) over this time was 1.36 infested fruit per tree per week, which was very similar

to orchard 19 (Isomate). The comparison between Isomate and Isomate Twintube in this trial might therefore not be sufficiently reliable and should be repeated in another trial.

2013/14 field trial:

Any product which is effective, if applied over a relatively large area (more than 1 ha), should reduce moth catches, simply due to its general suppression of the FCM population. However, if mating disruption is effective, virtually no moths should be caught, even before population suppression occurs. This is because moths should not be able to orientate themselves on the trap, due to the saturation of the the general environment with pheromone. Last Call and Last Call plus Cryptogran appeared to suppress trap catches for most of the period during which monitoring was conducted (Fig. 3.2.2.13). However, a spike in catches during the last couple of weeks, virtually nullified this difference, when one considered the total moths caught in the season (Table 3.2.2.9). As one might expect, no moths whatsoever were caught in the mating disruption overkill treatment (Isomate plus Checkmate). Notably fewer moths were also caught in the Isomate (alone) block and the two experimental product blocks.

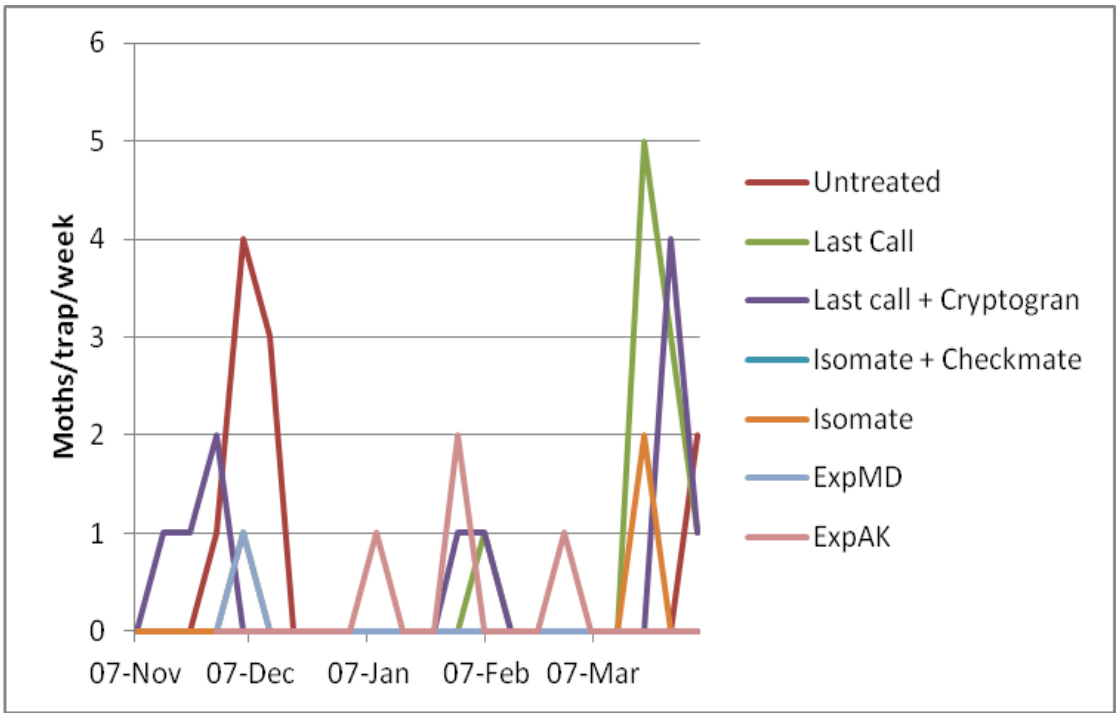

Figure 3.2.2.13. Adult male FCM caught per pheromone trap per week in the different treatment blocks.

Table 3.2.2.9. Total adult male FCM caught per pheromone trap for each treatment over the full duration of the trial (7 November 2012 to 3 April 2013).

Treatment	Total moths caught per trap
Untreated	10
Last Call	11
Last Call + Cryptogran	11
Isomate + Checkmate	0
Isomate	2
ExpMD	1
ExpAK	4

Of course, the most important thing is for an effective product to reduce FCM infestation, rather than just moth catches. When monitoring of FCM was initiated early in January, a high level of infestation was recorded (Fig. 3.2.2.14), probably due to a generational peak or the tail-end of such. The next peak was observed on 20 February. Over the full period monitored, FCM infestation was not high, averaging 0.11 infested fruit per tree per week in the untreated control (Table 3.2.2.10). Last Call was incapable of reducing infestation and in fact strangely, FCM infestation was almost four times higher in the Last Call-treated orchard than the untreated orchard (in synchrony with the moth catches). This must have been indicative of

an FCM "hot-spot", as even though the addition of Cryptogran reduced infestation by 61%, infestation remained higher than the untreated control. The most effective treatments were Isomate plus Checkmate and ExpMD. However, one must bear in mind the heterogeneity of FCM pressure within a trial site, particularly when one is compelled to use large blocks as is the case in a mating disruption and attract and kill trial.

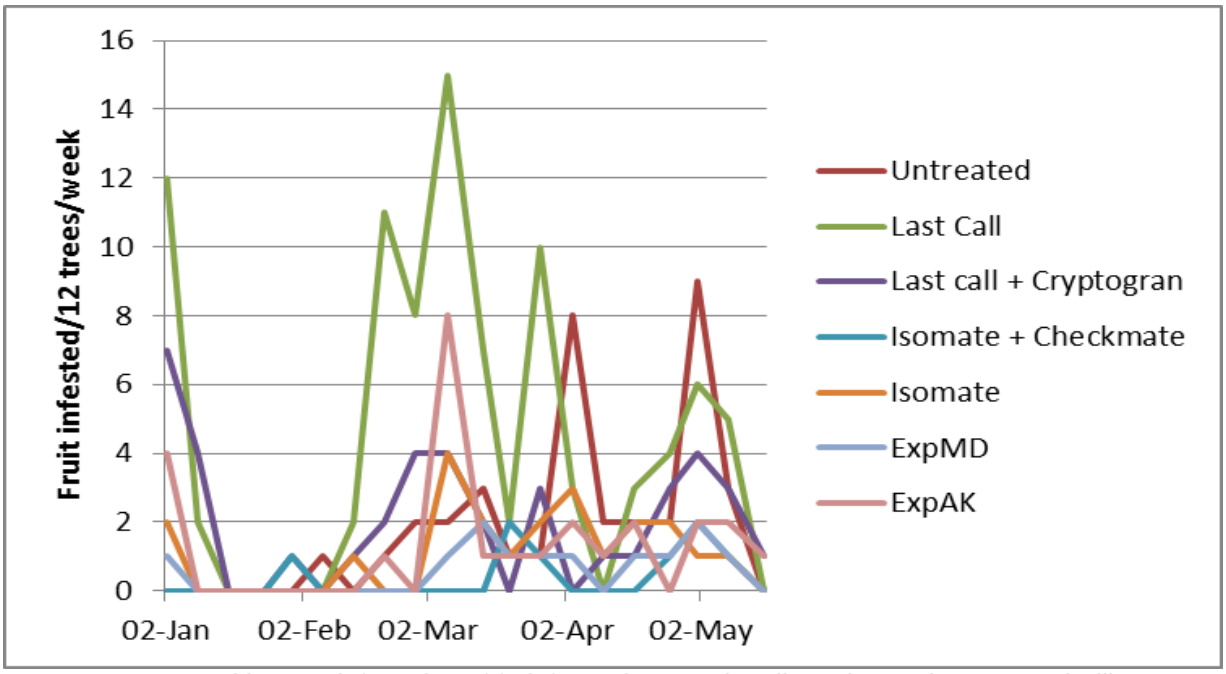

Figure 3.2.2.14. Weekly FCM infestation of fruit for various mating disruption and Attract and Kill treatments.

Table 3.2.2.10. Mean FCM infestation of fruit for each treatment over the full duration of the trial (2 January to 16 May 2013).

Treatment	Average fruit infested with FCM/tree/week
Untreated	0.15
Last Call	0.37
Last Call + Cryptogran	0.17
Isomate + Checkmate	0.03
Isomate	0.09
ExpMD	0.05
ExpAK	0.11

Comparison of different pheromone blends

In the first Y-tube olfactometer trial, the control males did not make choices in any sort of pattern, they were just as variable at deciding between the female and the empty vial as the males that were treated. Time ranged from 3 seconds to 45 minutes for the control and the treated moths to make a decision, but at times no decision was made after an hour. Males also got stuck in the vial and could not find the opening to the Y-tube. Variable temperature and humidity might also have been a problem.

The second trial with the Y-tube olfactometer showed the same sort of variability observed in the first trial and was therefore terminated after 1 hour.

Even the two no-choice trials did not produce results. It was concluded that the final design did not work as there was no airflow through the flasks. Some males stayed in the flask, others moved towards the line but stopped before crossing it. Only 3 out of the 10 males moved over the decision line.

This aspect of the project has been terminated as the experimental design failed numerous times, with no alterations having any positive effect and therefore no meaningful data was collected.

Conclusion

A rudimentary, yet appropriate dispenser was developed for use as a mating disruption tool for FCM. However, as the release rate from this dispenser was lower than that for the commercial product, Isomate, efficacy appeared to be slightly less than that of Isomate. Ultimately, the most effective pheromone-based control measure for FCM appeared to be a combination of Isomate and Checkmate, applied very frequently and thus termed mating disruption "overkill". This approach is currently being investigated further in a new and separate project. No further work is planned on this project.

Technology Transfer

None yet.

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Witzgall, P, Stelinski, L, Gut, L, & Thomson, D. 2008. Codling moth management and Chemical ecology. *Annual Review of Entomology*. 53:503-522.

3.2.3 PROGRESS REPORT: Development of mechanisms for the postharvest detection of cryptic pests in citrus fruit

Project 976 (April 2010 – March 2015) BY Wayne Kirkman & Sean Moore (CRI)

Summary

The objective of this study is to investigate post-harvest techniques to detect cryptic pests in citrus fruit. In previous studies using the Nuclear Energy Corporation of South Africa's (NECSA) Microfocus X-ray unit, the quality of the images were optimised. Subsequent trials have shown that abbreviated cold treatment kills most of the smaller larvae, so it might not be necessary to detect the very small areas of damage caused by these larvae. Emphasis was placed on speed of the scans. Time per scan was reduced by reducing the readout rate (binning), number of projections, energy and exposure time. Various combinations of these variables were tested, and image quality was measured by Signal to Noise Ratio, Contrast Ratio and Michelson's Contrast Ratio. In a study conducted on Satsuma mandarins, 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 8, 12, 14 and 16 days after infestation, using the new settings. Further refining of the technique resulted in a scan lasting only 34 seconds. The efficacy of this scan will be evaluated. Imaging algorithms are being developed in collaboration with NECSA, Maf-Roda and Greefa. Maf-Roda and Greefa have made sorting units and expertise available to CRI. Claims were made that an automatic sorting unit could detect FCM in fruit. The unit was tested. Results were disappointing, but the technology looks promising. Further trials will be conducted in 2014. Collaborative studies continued with Tastetech to investigate detection of known compounds using near infrared spectroscopy.

Opsomming

Die doel van die studie is om tegnieke te ondersoek wat peste binne sitrusvrugte na-oes kan opspoor. In vorige proewe met die Mikrofokus X-straaleenheid van die Nuclear Energy Corporation of South Africa (NECSA) is beeldkwaliteit geoptimiseer. Onlangse proewe het getoon dat verkorte kouebehandeling meeste van die kleiner larwes doodmaak. Dit is dus moontlik nie nodig om die klein penetrasies wat deur hulle veroorsaak word, op te spoor nie. Daar is op die spoed van skanderings gefokus. Die tyd per skandering is verkort deur die binning, aantal projeksies, energie en die tydperk van blootstelling te verminder. Verskeie kombinasies van hierdie veranderlikes is getoets, en beeldkwaliteit is gemeet met behulp van Signal to Noise Ratio, Contrast Ratio en Michelson se Contrast Ratio. In ŉ studie wat op Satsuma mandaryne gedoen is, is skanderingstyd van 35 minute tot 1 minuut 26 sekondes verminder. Met die nuwe verstellings is een honderd persent van VKM-penetrasies is opgespoor op vrugte 8, 12, 14 en 18 dae na besmetting. Die tegniek is verder verfyn, wat skanderingstyd tot 34 sekondes verminder het. The effektiwiteit van dié skandering sal geevalueer word. Beeld algoritmes word in samewerking met NECSA, Maf-Roda en Greefa ontwikkel. Maf-Roda en Greefa het sorteer-eenhede en kundigheid aan CRI beskikbaar gestel. Verklarings is gemaak dat sekere outomatiese sorterings-eenhede VKM in vrugte kan opspoor. Die toerusting is getoets,

maar die resultate was swak, hoewel die tegnologie belowend lyk. Verdere toetse sal gedurende 2014 uitgevoer word. Samewerking tussen CRI en Tastetech word voortgesit om chemiese stowwe deur middel van naby-infrarooi spektroskopie op te spoor.

3.2.4 PROGRESS REPORT: Late season releases of *Trichogrammatoidea cryptophlebiae* for suppression of FCM

Project 1021 (Apr 2012 – March 2015) by Sean Moore, Wayne Kirkman (CRI), Wayne Mommsen, Lezel Beetge & Hannah Otto (Du Roi IPM)

Summary

Studies on the effectiveness of the FCM egg parasitoid, *Trichogrammatoidea cryptophlebiae*, were conducted a number of years ago, showing that FCM infestation could be reduced by up to 61%. However, this was a result of early season parasitoid releases. Parasitism by naturally occurring *T. cryptophlebiae* generally builds up from December and reaches a peak in January or February. Any releases at and shortly before this time gave negligible benefit over and above that of the naturally occurring parasitoids. Currently such early releases are near impossible in the industry, due to the application of a series of pesticides for control of thrips and other pests and diseases during the first half of the season. *Trichogrammatoidea cryptophlebiae* is very sensitive to many of these pesticides. Where a chemical orientated pest control programme is followed, an FCM parasitoid vacuum is often created, exacerbating this increase in FCM infestation shortly before harvest. This study aims to determine whether mid to late season releases would still be of benefit in reducing FCM infestation, even if the effect is only seen shortly before harvest. During the 2012/13 season parasitoids were released at four sites – all Navel oranges – at 100 000 parasitoids per hectare, as monthly releases of 25 000 parastioids from January to April. Thereafter, FCM presence (moths, eggs and larval infestation of fruit) and egg parasitism were evaluated weekly in release and comparable control blocks. At three out of the four trial sites, FCM levels were too low to obtain meaningful results. At the fourth site, FCM levels were very high. However, so too was natural parasitism, thus obscuring any impact which the released parasitoids might have had. The study was repeated during the 2013/14 season at four different sites in the same region. FCM egg parasitism and fruit infestation has been evaluated weekly since 7 February in release and control blocks at all sites. Only at one of the sites is parasitism markedly higher in the release block. To date there is little difference in FCM infestation between release and control blocks. Evaluations will continue until harvest.

Opsomming

Studies oor die doeltreffendheid van die VKM eier parasiet, *Trichogrammatoidea cryptophlebiae*, is ŉ paar jaar gelede uitgevoer en het gewys dat VKM besmetting met tot 61% verminder kon word. Hierdie is egter as gevolg van vroeë seisoen loslatings. Parasitisme deur *T. cryptophlebiae* wat natuurlik voorkom, bou gewoonlik van omtrent Desember op en bereik ŉ piek in getalle in Januarie of Februarie. Enige loslating op hierdie stadium en kort voor hierdie stadium het weglaatbaar min voordeel gegee oor dié van die parasiete wat natuurlik voorgekom het. Tans is sulke vroeë loslatings amper onmoontlik in die bedryf as gevolg van toediening van ŉ reeks plaagdoders vir beheer van blaaspootjie en ander plae en siektes geduurende die eerste helfte van die seisoen. *Trichogrammatoidea cryptophlebiae* is baie sensitief vir baie van hierdie plaagdoders. Waar ŉ chemies-gëorienteerde plaagbestrydingsprogram uitgevoer word, veroorsaak dit gereeld ŉ VKM-parasiet-vakuum, wat ŉ styging in VKM besmetting kort voor oes kan veroorsaak. Die doel van hierdie studie is om te bepaal of mid tot laat seisoen loslatings nogsteeds VKM besmetting sal verminder, selfs as die effek eers kort voor oes gesien word. Gedurende die 2012/13 seisoen is parasiete is by vier persele losgelaat – alles Nawellemoene – teen 100 000 parasiete per hektaar. Maandeliks van Januarie tot April is 25 000 parasiete per hektaar losgelaat. VKM teenwoordigheid (motte, eiers en larwe besmetting van vrugte) en eier parasitisme is daarna weekliks in loslatings blokke en vergelykbare onbehandelde kontrole blokke geëvalueer. By drie uit die vier proefpersele is VKM vlakke te laag om waardevolle resultate te kry. By die vierde perseel is VKM vlakke baie hoog. Natuurlike parasitisme was egter ook hoog, en het dus enige impak wat die losgelate parasiet kon gehad het verduister. The study was repeated during the 2013/14 season at four different sites in the same region. VKM eierparasitisme en vrugbesmetting is vanaf 7 Februarie ontleed in albei loslatings en kontrole blokke by alle persele. Net by een van die persele is parasitisme beduidend hoër in die loslatingsblok. Op hierdie stadium is daar min verskil in VKM besmetting tussen loslatings en kontrole blokke. Evaluasies sal tot oestyd voortduur.

3.2.5 **FINAL REPORT: Post-harvest Phytosanitary Disinfestation of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in Export Citrus Fruit from South Africa: Validation of a Cold and Ionizing Radiation Combination Treatment**

Project IBB02/12 (April 2012 – March 2013) by Hendrik & Marsheille Hofmeyr (Ibbenbüren Navorsing)

Opsomming

Die voordeel daarvan om behandelingtipes wat teen suboptimale vlakke toegedien kan word om gesamentlik fitosanitêre sekuriteit teen valskodlingmot *Thaumatotibia leucotreta* te verskaf, is bespreek. Vorige studies het daarop gedui dat kombinasiebehandelings van ioniserende bestraling en koue *T. leucotreta* larwes en die daaropvolgende stadia beter as elke behandeling opsigself kan onderdruk. ŉ Proef om die doeltreffendheid te bewys van ŉ kombinasiebehandeling van 60 Gy ioniserende bestraling met opeenvolgende koue-opberging vir 16 dae teen 2.5°C, is vervolgens op probit-9 vlak (minstens 93 613 larwes) uitgevoer. ŉ Totaal van 104 617 5de instar larwes is behandel. Meer as 99.7% van die behandelde larwes en 50,2% van die oorblywende papies het gevrek. ŉ Totaal van 143 motte, met ŉ geslagsverhouding van een wyfie vir elke 7.9 mannetjies, het uiteindelik ontwikkel. Slegs 4.8% van die motte kon vlieg. Paringstudies het daarop gedui dat die potensiële vrugbaarheid van die motte volkome onderdruk was – geen eiers is gelê nie. Die doeltreffendheid van die kombinasiebehandeling is dus vir *T. leucotreta* bewys. Daar word voorgestel dat die benadering vir die instandhouding van fitosanitêre sekuriteit in uitvoersitrus oorweeg word.

Summary

The advantage of combining treatment types that could be applied at suboptimal levels for phytosanitary security to larvae of false codling moth, *Thaumatotibia leucotreta*, was discussed. Previous experiments had indicated that combination treatments that involved applications of ionizing radiation and cold exposure suppressed *T. leucotreta* larvae and their successive development stages more effectively than the constituents separately. An experiment to evaluate the efficacy of a treatment combination comprising 60 Gy of ionizing radiation followed by cold exposure for 16 days at 2.5°C was subsequently conducted at probit-9 level (at least 93 613 insects). A total of 104 617 mature, 5th instar larvae was treated. More than 99.7% of the treated larvae were killed and 50.2% of the subsequent pupae died. A total of 143 moths with a gender ratio of 1 female to 7.9 males eventually eclosed. Only 4.8% of the moths were able to fly. Mating studies were conducted and it was established that fecundity of the moths was totally suppressed – no eggs were produced. The efficacy of the combination approach was thus validated for false codling moth. It is proposed that the approach be considered for the maintenance of phytosanitary security in export citrus fruit.

Introduction

The pre- and post-harvest risks presented by false codling moth, *Thaumatotibia leucotreta*, on citrus were described in a previous article (Hofmeyr *et al*. 2013e, in press). The reasons for assessing alternatives to the prevailing cold disinfestation treatment in use for the post-harvest phytosanitary disinfestation of *T. leucotreta* were also discussed. The cold disinfestation protocol required for oranges to be exported to, for example the U.S.A., at -0,6°C for 22 days, can be hazardous for fruit quality (Hattingh, pers. com.). Similarly, the high dose uniformity ratio encountered when pallets fully stacked with boxes of oranges are treated with ionizing radiation (IoR), can impact on fruit quality (Barry *et al*. 2005, unpub. data). Combining two variably problematic practices that are applied at suboptimal levels to eliminate *T. leucotreta* as a phytosanitary hazard and also decrease the impact of these methods on fruit quality, was regarded as a potential improvement to commodity safety.

Doses of 60-100 Gy IoR were previously shown to totally supress the fecundity or fertility of moths developing from treated 5th instar larvae (Hofmeyr & Hofmeyr 2013b&e in press). It was consequently decided to reduce these doses to a sub-sterilizing level to allow combination with a cold treatment that would not cause total mortality of larvae independently, the colloquially called “combination approach”. A series of 7 experiments was conducted to determine optimal levels for the 2 respective measures. It was established that the combination of a 60 Gy IoR treatment followed by a cold treatment of 2.5°C for 16 days would largely reduce a larval population, suppress flight ability and render the survivors totally sterile. The combined effect of the 2 selected treatments was greater than the respective effect of the treatments separately. Here we report on the assessment of that combination treatment at probit-9 level.

Materials and methods

1. **Achieving treatment success**: The study was aimed at the assessment of a treatment that would totally prevent the ability of *T. leucotreta* to pose a phytosanitary threat to export fruit. To be successful the treatment would have to be 100% effective for one or more of the following aspects:

- total mortality of larvae or pupae,
- moths incapable of flying and thus unable to locate mates for reproduction, or
- totally sterile moths.

2. **Treatment selection**: A treatment combination of 60 Gy IoR followed by a cold application of 2.5°C for 16 days was selected for the experiment. An IoR treatment of 60 Gy sterilized all moths in 2 experiments (Hofmeyr *et al*. 2013b, in press; CRI annual report for 2010-11, table 3.2.4.10), but failed narrowly to do so twice in later experiments (CRI annual report for 2012-13, tables 3.2.2.5 and 3.2.2.7). These data were considered sufficient reason to include the dose as the first element of the combination approach. Additionally, in a previous experiment a cold treatment at 2.5°C for 22 days killed all larvae; at 12 days a large percentage of larvae survived the treatment (CRI annual report for 2010-11, exp. 4). At 16 days the number of survivors was reduced to 8% that of the control (Hofmeyr *et al*. 2013d, in press). This level of suppression also fulfilled the stated objective of the study and was included as the second element of the combination approach.

3. **Test material**: The experiments culminating in, and including, this probit study, were conducted with artificially reared *T. leucotreta*. In an experiment preceding the above research, the relative radiotolerance of larvae in synthetic diet was compared to that of feral larvae occurring in naturally infested oranges (Hofmeyr *et al*. 2013c, in press). It was established that the insectary reared larvae were at least as radiotolerant as feral larvae and thus justified their use in treatment validation experiments.

The required number of *T. leucotreta* larvae was reared in the laboratories of Citrus Research International (Pty) Ltd (CRI) in Citrusdal, Western Cape, South Africa. Eggs for this purpose were supplied by the sterile insect rearing facility, Xsit (Pty) Ltd, in Citrusdal. The gene pool of the insects reared in the facility was replenished by introducing feral *T. leucotreta* into the culture on a seasonal basis. The test insects were reared on a diet consisting predominantly of corn flour (Moore 2002) in 500 ml wide-necked glass rearing jars with membrane-fitted screw-on metal lids (Hofmeyr, unpub. data). Seven hundred to 800 24-h old eggs on wax paper were inoculated into each jar, incubated at 26°C and 5th (final) instar larvae were obtained approximately 14 days later. In the control treatment, rolled-up strips of single-sided corrugated cardboard, 30 mm wide, replaced the diet jars' metal lids to provide pupation sites. The cardboard strips were pulled apart to collect the pupae. In the IoR/cold treatment the pupae were hand-collected from the jars on a daily basis.

4. **Experimental design**: To achieve probit-9 mortality at the 95% confidence level, a minimum of 93 613 individuals had to be tested with no survivors (Follet and Neven, 2006). It was accepted that this stringent level of efficacy would also apply for any other factor than mortality, such as sterility. From previous experience it was expected that at least 500 test insects would be obtained per rearing jar. The required number of larvae due for treatment was obtained in 3 consecutive replicates of unequal size, 4 weeks apart. The sizes of replicates 2 and 3 were adjusted to provide similar numbers of larvae. The jars in each replicate were allocated as follows:

(a) *Replicate 1*: Seventy five jars were allocated at random, *viz*. one jar to establish instar ratio; 8 jars as an untreated control; and 66 jars for the combination treatment assessment.

(b) *Replicates 2 and 3*: Fifty jars were allocated at random in each of the 2 replicates, *viz*. one jar to establish instar ratio; 8 jars as an untreated control; and 41 jars for the combination treatment assessment.

The number of larvae developing in a diet jar could not be established *in situ* or the larvae be removed from a jar, counted and replaced without injury and/or shock resulting in unavoidable unnatural mortality. Larvae in the control and IoR/cold treatments were therefore allowed to pupate; the pupae were collected and their numbers compared. Possible natural mortality of larvae in the diet jars was ignored. The diet jars containing larvae were allocated at random to the treatments. The mean number of pupae developing per jar in the control could therefore be regarded as indicative of the number of larvae that would have pupated in the treated jars in the absence of treatment.

5. **Instar assessment**: Previous studies had indicated 5th instar larvae to be most radiotolerant when compared to younger instars and they were consequently used in this experiment (Hofmeyr *et al*. 2013b, in press). To confirm instar category, 60 larvae were removed at random from a diet jar in each of the three

replicates on the day of treatment (see section 4: *Experimental design*). A 'random' sub-sample of 20 of these larvae, including the smallest larvae obvious in the sample, was collected from each sample. The balance of the sub-sample was collected at random for assessment. The width of the larval head capsules was measured with the aid of an electronic digital calliper, allowing larvae to be allocated to a particular instar category (Daiber, 1979). This method, however, created difficulties as gaps existed between each instar category that were not allocated to any particular group. For example, the gap between 4th and 5th instar larvae extended from 1.08 mm to 1.24 mm (Table 3.2.5.1). Head capsule widths sometimes matched these gaps. For practical reasons we consequently divided the non-allocated areas evenly between categories to allow allocation of all larvae to an appropriate instar group.

Table 3.2.5.1. Allocation of *T. leucotreta* larvae to instar categories based on head capsule width.

Instar	Head capsule width (mm) according to Daiber	Adjusted instar categories (mm)
1	0.17 – 0.25	0.00 – 0.28
2	0.32 – 0.43	0.29 – 0.46
3	0.50 – 0.72	0.47 – 0.77
4	0.82 – 1.07	0.78 – 1.16
5	1.25 – 1.49	1.17 and wider

6. **Application of IoR treatments**: A single IoR treatment was applied to each replicate as soon as the first larvae started leaving the diet to pupate. Dosimetry and irradiation were conducted as follows:

(a) ***Irradiator design***: A Cobalt-60 point source, panoramic irradiator at the *T. leucotreta* rearing facility of Xsit (Pty) Ltd in Citrusdal, Western Cape, South Africa, was used. The targets were placed on 8 secondary turntables, each 300 mm in diameter that were located on a primary turntable, 1 370 mm in diameter. The primary turntable rotated at 2 r.p.m. and the secondaries at 15 r.p.m.

(b) ***Irradiation container***: Artificially reared larvae were treated in the glass rearing jars as the container sides provided sufficient build-up. The set-up for dosimetry and treatment applications consisted of a stack of 3 jars with larvae placed on each of the secondary turntables.

(c) ***Initial calibration***: The initial calibration was done using Fricke dosimeters with standard G-value for Fe^{3+} = 15.5/100 eV radiation energy absorbed. The Molar extinction coefficient for a HP diode array spectrophotometer was determined at 304 nanometre using spectrosol grade Fe^{3+} solutions (value 2081 ℓ/Mole/cm). This was to convert optical density readings made using a quartz glass flow cell – optical path 10 mm and reflecting [Fe^{3+}] concentrations into J/kg (Gray) values. The dosimeter response was checked in a Cobalt-60 radiation field – set-up 6 mm build-up and 50 mm backscatter in a 300 mm x 300 mm collimated field. The output factor was determined for the above using a Farmer tissue equivalent ionization chamber calibrated in a standard field of National Metrology Laboratory (C.S.I.R). Routine consistency checks were conducted on newly prepared Fricke solutions using 6 MV X-rays in a 300 mm x 300 mm field of a clinical photon therapy linear accelerator. Calibration factors relative to the National Standard as above applied.

(d) ***Dose mapping***: Dose mapping was conducted at the top and bottom of the rearing jar stack. The dose uniformity varied 17% from top to bottom. No reference position was used; the dosimetry was conducted for various positions in the irradiation set-up and the mean was calculated. The dose rate was updated before every irradiation procedure using the decay half-life of Cobalt-60 at 63.26 months.

(e) ***Uncertainties***: Repeated readings were taken at each position with differences less than 0.5%. Each radiation set-up was calibrated using a range of doses that included the radiation levels to be used. Uncertainties in the jar stack from repeated dose rate readings were less than 4%.

(f) ***IoR dose***: The IoR treatment in the validation study consisted of one dose only, *viz*. 60 Gy. Due to the dose uniformity variation of 17% in the jar stack, this dose had to be regarded as a mean; the applied dose varied 8.5% above and below the specified, *viz*. from 54.9 Gy to 65.1 Gy.

7. **Application of cold treatments**: Cold treatments were applied subsequent to the IoR applications. The CRI cold room in Citrusdal was used throughout the experiment. At least 72 hours before the treatments were initiated 3 diet jars with mature larvae corresponding in age to the test insects, were obtained from Xsit. A K-type thermocouple from a calibrated Squirrel 800 data logger was inserted into the diet of each jar and placed on a stainless steel bench in the cold room. Adjustments were made to the cooling equipment until the required diet temperature had stabilized. The test jars were placed into the cold room within one hour

after the IoR treatments had been applied. They were exposed in a single layer on stainless steel wire frame rearing containers holding 25 diet jars each. The thermocouples were transferred from the calibration jars to each of 3 randomly-selected test jars. Temperatures for each replicate were recorded every 30 minutes for the duration of the experiment. The larvae were cold treated for 16 days, then removed and incubated in a laboratory at 26°C until the efficacy assessments were concluded.

8. **Evaluation of treatments**

(a) **Untreated control**: The following factors were assessed using the 8-jar batches mentioned in section 4 (a) and (b) above.

(i) *Larval mortality*: Natural mortality of the larvae in the control jars could not be assessed [see *Materials and methods*, section 4: *Experimental design*].

(ii) *Pupal mortality*: All pupae from the 8 jars allocated for each control replicate were collected separately and counted. A sample of 60 pupae was removed at random from each jar and placed individually into glass vials, 50 mm x 15 mm, with foam rubber stoppers. All moths were sexed as they eclosed and the number of dead pupae was recorded 7 days after the last moth had emerged.

(iii) *Flight tests*: A sample of 5 female and 5 male moths eclosing from the remainder of each sample of 60 pupae was used for flight tests out-of-doors. Moths were released individually from a height of 2.5 m above ground level. Moths that flew strongly were considered to be flight capable. Moths unable to gain height and invariably descending involuntarily, or falling directly to the ground, were regarded to be unable to fly.

(iv) *Reproductive potential*: The first 5 pairs of moths to eclose from each sample of 60 pupae were used to assess reproductive potential; a newly eclosed female and male were placed into each of 5 x 100 ml plastic oviposition cages. Only inbreeding was assessed, *i.e*. untreated female x untreated male. Ovipositing was allowed for 5 days. The females were then collected, dissected and examined for the presence of spermatophores in their *bursae copulatrix* to confirm mating status. The eggs were incubated and all hatched and dead eggs were recorded per oviposition cage 14 days later.

(b) **Probit assessment**: In contrast to the control, larval mortality could be assessed by comparing the number of pupae from larvae surviving the combination treatment to the total number of pupae produced in the control replicates. Pupal mortality, flight ability and reproduction were assessed as in the control treatment (section 8a above). The extent of these studies were limited due to treatment effect and they could not be examined at a level similar to the control. All pupae were hand-collected from the respective numbers of test jars for each replicate until no more pupae became available or live larvae were noticed for 7 days. All collected pupae were placed into glass vials for eclosion [section 8(a) – *Pupal mortality*], but their numbers were to small to be grouped into replicates. In the reproductive study inbreeding only was assessed, *i.e*. treated female x treated male.

Results and discussion

1. **Numbers and instars of larvae treated**: The head capsules of all larvae in the 3 replicates were wider than the upper threshold (1.07 mm) for 4th instar larvae according to Daiber's classification (Fig. 3.2.5.1).

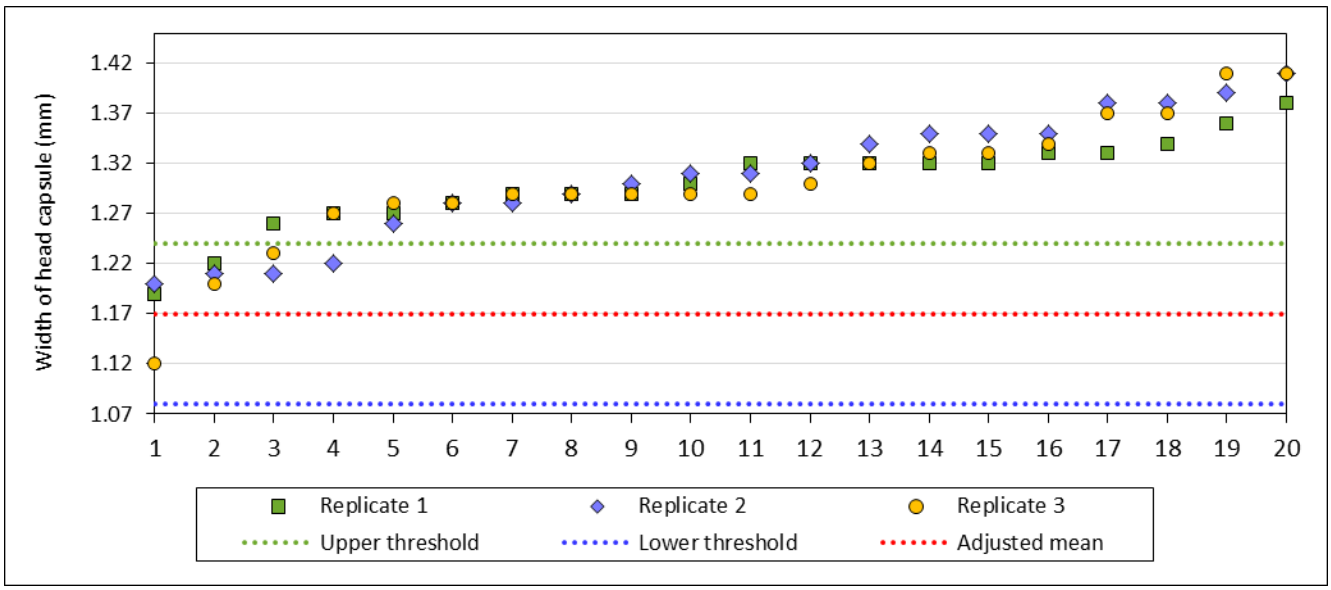

Fig. 3.2.5.1. Instar distribution of *T. leucotreta* larvae treated in 3 replicates used for probit-9 assessment. The gap between lower and upper thresholds represents a non-allocated area as described by Daiber (1979) in the instar categories for 4th and 5th instar larvae respectively.

Nine of the 60 larvae matched the non-allocated area between 4th and 5th instar. The head capsules of 8 of these larvae exceeded the adjusted minimum mean width for 5th instar, *i.e*. 1.17 mm. Only one larva in the 3rd replicate was smaller, *i.e*. 1.12 mm and was regarded as a 4th instar. The total number of larvae recorded in the 3rd replicate was consequently reduced by 5% (Table 3.2.5.2).

A total of 104 617 5th instar larvae was treated in the experiment. This exceeded the minimum number of insects required for probit-9 (93 613) by 11 004 (11.8%) (Table 3.2.5.2).

Table 3.2.5.2. Numbers of *T. leucotreta* larvae[1] treated in the 3 replicates of the validation study.

	Replicate 1	**Replicate 2**	**Replicate 3**
Mean number of pupae per jar in untreated control	862.6	559.6	635.2
Number of jars treated per replicate	66	41	41
Number of larvae per treatment replicate	56 932	22 944	24 741[2]
Number of *T. leucotreta* larvae treated	104 617		

[1]Equivalent to the total number of pupae produced in the untreated control.
[2]Total number of larvae reduced by 5% to compensate for 4th instars.

2. **Treatment temperatures**: The measured treatment temperatures in the 3 replicates were on average 0.16°C lower than the required and were regarded as acceptable. Temperatures varied from 2.31°C (Fig. 3.2.5.2) to 2.32°C (Fig. 3.2.5.3) to 2.4°C (Fig. 3.2.5.4).

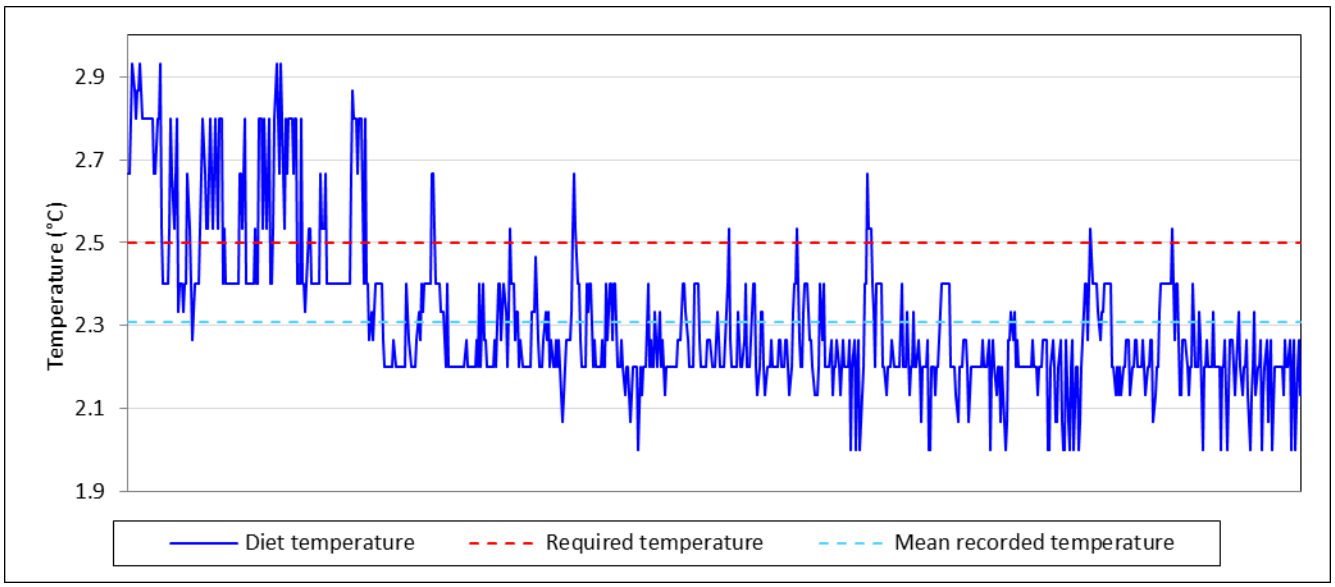

Fig. 3.2.5.2. Replicate 1: Temperatures recorded at 30 minute intervals in a 16 day period. The required temperature was 2.5°C and the mean recorded temperature was 2.31°C.

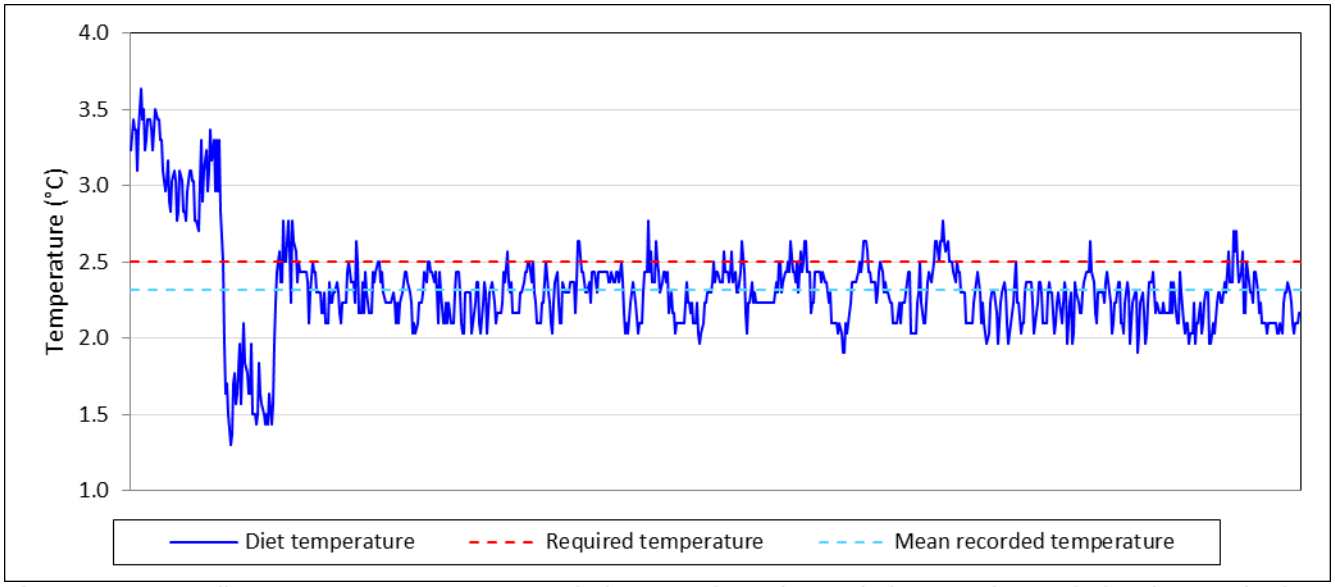

Fig. 3.2.5.3. Replicate 2: Temperatures recorded at 30 minute intervals in a 16 day period. The required temperature was 2.5°C and the mean recorded temperature was 2.32°C.

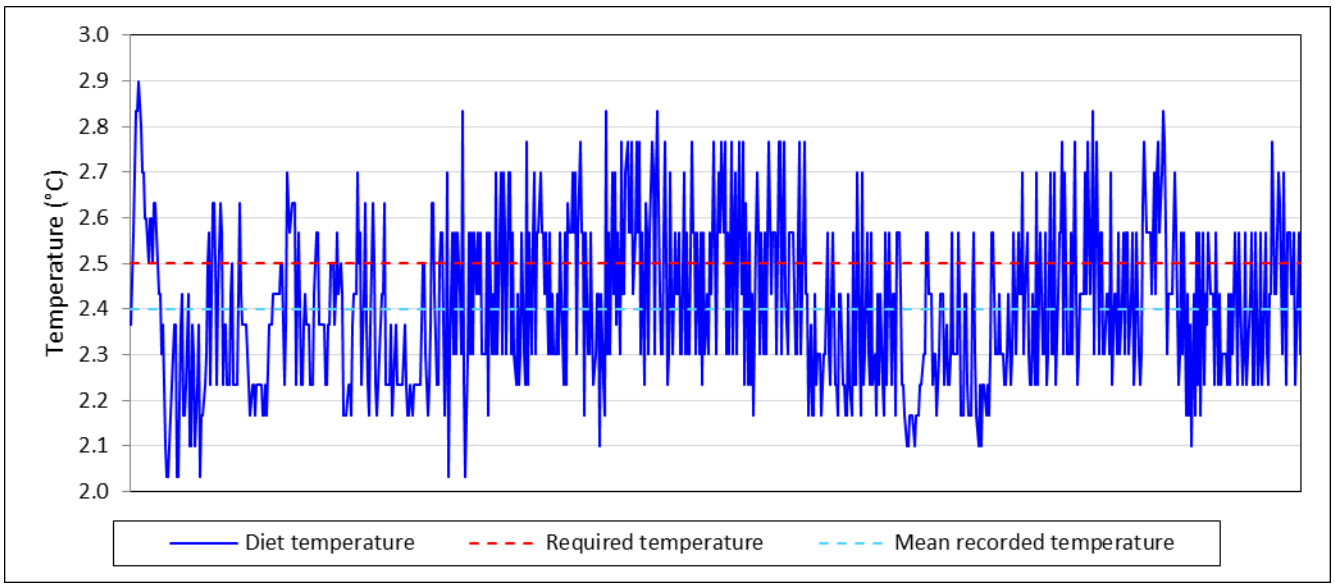

Fig. 3.2.5.4. Replicate 3: Temperatures recorded at 30 minute intervals in a 16 day period. The required temperature was 2.5°C and the mean recorded temperature was 2.4°C.

3. **Larval and pupal mortality**: Very small numbers of larvae survived the combination treatment to pupate (Table 3.2.5.3). Although cold exposure was mainly responsible for larval mortality, the IoR element increased the overall lethality of the combination treatment (Hofmeyr 2013d, in press). The surviving larvae did not form cocoons but pupated unprotected on the diet surface. This phenomenon is entirely due to the effect of the cold treatment as it had never been observed in many experiments involving IoR treatments only (Hofmeyr, unpub. data). In practice such an unprotected larva could possibly be exposed to an environment not conducive to survival for a variety of reasons such as unfavourable ambient conditions and predators. This possibility is, however, of more hypothetical than practical value in a commercial situation.

The combination treatment killed a mean of 99.7% of the larvae and so weakened many survivors that the subsequent pupae died before moth eclosion (Table 3.2.5.3). Despite these severe effects, the collective larval and pupal mortality was insufficient to validate the combination treatment as a stand-alone control measure at probit-9 level.

Table 3.2.5.3. Acute and indirect effects of a treatment combination of 60 Gy ionizing radiation followed by cold exposure for 16 days at 2.5°C on the survival of 5th instar *T. leucotreta* larvae and subsequent pupae.

Replicate	**Combination treatment: No. of 5th instar larvae**		**% pupal mortality in**		**No. of moths to eclose in**	
	treated	**to pupate**	**untreated control**	**combination treatment**	**untreated control**	**combination treatment**
1	56 932	237	3.3	48.5	55 053	122
2	22 944	14	4.4	71.4	21 934	4
3	24 741	38	3.3	52.6	23 925	18
Total	**104 617**	**289**	-	-	**100 912**	**144**

4. **Flight tests**: Most moths in the pooled control replicates flew normally and 95% qualified as flight-able. A restricted number was available for flight tests in the combination treatment as the reproductive assessment received preference and a maximum of respectively 100, 2 and 3 moths was eventually flight tested. Only 5 moths from the 105 moths were able to fly (4.8%), but nonetheless disqualified flight disability as a possible validation measure.

5. **Reproductive potential**: The comprehensive effects of the combination treatment on *T. leucotreta* development severely restricted the number of mating pairs that could be assessed in the experiment (Table 3.2.5.4):

(i) The gender ratio in the combination treatment was prominently male biased at 8 males for each female.

(ii) The larvae's protracted pupation/moth eclosion period – more than 40 days in the combination treatment compared to approximately 10 days in the control – prevented complete overlap in female and

male eclosion. None of the 3 males that eclosed in replicate 2 could consequently be paired with the one female. All females could be used to assemble mating pairs in replicates 1 and 3.

(iii) Successful mating was largely suppressed in the 15 pairs that could be assembled and spermatophores were found in only 4 females.

Table 3.2.5.4. Effects of a combination of 60 Gy ionizing radiation followed by cold exposure for 16 days at 2.5°C on the mating of moths from treated 5th instar *T. leucotreta* larvae.

Replicate	Gender ratio (♀:♂) of moths in		No. of mated females in	
	untreated control[1]	combination treatment	untreated control (n=40)	combination treatment (n=mating pairs)
1	1 : 1.2	1 : 10.1	39	2 (11)
2	1 : 1.1	1 : 3.0	39	0 (0)
3	1 : 1.3	1 : 3.5	39	2 (4)

The moths' fecundity was totally suppressed and no eggs were produced (Table 3.2.5.5).

Table 3.2.5.5. Effects of a combination of 60 Gy ionizing radiation plus 2.5°C for 16 days on the fecundity and fertility of moths from treated 5th instar *T. leucotreta* larvae.

Replicate	Fecundity: Total no. of eggs produced in		Fertility: % of eggs hatched in	
	untreated control	combination treatment	untreated control	combination treatment
1	13 596	0	90.0	No eggs
2	15 816	0	85.7	No eggs
3	14 014	0	83.7	No eggs

Conclusion

The study was conducted on a scale 11.8% larger than the minimum required for a probit-9 assessment. The results showed that the acute effects from the combination treatment would almost totally reduce a *T. leucotreta* population due to larval and pupal mortality. Most of the remaining moths would be unable to fly. The gender ratio would be extremely male biased, and an extended eclosion pattern would reduce the chances of mating. Ultimately, the moths would be sterile and if mating did occur, no eggs would be produced. A combination treatment for *T. leucotreta* consisting of 60 Gy of ionizing radiation followed by cold exposure for 16 days at 2.5°C is therefore regarded as adequate to maintain phytosanitary security in oranges.

Technology Transfer

This work was presented at a workshop at the IAEA in Vienna in 2014.

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3.2.6 PROGRESS REPORT: The use of entomopathogenic fungi to control the soil-dwelling life stages of false codling moth

Project 1024 (Jan 2011 – Dec 2012) by Candice Coombes, Martin Hill, Jo Dames (RU) and Sean Moore (CRI)

Summary

The control of FCM is essential in the citrus industry due to the financial loss which can occur largely as a result of the phytosanitary status associated with FCM. Entomopathogenic fungi (EPF) can potentially serve as an additional biological control option for the control of FCM, specifically the soil-dwelling life stages (late fifth instar, pre-pupae and pupae). In an earlier study, 62 fungal isolates were isolated from soil samples collected from citrus orchards within the Eastern Cape Province of South Africa. Twelve were identified as showing good control potential. Further laboratory analysis revealed three of these isolates (*M. anisopliae* G 11 3 L6, *M. anisopliae* FCM Ar 23 B3, and *B. bassiana* G Ar 17 B3) as having the most potential against the soil-dwelling life stages of FCM. In general, a dose-dependent relationship was found with an increase in mortality associated with an increase in conidial concentration, with LC_{50} values of 6.26 x 10^5, 1.92 x 10^6 and 1.98 x 10^5 conidia/ml calculated for isolates G 11 3 L6, FCM Ar 23 B3, and G Ar 17 B3. Likewise, an increase in mortality was observed with an increase in the amount of time (in days) the larvae were exposed to the fungus. At a fungal concentration of 1 x 10^7 conidia/ml, it was estimated that late fifth instar larvae are required to be exposed to the fungal isolates for a period of nine days to ensure 90% mortality as a result of fungal infection. Results also indicated the better performance of these isolates over two commercial isolates tested and their ability to persist whilst still remaining infective towards FCM fifth instar larvae over a six month period in a citrus orchard. Whether this is true persistence however remains to be determined. To test the performance of these isolates in the field, a large scale field trial in conjunction with a smaller caged trial was initiated. Results of the large scale field trial suggest the better performance of the *B. bassiana* isolate with a reduction in FCM infestation by approximately 80%. In light of this, an additional field trial using only isolate G Ar 17 B3 was initiated on 13 March 2014. No data is yet available to report. Caged trials were ended on 27 March 2014. Data are currently being collected.

Opsomming

Die beheer van VKM in die sitrusbedryf is noodsaaklik vanweë die finsiële verlies wat hoofsaaklik as gevolg van die fitosanitêre status geassosieer met VKM kan gebeur. Entomopathogeniese swamme (EPF) kan moontlik as ʼn addisionele biologiesebeheer opsie teen VKM, spesifiek die grond-lewensstadiums (laat vyfde instar, voor-papies en papies), dien. ʼn Vorige studie het 62 swam isolate geïsoleer uit grond opnames wat versamel is van sitrus boorde in die Oos-Kaap Provinsie van Suid-Afrika. Twaalf is geïdentifiseer as isolate wat die meeste potensiaal gewys het. Verdere laboratorium-ontleding het gewys dat drie van hierdie isolate (*M. anisopliae* G 11 3 L6, *M. anisopliae* VKM Ar 23 B3, en *B. bassiana* G Ar 17 B3) die meeste potensiaal teen die grond-lewensstadiums van VKM het. Oor die algemeen, is ʼn dosis-afhangende verhouding tussen ʼn toename in mortaliteit en swam konsentrasie waargeneem, met LC_{50} waardes van 6,26 x 10^5, 1,92 x 10^6 en 1,98 x 10^5 spore/ml bereken vir isolate G 11 3 L6, VKM Ar 23 B3, en G Ar 17 B3 onderskeidelik. Net so, is ʼn verhoging in mortaliteit waargeneem met ʼn verhoging in tydsduur (dae) wat larwes aan die swam blootgestel is. Swam nawerkingsproewe het getoon dat hierdie drie isolate die vermoë het om oor ʼn ses maande typerk nog infeksie in vyfde instar VKM larwes te veroorsaak. Teen ʼn swam konsentrasie van 1 x 10^7 spore/ml, is dit beraam dat laat vyfde instar larwes vir ʼn tydperk van 9 dae blootgestel moet word om 90% mortaliteit as gevolg van swam infeksie te verseker. Resultate het ook aangedui dat hierdie isolate beter as twee kommersiële isolate presteer het en die vermoë het om oor ʼn 6 maande typerk in ʼn sitrusboord doeltreffend teen vyfde instar VKM larwes te bly. Of hierdie ware nawerking is of nie moet nog bepaal word. Om die werking van hierdie isolate in die veld te toets, is ʼn grootskaalse veldproef saam met ʼn kleiner hok-proef gëinisieer. Resultate van die grootskaalse veldproef dui aan dat die *B. bassiana* isolaat beter presteer, met ʼn vermindering in VKM besmetting van omtrent 80%. As gevolg hiervan is nog ʼn veldproef met net die G Ar 17 B3 isolaat op 13 Maart 2014 begin. Nog geen data is beskikbaar nie. Hok proewe is 27 Maart voltoei; data word nou ingesamel.

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

Project 1039 (April 2012 – March 2015) by Sean Moore, Wayne Kirkman 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, an ad hoc trial on the effect of abbreviated cold treatments on FCM survival was initiated in 2012. This study was continued in 2013. FCM larvae of all instars in both fruit and artificial diet were exposed to time-temperature regimes of 1°C for 14 days, 1°C for 16 days and 2°C for 18 days. The latter two treatments had similar efficacy and were more effective than 1°C for 14 days. At 2°C for 18 days, no 1st and 2nd instar larvae survived. Survival in fruit was slightly higher than in diet, with 4.35% and 0.04% of 5th instars surviving in each medium respectively. Surviving larvae were retained and it was determined that an average of 29% of larvae completed development to adulthood. However, when surviving male and female adults were paired, only a small minority produced fertile eggs.

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 ad hoc proef op die effek van verkorte koue behandelings op VKM oorlewing in 2012 gëinisieer. Hierdie studie is in 2013 voortgesit. VKM larwes van alle instars in albei vrugte en kunsmatige diet is aan tyd-temperatuur behandelings van 1°C vir 14 dae, 1°C vir 16 dae en 2°C vir 18 dae blootgestel. Die laaste twee behandelings was ewe doeltreffend en is meer doeltreffend as 1°C vir 14 dae. Teen 2°C vir 18 dae was daar geen oorlewing van 1ste of 2de instar larwes nie. Oorlewing in vrugte is effens hoër as in dieet, met 4.35% en 0.04% oorlewing van 5de instars op die twee middels ondeerskeidelik. Oorlewende larwes is behou en dit is bepaal dat 29% van die larwes hulle ontwikkeling tot volwassendheid voltoei het. Waar oorlewende mannetjie en wyfie motte gepaar is, het net ŉ klein minderheid vrugbare eiers gelê.

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

Project 1042 (Sep 2011 – March 2015) by Sean Moore (CRI), Ralf-Udo Ehlers (e-nema), Aruna Manrakhan, Martin Gilbert, Wayne Kirkman and John-Henry Daneel (CRI)

Summary

A total of three field trials were conducted with entomopathogenic nematodes (EPNs) (a commercial formulation of *Heterorhabditis bacteriophora*) in citrus orchards during the 2013/14 season: one in each of the Eastern Cape, Western Cape and Mpumalanga. The trials were designed to compare the efficacy against false codling moth (FCM) of a once-off soil application in spring with that of monthly applications for four months (from September to December) at 10 and 5 infective juveniles (IJs) per cm^2. This comparison was made mainly due to the variable results achieved with once-off spring (or sometimes autumn) applications during the preceding two seasons. EPNs were applied either through the irrigation system, using microsprinklers or with the use of a spray machine, aiming the bottom three nozzles on either side of the machine to spray underneath the tree canopy. Survival and persistence of EPNs in the soil were monitored by collecting 50 soil samples from each treatment on a monthly basis, baiting these with two larvae (5th instar FCM) each and evaluating EPN infestation of larvae one week later. Population suppression of FCM was measured using a pheromone trap in the dead centre of each treatment orchard. Damage reduction was evaluated by inspecting fallen fruit from data trees at weekly intervals from January. Survival and persistence of EPNs at all three sites was better with the monthly applications than the once-off treatment. EPN recovery from the 5 IJ/cm^2 treatments (applied at two of the sites) was almost as good as for the 10 IJ/cm^2 treatments. FCM levels at the Western Cape and Mpumalanga trial sites were extremely low, precluding any evaluation of pest control. At the Eastern Cape trial site, FCM infestation of fruit was reduced by 72% by the monthly 10 IJs/cm^2 treatment, whereas the once-off application only reduced infestation by 28%. FCM infestation in the 5 IJ/cm^2 orchard was extremely high. This anomaly can only be attributed to an incomparably high natural level of FCM occurrence in this orchard. Monitoring will continue until harvest. Fruit fly will also be monitored in trial orchards. Finally, an additional trial will be conducted in each region to test the corrective efficacy of an EPN application against a high level of FCM activity late in the season.

Opsomming

In totaal is drie veldproewe met entomopatogeniese nematodes (EPNs) ('n kommersiele formulasie van *Heterorhabditis bacteriophora*) in sitrusboorde gedurende die 2013/14 seisoen uitgevoer: in elk van die Oos-Kaap, Wes-Kaap en Mpumalanga provinsies. Die proewe is ontwerp om die effektiwiteit teen valskodlingmot (VKM) van 'n eenmalige behandeling in die lente met dié van maandelikse toedienings vir vier maande (September tot Desember) teen 10 en 5 nematodes (IJs) per cm^2 te vergelyk. Hierdie vergelyking is hoofsaakliks gemaak omrede die variasie in resultate van eenmalige toedienings in lente (of soms herfs) gedurende die vorige twee seisoene. EPNs is toegedien of deur die besproeingsstelsel, met die gebruik van mikrobesproeiers, of met die gebruik van 'n spuitmasjien met die onderste drie neuse aan albei kante gemik onder die boom kappie. Oorlewing en nawerking van EPNs in die grond is gemoniteer deur versameling van 50 grondmonsters van elke behandeling op 'n maandelikse basis, die gebruik van twee 5de instar VKM larwes per monster as lok-insekte en ontleding van EPN infestasie van dié larwes na een week. Populasie onderdrukking van VKM is gemeet deur gebruik van 'n feromoonlokval wat in die middel van elke behandelingsboord geplaas is. Skade vermindering is geëvalueer deur inspeksie van gevalde vrugte van databome op 'n weeklikse basis van Januarie. Oorlewing and nawerking van EPNs by al drie persele was beter met die maandelikse toedienings as die eenmalige behandelings. EPN herkryging van die 5 IJ/cm^2 behandelings (toegedien by twee van die persele) is amper so goed soos vir die 10 IJ/cm^2 behandelings. VKM vlakke by die Wes-Kaap en Mpumalanga proefpersele is besonders laag, wat evaluasie van plaagbeheer verhoed het. By die Oos-Kaapse proefperseel is VKM besmetting van vrugte met 72% verminder met die maandelikse 10 IJs/cm^2 behandeling, waar die eenmalige behandeling besmetting met net 28% verminder het. VKM besmetting in die 5 IJ/cm^2 boord was besonders hoog. Hierdie anomalie kan net aan 'n onvergelykbare hoë natuurlike vlak van VKM in hierdie boord toegeskryf word. Monitering sal tot oestyd toe voortgesit word. Vrugtevlieg sal ook in proefboorde gemoniteer word. Ten laaste sal 'n verdere proef in elke streek uitgevoer word om die korrektiewe effektiwiteit van EPN toedieining teen 'n hoë vlak van VKM aktiwiteit laat in die seisoen te toets.

3.2.9 PROGRESS REPORT: Gene expression analysis of *Thaumatotibia leucotreta* as result of different isolates of *Cryptophlebia leucotreta* granulosis virus

Project 1049 (2012/01 – 2014/12) by A.E. Timm & J. Ridgeway (RU)

Summary

The biotic response of *Thaumatotibia leucotreta* to Cryptophlebia leucotreta granulovirus (CrleGV) has been researched at an organism level. However, the genes involved in this interaction are not well known. This project looks at the differences in *T. leucotreta* response to CrleGV at genomic level to determine the degree of genetic differences elicited by different time and temperature conditions. The current best method of determining genetic interaction is by using RT-qPCR. As outlined by the MIQE guidelines, two reference/housekeeping genes are required to validate the gene expression level using RT-qPCR. Thirty-one genes were evaluated under different conditions (temperature and virus stress, using three different tissue types) in order to identify the best two housekeeping genes for evaluating interactions between *T. leucotreta* and CrleGV. It was concluded that the most stable genes for analysis were Elongation factor 1 and Alpha kinase. These genes will therefore be used as housekeeping genes for evaluating the interaction between *T. leucotreta* and CrleGV.

Opsomming

Die biotiese reaksie van *Thaumatotibia leucotreta* teenoor Cryptophlebia leucotreta granulovirus (CrleGV) op organisme vlak is wel bekend. Inteendeel is die gene wat betrokke is in hierdie interaksie nie goed bekend nie. Hierdie projek kyk na die verskille in *T. leucotreta* reaksie teenoor CrleGV op genomiese vlak om die graad van genetiese verskille wat ontlok is deur verskillende tyd en temperatuur toestande te bepaal. Tans is RT-qPCR die beste metode om genetiese interaksie te ontleed. Soos uiteengesit in die MIQE riglyne is twee verwysingsgenes nodig om die uitdrukking van genes met behulp van RT-qPCR te bekragtig. Een-en-dertig genes is gëevalueer onder verskillende toestande (temperatuur en virus stres met gebruik van drie verskillende tipes weefsel) om die beste twee verwysingsgenes vir die evaluering van interaksies tussen *T. leucotreta* en CrleGV te identifiseer. Die mees stabiele gene vir ontleding was verlenging faktor 1 en alpha kinase. Hierdie genes sal dus gebruik word as verwysingsgenes vir die evaluering van die interaksie tussen *T. leucotreta* en CrleGV.

3.2.10 **PROGRESS REPORT: Behaviour, biology and survival of pupating false codling moth**

Project 1059 (April 2013 – March 2015) by Claire Love, Martin Hill (RU) and Sean Moore (CRI)

Summary

This project aims to fulfil the need to improve the current understanding of false codling moth (FCM) biology, behaviour and survival of the wandering larvae, pre-pupal and pupal life stages of this important citrus pest in the soil environment. Experiments are being conducted to examine the influence of factors such as soil type, soil moisture, soil compaction and air temperature on pupation. Two additional experiments examining the influence of groundcover and shade will also be included. Increased knowledge in this area may then be used to improve control of FCM in the field through direct soil manipulation, improvement of orchard sanitation or indirectly through the use of this knowledge for more effective use of entomopathogenic fungi and entomopathogenic nematodes. Laboratory experiments are currently underway, including filming of larval biology and behaviour. Two of the experiments have been completed thus far, with the first determining changes in larval biology, behaviour and survival in different soil types or textures (sandy loam, silt loam and silt clay loam) and the second determining the impact of organic groundcover on pupation. A change to the project was also decided upon, with the planned field trials being replaced by further laboratory trials, as these may be more valuable at this stage. Data analysis of the completed experiments is currently underway. Preliminary analysis of the soil type data indicates that soil type does not significantly impact amount of time wandered ($H = 3.092$; $P = 0.213$) or the amount of time taken to initially form the pupal case ($H = 0.597$; $P = 0.970$) of FCM ($n = 90$). Overall, survival rates were highest for sandy loam soil with 83% of adults successfully eclosing, while only 53% and 57% of FCM adults successfully eclosed for silt loam and silty clay loam respectively. Data collected for the groundcover experiment is currently being analysed.

Opsomming

Die doelwitte van hierdie projek is om die huidige kennis van valskodlinmot (VKM) biologie, gedrag en oorlewing van die bewegende larwes, die voor-papie en die papie stadiums van hierdie belangrike sitrusplaag te verbeter. Die invloed van faktore soos grondtipe, grondvog, grondkompaksie en lugtemperatuur op papievormende VKM sal ook getoets word. Twee addisonele proewe wat die invloed van grondbedekking en skaduwee gaan ondersoek sal ook uitgevoer word. ʼn Beter kennis op hierdie onderwerp sal gebruik word om die beheer van VKM in die veld te verbeter deur direkte manipulasie van die grond omgewing, verbetering van boordsanitasie, of indirek deur om hierdie inligting te gebruik vir meer doeltreffende toediening van entomopatogeniese swamme en entomopatogeniese nematodes. Laboratorium proewe, wat die verfilming van die larwe se bedrag en biologie insluit, is tans aan die gang. Twee van die eksperimente is voltooi. Die eerste een het die invloed van groendtipe of tekstuur (sandleem, slikleem en slikkleileem) op larwes se biologie, gedrag en oorlewing bepaal en die tweede eksperiment het die invloed van organiese grondbedekking op papievormende VKM ondersoek. Daar is ook ʼn verandering aan die projek gemaak, waar beplande veldproewe met verdere laboratorium proewe vervang is omdat laboratorium proewe op hierdie stadium meer waardevol vir die projek sal wees. Ontleding van data van proewe wat voltooi is het al begin. Voorlopige ontleding van die grondtipe data ($n = 90$) dui aan dat grondtipe nie ʼn beduidende invloed het op tydsduur van ronddwaal voor papievorming ($H = 3.092$; $P = 0.213$), of die tydsduur om die kokon te vorm nie ($H = 0.597$; $P = 0.970$). Oorlewing gemeet deur suksesvolle ontpopping van VKM volwassenes was hoogste vir sandleem grond met 83%, terwyl net 53% en 57% vir slikleem en slikkleileem onderskeidelik ontpop het. Data vir die grondbedekking eksperiment word tans ontleed.

3.2.11 **PROGRESS REPORT: A feasibility study of postharvest vapour heat for FCM control**

Project 1060 (2013/4) by T G Grout and P R Stephen (CRI)

Summary

Thailand is one of the few countries in the world where vapour heat or high temperature forced air is being used as a postharvest treatment on citrus to control *Bactrocera dorsalis* in pomelos being exported to Japan. Although the USDA-APHIS has treatment schedules for such treatments on citrus and several papers were published in the 1980s and 90s that said it could be done safely, only Thailand is risking this treatment on a commercial basis. Their treatment is at 43°C and 50-65% relative humidity (RH) and the fruit is held at 5-10°C afterwards. We evaluated various time-temperature combinations on FCM fifth instars in media in an environmental chamber and sometimes had oranges present to determine whether there was any visible damage to the fruit several days later. Six hours at 44°C and around 70% RH caused 75% mortality when the temperature was increased rapidly, but only 47% mortality when the temperature was increased more gradually. The same time-temperature combination caused 97% mortality at 35-55% RH, but this may be more likely to damage the fruit. Increasing the time at 44°C to 8 h did not increase mortality. A temperature

of 46°C for 6 h at 53% RH resulted in 100% mortality of 448 larvae but this may be a risky treatment for fruit quality. A treatment at 43°C for 4 h followed by a short cold treatment at 1°C for 7 d resulted in similar mortality to the cold treatment alone, so this heat treatment provided no additional benefit. We will conduct a similar trial with a short cold treatment after a heat treatment of 46°C for 4 h before deciding whether this work should be taken further with infested fruit.

Opsomming

Thailand is een van die min lande in die wêreld wat damp hitte of lug teen ŉ hoë temperatuur en druk gebruik as ŉ na-oes behandeling vir sitrus om *Bactrocera dorsalis* in pomelos te beheer, vir uitvoer na Japan. Alhoewel die USDA-APHIS behandeling schedules vir die bogenoemde behandelings op sitrus in verskeie artikels gepupliseer is in die 1980s en 90s wat bevestig dat die behandelings veilig uitgevoer kan word, is Thailand die enigste land wat dit waag om die behandelings op kommersiele vlak te gebruik. Die behandelings wat hulle gebruik is teen 43°C en 50-65% relatiewe humiditeit (RH) uitgevoer, waarna die vrugte teen 5-10°C gestoor word. Ons het verskeie tyd-temperatuur kombinasies getoets op 5de instar VKM larwes in media in ŉ omgewingsbeheerde kamer wat soms ook lemoene in gehad het om te bepaal of daar enige sigbare skade aan vrute was na verskeie dae. Ses ure teen 44°C en ongeveer 70% RH het 75% mortaliteit veroorsaak toe die temperature spoedig verhoog is, maar slegs 47% mortaliteit is verkry waneer die temperature geleidelik verhoog is. Dieselfde tyd-temperatuur kombinasie het 97% mortaliteit veroorsaak teen 35-55% RH, maar die toestande sal moontlik meer skade aan vrugte veroorsaak. Mortaliteit het nie verhoog toe die tyd vir die 44°C behandeling na 8 h verhoog is nie. ŉ Temperatuur van 46°C vir 6 h teen 53% RH het 100% mortaliteit van 448 larwes veroorsaak, maar dié hoë temperatuur sal moontlik vrug kwaliteit belemmer. ŉ Behandeling van 43°C vir 4 h gevolg deur ŉ koue behandeling vir 7 dae het soortgelyke mortaliteit as slegs kouebehandeling veroorsaak en bied daarom geen addisionele voordeel aan nie. Ons wil ŉ soortgelyke experimentele toets uitvoer waar ŉ kort koue behandeling gevolg word deur ŉ hitte behandeling van 46°C vir 4 h, voordat ons besluit of die studie verder gevat moet word met besmette vrugte.

3.2.12 PROGRESS REPORT: Evaluation of 7-Vinyl-Decyl Acetate 1 for mating inhibition in FCM

Project 1063 (Sep 2011 – March 2015) by Sean Moore, Wayne Kirkman (CRI) 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. Twelve cages were erected over small Wasington Navel trees on Arundel Farm in the Sundays River Valley (two trees per cage). Six treatments were used (two cages per treatment): untreated control, Isomate (two dispensers per cage), and 7-VDA at 1, 2, 4 and 8 dispensers per cage. The 7-VDA dispensers were small polyethylene vials covered with breathable fabric with 10 µl per dispenser. On 13 December treatments were hung on trees in cages and four and a half hours later, 10 virgin male and virgin female moths were released into each cage. FCM infestation of dropped fruit was evaluated from three weeks after this date for a five-week period. FCM infestation was very low throughout. However, there was no FCM infestation where Isomate was hung. It thus appeared that the efficacy of the experimental compound was inferior. Similarly disappointing results were obtained in a small laboratory trial where mating was markedly reduced by Isomate but not 7-VDA. Through execution of release rate trials in an incubator thereafter, it was established that the problem was an inferior release rate. An improved dispenser was acquired. However, it was subsequently established that even in identical dispensers, release rate of 7-VDA was lower than that of the FCM pheromone. This will be countered for by using more 7-VDA dispensers in future trials in order to get an equivalent release quantity to that of the FCM pheromone (including Isomate).

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. Twaalf hokke is oor klein Washington Nawelbome op Arundel Plaas in die Sondagsriviervallei opgeheg (twee bome per hok). Ses behandelings is gebruik (twee hokke per behandeling): onbehandelde kontrole, Isomate (twee vrystellers per hok), en 7-VDA teen 1, 2, 4 en 8 vrystellers per hok. Die 7-VDA vrystellers is klein polietileen buisies gedek met deurlaatbare kleedstof met 10 µl per vrysteller. Op 13 Desember is behandelings op bome in hokke gehang en vier en ŉ half ure later is 10 ongepaarde mannetjie en wyfie motte in elke hok losgelaat. VKM besmetting van gevalde vrugte is van drie weke daarna vir ŉ vyf-weke tydperk gëevalueer. VKM besmetting is orals baie laag, maar geen besmetting is in die Isomate hok gekry nie. Dit het dus voorgekom dat die werking van die eksperimentele

stof laer was. Soortgelyke teleurstellende resultate is in ŉ klein laboratorium proef gekry waar paring van motte beduidend verminder is deur Isomate maar nie 7-VDA nie. Deur die uitvoer van loslatingskoers proewe is dit bepaal dat die probleem ŉ laer loslatingskoers is. ŉ Verbeterde vrysteller is gekry maar dit is daarna bepaal dat selfs in identiese vrystellers is die loslatingskoers van 7-VDA laer as dié van die VKM feromoon. Hierdie sal teengestaan word deur gebruik van meer 7-VDA vrystellers in toekomstige proewe om ŉ ekwivalente loslating as VKM feromoon (insluitend Isomate) te kry.

3.2.13 PROGRESS REPORT: Determination of reapplication frequency of the *Cryptophlebia leucotreta* granulovirus to provide protection against FCM infestation of citrus.

Project NMMU-1065 by Patrick Mwanza, Gill Dealtry, Mike Lee (NMMU) and Sean Moore (CRI)

Summary

The use of baculoviruses as biopesticides continues to gain popularity as they appear to provide a more advantageous approach to combat crop pests than do chemical pesticides. As such they have become important in the agricultural economy. The *Cryptophlebia leucotreta* granulovirus (CrleGV) has been formulated as a biocontrol agent against the false codling moth, (FCM). FCM is a common pest of citrus. One of the major setbacks with the use of baculoviruses as biopesticides has been their susceptibility to ultraviolet radiation (UV) from the sun. In this study the effect of UV on CrleGV is being evaluated. Laboratory experiments simulating field conditions are being conducted to determine the nature of the damage caused by UV on the structure of the granulovirus. Field experiments have been carried out and processing and analysis of samples obtained from the field is currently underway. The frequency of reapplication of the formulated products Cryptogran® (River Bioscience) and Cryptex (Andermatt) will also be determined from this study.

Opsomming

Die gebruik van bakuloviruse as biologiese plaagdoders word meer gewild omdat hulle ŉ meer voordelige benadering tot die bestryding van gewasplase in vergelyking met chemiese plaagdoders voorsien. As sulks het hulle belangrik geword in die landbou eknomie. Die *Cryptophlebia leucotreta* granulovirus (CrleGV) is as ŉ biologiesebeheer agent teen die valskodlingmot (VKM) geformuleer. VKM is ŉ algemene plaag van sitrus. Een van die terugslae met die gebruik van bakuloviruse as biologiese plaagdoders is hulle vatbaarheid vir ultraviolet bestraling van die son. In hierdie studie word die effek van UV op CrleGV ontleed. Laboratorium eksperimente wat veld omstandighede simuleer word uitgevoer om die geaardheid van die UV skade op die struktuur van die granulovirus te bepaal. Veldproewe is uitgevoer en monsters van dié proewe word tans geprosesseer en ontleed. Gereeldheid van hertoediening van die geforumuleerde produkte, Cryptogran (River Bioscience) en Cryptex (Andermatt) sal ook van hierdie studie bepaal word.

3.2.14 PROGRESS REPORT: Using the larval parasitoid, *Agathis bishopi*, for detection of FCM infested fruit.

Project number 1066 (2013/4 – 2015/3) by Kennedy Zimba, Martin Hill (RU) and Sean Moore (CRI)

Summary

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Totricidae), is one of the major citrus pests in South Africa. It is a phytosanitary pest and its detection in export markets can result in loss of income. *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) is the dominant species of larval parasitoid attacking FCM; it is particularly predominant in the Eastern Cape, with parasitism of up to 34% of larvae in fruit. It has thus been considered to possess meaningful biocontrol potential for FCM. From FCM larvae collected from the field, second instars yielded the highest number of parasitoids. Therefore, the host seeking behaviour of *A. bishopi* for early instars of their host could indicate FCM infested fruit. The study will examine the behavioural response of female *A. bishopi* to volatiles from FCM infested fruit. If found effective, this study will demonstrate an innovative way of timeously detecting FCM infested fruit before shipment to export markets, potentially saving the citrus industry considerable income.

Opsomming

Valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is een van die hoof sitrusplae in Suid-Afrika. Dit is ŉ fitosanitêre plaag en sy opsporing in uitvoermarkte kan ŉ verlies in inkomste veroorsaak. *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) is die dominante spesie van larwe parasiet wat VKM aanval. Dit is veral in die Oos-Kaap algemeen met parasitisme van tot 34% van larwes in vrugte. Dit word dus beskou dat dit beduidende belofte inhou vir biologiese beheer van VKM. Van VKM larwes wat van die veld versamel is het tweede instars die hoogste getalle parasiet opgelewer. Daarom is

dit moontlik dat die gasheer soekende gedrag van *A. bishopi* vir vroë instars van sy gasheer, VKM besmette vrugte kan aandui. Die studie sal die gedrags respons van wyfie *A. bishopi* teenoor vlugtige stowwe van VKM besmette vrugte ondersoek. As suksesvol, sal hierdie studie ŉ inoverende manier van opsporing van VKM besmette vrugte voor verskeping na uitvoer markte demonstreer, wat moontlik die sitrusbedryf geweldige inkomste kan bespaar.

3.2.15 FINAL REPORT: Effect of molasses as a baculovirus additive on the behaviour of neonate false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) larvae

Project not funded by CRI (Jan-Dec 2013) by Storm Hilliar, Martin Hill (RU) and Sean Moore (CRI)

Summary

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (1912) (Lepidoptera: Tortricidae), is one of the most important pests of citrus in southern Africa and may be difficult to control. To comply with the requirements of strict export regulations, various methods to control FCM have been developed. However, the pest still causes economic losses to the citrus industry of South Africa annually. Among the control methods, *Cryptophlebia leucotreta* granulovirus (CrleGV), registered under the names Cryptogran, Cryptex and Gratham, has had success in controlling FCM in the field, however its efficiency is determined by the small frame within which FCM neonate larvae feed before penetration into the fruit. The aim of this study was to investigate the behaviour of neonate FCM larvae in the presence of molasses, as a baculovirus additive, on the surface of Navel oranges. In previous experiments conducted using visual observation, the addition of molasses induced feeding in patches on the surface of the fruit, as well as a decrease in total movement and total penetration due to surface feeding. This continuation in behavioural investigation made use of video cameras to accurately observe and record the behaviour of FCM neonate larvae when exposed to concentrations of molasses as well as to scientifically quantify their movement, time of feeding and penetration between each treatment. In analysing the effect molasses has on FCM neonate behaviour with the use of both observational and video recording, the data showed that molasses resulted in decreased larval movement (by distance) on the fruit surface, increased time spent on the fruit surface (due to increased feeding) and reduced penetration of fruit. Implications are that molasses acts as a phagostimulant, potentially increasing the probability that a lethal virus dose will be ingested before attempted penetration into the fruit. Additionally, the increased time spent on the fruit surface could increase larval exposure to potential mortality factors such as wind, dust, parasitism or predation.

Opsomming

Valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (1912) (Lepidoptera: Tortricidae), is een van die belangrikste sitruplae in Suid-Afrika en mag moeilik wees om te beheer. Om die behoeftes van streng uitvoer regulasies na te kom is verskeie metodes om VKM te beheer ontwikkel. Nietemin veroorsaak die plaag nogsteeds ekonomiese verliese vir die Suid-Afrikaanse sitrusbedryf elke jaar. Tussen die beheer metodes bestaan die *Cryptophlebia leucotreta* granulovirus (CrleGV), geregistreer onder die name Cryptogran, Cryptex en Gratham, wat suksesvol VKM in die veld kan beheer. Sy werking word bepaal deur die klein venster waar pasuitgebroeide VKM larwes voed voor penetrasie tot in die vrug. Die doel van hierdie studie was om die gedrag van pasuitgebroeide larwes te ondersoek in die teenwoordigheid van molasse, as ŉ bakulovirus byvoegsel op die oppervlak van Nawellemoene. In vorige eksperimente, met die gebruik van visuele opmerking, het die byvoeging van molasse voeding in kolle op die vrugoppervlak veroorsaak, sowel as ŉ vermindering in totale beweging en penetrasie as gevolg van voeding op die oppervlak. Hierdie verdere ondersoek van gedrag het gebruik gemaak van videokameras om akkuraat die gedrag van pasuitgebroeide VKM larwes waar te neem (en op te neem) wanneer hulle aan molasse konsentrasies blootgestel word. Dit sluit in die wetenskaplike kwantifisering van beweging, tydsduur van voeding en penetrasie vir die verskillende behandelings. Analise van die data oor die effek van molasses op pasuitgebroeide VKM larwes – albei visuele waarneming en video opnames – het molasse beweging van larwes op die vrug oppervlak verminder (gemeet in afstand), tydsduur op die vrug oppervlak verleng (as gevolg van verhoogde voeding) en het vrugpenetrasie verminder. Implikasies is dat molasse as a voedingsstimulant werk wat potensiaal die waarskynlikheid van inname van ŉ letale virus dosis voor vrugpenetrasie vermeerder. Verder kan die langer tydsduur op die vrugoppervlak blootstelling van larwes aan ander moontlike mortaliteits faktore blootstel, soos wind, stof, parasitisme of predasie.

Introduction

Baculovirus-based biopesticides such as Cryptogran and Cryptex, which are formulated with an isolate of the *Cryptophlebia leucotreta* granulovirus (CrleGV), are popular biological control options used by farmers in southern Africa. CrleGV, of the family *Baculoviridae*, is host specific to FCM and are unique due to the

presence of occlusion bodies (OBs) which contribute to both the stability and persistence of the virus within the environment (Moore 2002). CrleGV is highly successful in its mode of action.

Unlike chemical insecticides which are soluble substances, viral insecticides such as CrleGV consist of solid particles (OBs) which need to be ingested to be effective. In order to increase effectiveness of CrleGV (in the form of Cryptogran) as a biological control agent suitable under IPM systems, is the possibility of a feeding stimulant as an alternative approach in stimulating ingestion of lethal doses of virus before actual penetration into the fruit. According to Moore *et al.* (2004) various trials have showed that the efficacy of Cryptogran is enhanced when sprayed in conjunction with molasses. The molasses was believed to act as a sticking agent preventing the virus from being washed off the fruit as well as a feeding attractant to FCM larvae, which could potentially cause increased virus ingestion (Moore *et al.* 2004). Due to its stickiness, molasses also may increase larval exposure to other potential external mortality factors such as wind, dust, parasitism or predation.

There is a very brief period between hatching of the larvae and their penetration into the fruit. Neonate larvae of FCM are required to ingest the virus for it to be effective and as long as the virus on the surface of the fruit remains viable, potential infection can occur. The feeding of neonate larvae before fruit penetration is a key aspect that restricts the effectiveness of CrleGV and additives that increase larval exposure and ingestion of OBs have the potential to improve viral efficacy. In a previous experiment conducted using visual observation the addition of molasses induced feeding in areas along the surface of the fruit; a statistically significant (F= 42.5; F-crit= 1.6; df= 2; p= 0.00899) decrease in total movement due to surface feeding as well as a decrease in total penetration was observed.

This continuation in behavioural investigation aimed, with the use of video cameras, to accurately observe and record the behaviour of FCM neonate larvae when exposed to concentrations of molasses. In addition to that, we aimed to scientifically quantify their movement, time of feeding and penetration between each treatment. A further aim, based on the addition of molasses as a phagostimulant, is to prolong surface feeding by FCM neonates, delaying penetration and increasing the potential for ingestion of a lethal dose of CrleGV before penetration into the fruit.

Materials and methods

Source of insects
FCM was reared as described by Moore (2002) and Moore et al. (2014), but at a temperature of approximately ±21ºC and 30 % RH. To obtain neonate larvae for trials, one square egg sheet (wax paper) each containing approximately 100 eggs, were placed in sterile honey jars which were left in a constant environment (CE) room at 28ºC and ~60% RH until eggs hatched.

Treatment preparation
In producing the individual treatments, 0.25% and 0.50% molasses concentrations were prepared in separately labelled 1000 ml beakers. 500 ml dH_2O was placed in each respective beaker and measured amounts of molasses syrup, according to concentration, were added and mixed with the dH_2O using a plastic dropper with suction bulb. The control was prepared by using 500 ml dH_2O on its own in a separate, labelled, 1000 ml beaker. Two individual oranges per treatment were submerged in the respective beakers and allowed to dry before the introduction of one FCM neonate larvae onto each treated fruit surface. This procedure was repeated three times for a total of three replicates.

Detached fruit bioassays
Navel oranges which had reached colour break were used in the bioassays and each exposed to different treatments: 1) the control, submerged in dH_2O, 2) treatment with a 0.25% concentration of molasses and 3) treatment with a 0.5% concentration of molasses. After the oranges were covered by the respective treatment and allowed to dry, a single orange was placed with navel ends to the side onto a pre-made stand in the centre of three video cameras placed 1) directly above 2) to the left in view of one diagonal half of the orange and 3) to the right in view of the remaining diagonal half of the orange (Fig. 3.2.15.1). Each video camera was measured and placed exactly 6.5 cm away from the base of the stand containing the test orange.

A single FCM neonate larva from the hatching jar was carefully removed and placed onto the top centre of the treated orange using a size 000 sterile paintbrush. All three video cameras were then simultaneously set to record for a period of half an hour. This process was then repeated for each replicate and neonate behaviour was monitored and recorded. In a first trial (semester 1), 10 replicates were used and in a second trial (semester 2: a repeat of the first), six replicates were used. Using imageJ software the distance

travelled by the neonates as well as penetration times from the video footage were then analysed and recorded.

Figure 3.2.15.1. Experimental setup including positioning and placement of individual video cameras for determining an accurate account of the effect an introduction of molasses has on FCM neonate larvae behaviour.

Results and discussion

Oranges were treated with water, 0.25% and 0.5% concentrations of molasses and once dry, three FCM neonate larvae were placed on each orange and observed. At regular time intervals larval movement was recorded with a dot and number to visually indicate movement patterns over the surface of each treated fruit.

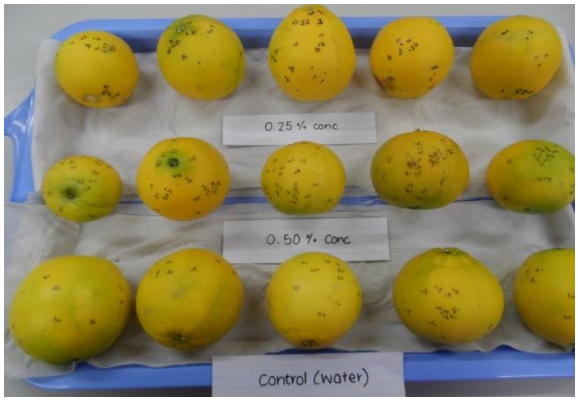


Figure 3.2.15.2. Visual display of the movement patterns by the FCM neonate larvae in replicate 1 from experiments conducted in semester 1.

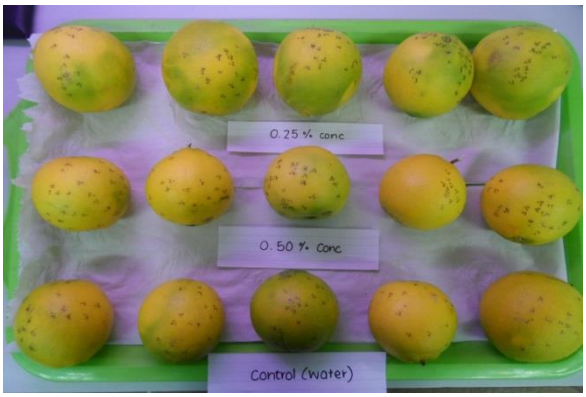
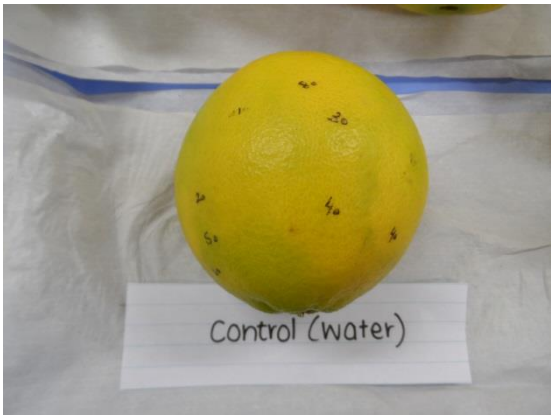
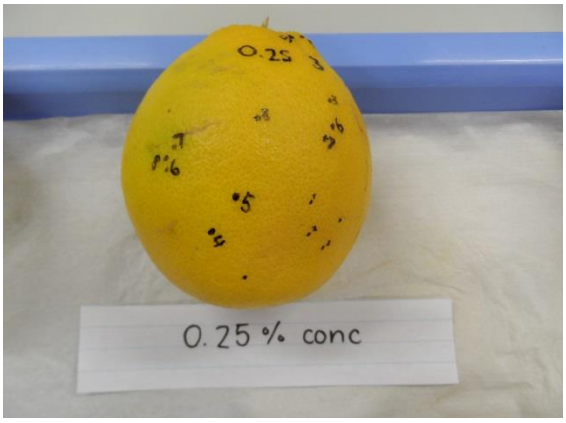


Figure 3.2.15.3. Visual display of the movement patterns by the FCM neonate larvae in replicate 2 from experiments conducted in semester 1.

In Figures 3.2.15.2, 3.2.15.3 and 3.2.15.4 the dots show the difference in movement patterns between each treatment. In the 0.25% treatment there is moderate grouping/ patches of movement in combination with dispersed movement over the surface of the fruit. In the 0.5% treatment less dispersed movement is seen over the surface of the fruit, with movement in more concentrated patches/ areas. In the water treatment movement in patches is a lot less and more even or dispersed movement over the surface of the fruit is seen.



A



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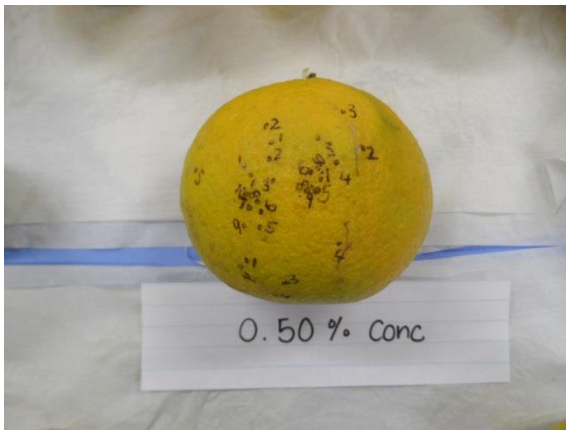

C

Figure 3.2.15.4. Samples taken from each treatment, A) water B) 0.25 % molasses and C) 0.50 % molasses concentrations, as a close up representation of the movement patterns by the larvae across the fruit surfaces.

Larval movement was visually recorded using video cameras and analyses were performed by imageJ software to accurately measure the distances each larva travelled over each treated fruit surface.

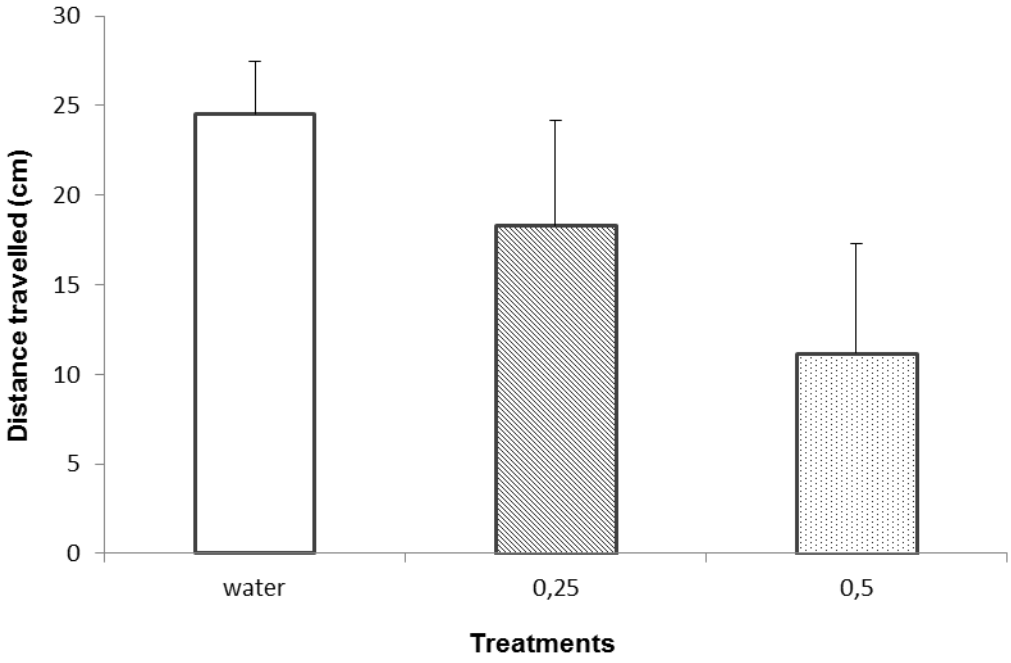

Figure 3.2.15.5. Total mean distance FCM neonate larvae travelled across all replicates treated with water (24,5 ± 3 n=18), 0,25 % (18,3 ± 5,9 n=18) and 0,5 % (11,2 ± 6,1 n=18) molasses**.**

The total mean distance travelled by FCM neonate larvae is the highest for oranges treated with water. For the oranges treated with 0.25 % and 0.5 % molasses concentrations, a decrease in distance travelled is evident indicating that with an increase in molasses concentration less movement by the larva occurs (Fig. 3.2.15.5).

The results from the experiment conducted in semester 1 were compared with the results obtained in semester 2 to identify any similarities or trends present in the data.

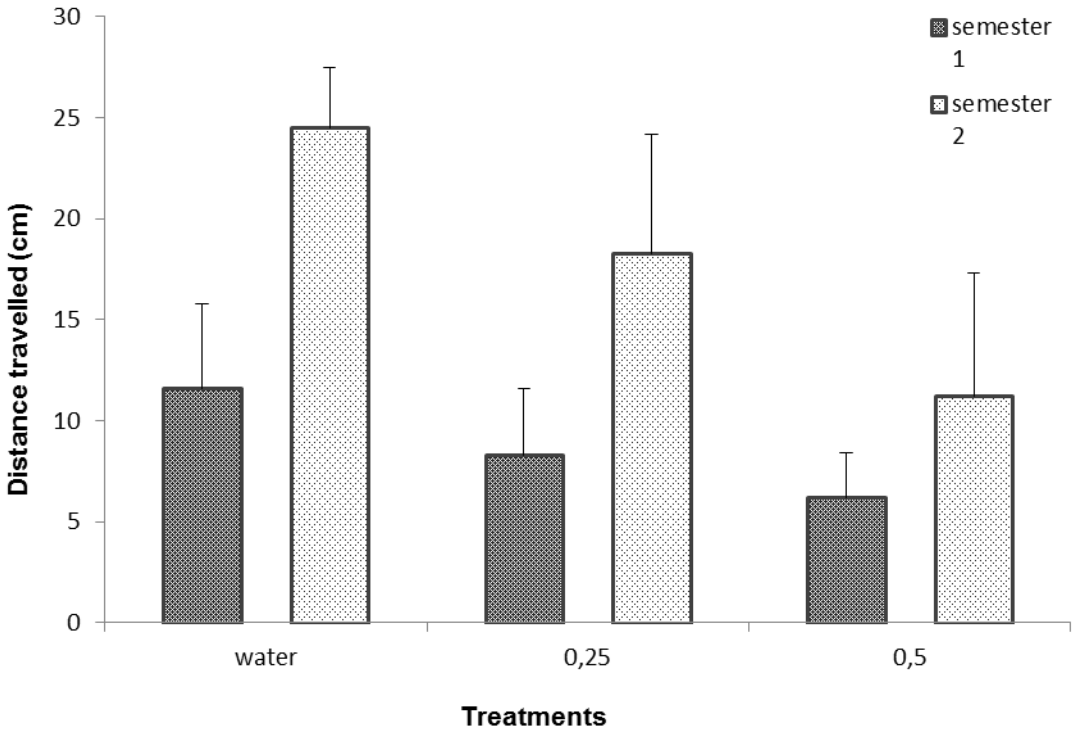

Figure 3.2.15.6. Total mean distance FCM neonate larvae travelled across all replicates treated with water (11,6 ± 4,2 n=30, 24,5 ± 3 n=18), 0,25% (8,3 ± 3,3 n=30, 18,3 ± 5,9 n=18) and 0,5% (6,2 ± 2,2 n=30, 11,2 ± 6,1 n=18) molasses concentrations.

The comparison of total mean distance travelled by larvae between semester 1 and semester 2 results indicate that a similar trend occurs in each experiment (Fig. 3.2.15.6). In both experiments the mean distance travelled by the larvae is highest on the oranges treated with water, and with the increase in molasses concentrations total mean distance travelled by the larvae decreases.

The distances travelled by the larvae on each treated orange were accurately analysed using imageJ software in order to record any differences in distance within the set time intervals between each treatment.

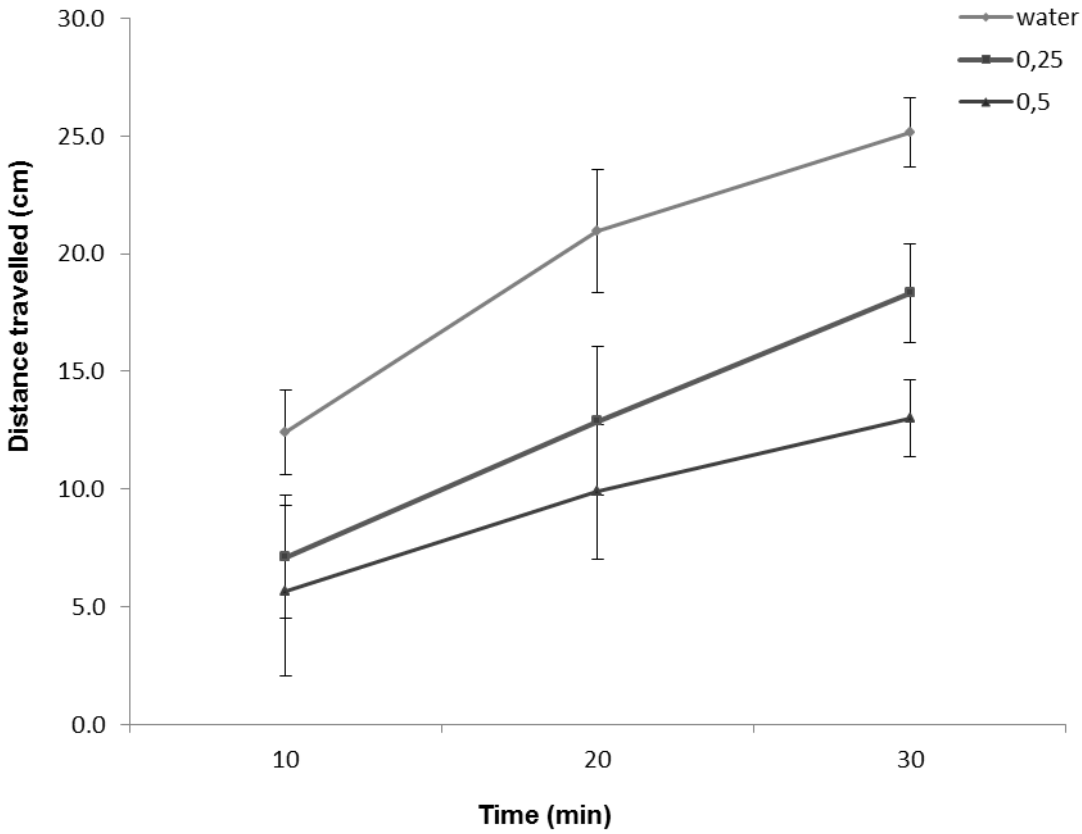

Figure 3.2.15.7. Mean total distance FCM neonate larvae travel within each time interval across the individual treatments.

The oranges treated with water show higher mean total distances travelled within all three time intervals when compared with the molasses treated oranges (Fig. 7). Within the water treatment, between 0-10 minutes mean distance (MD) travelled by the larvae was 12.4 cm, 10-20 minutes MD=8.6 cm and 20-30 minutes MD=4.2 cm. Within the 0.25 % molasses treatment, between 0-10 minutes MD=7.1 cm, 10-20 minutes MD=5.8 cm and 20-30 minutes MD=5.4 cm. Within the 0.5 % molasses treatment, between 0-10 minutes MD=5.7 cm, 10-20 minutes MD=4.2 cm and 20-30 minutes MD=3.1cm.

Table 3.2.15.1. Total number of penetration events by the larvae across all replicates within each time interval.

Time (min)	Semester 1			Semester 2		
	water	**0.25 %**	**0.50 %**	**water**	**0.25 %**	**0.50 %**
0-10	0	0	0	0	0	0
10-20	3	2	0	0	0	0
20-30	5	2	1	4	1	0
30-40	2	3	3	-	-	-
40-50	1	4	2	-	-	-
50-60	6	1	3	-	-	-
Sum	**17**	**12**	**9**	**4**	**1**	**0**

Penetration events for the water treatment were most frequent within earlier time intervals compared with the molasses treatments which show delayed penetration as penetration events occur within later time intervals. The total number of penetration events was highest for the water treatment which then decreased with the addition of molasses (Table 3.2.15.1).

Using the video footage and imageJ software, penetration by the larvae into the treated oranges was analysed and time of penetration was recorded.

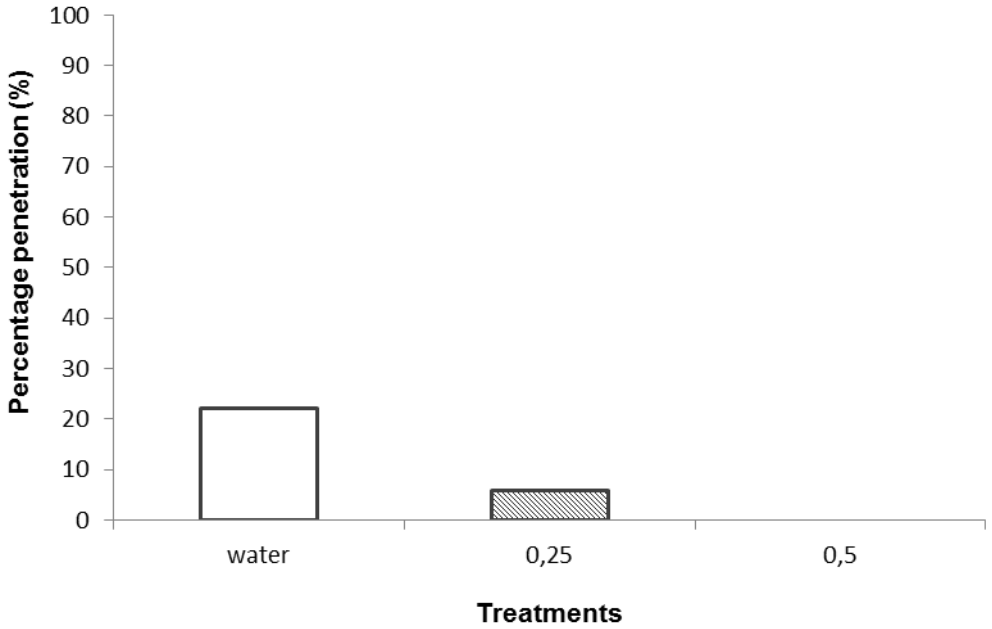

Figure 3.2.15.8. Total percentage penetration of larvae from all replicates between each treatment.

The total percentage penetration was highest in the water treatment with penetration events decreasing with an increase in molasses concentration. Penetration did occur in the 0.25 % water treatment however, there were fewer penetration events than the water treatment and in the 0.5 % molasses treatment no penetration occurred (Fig. 3.2.15.8).

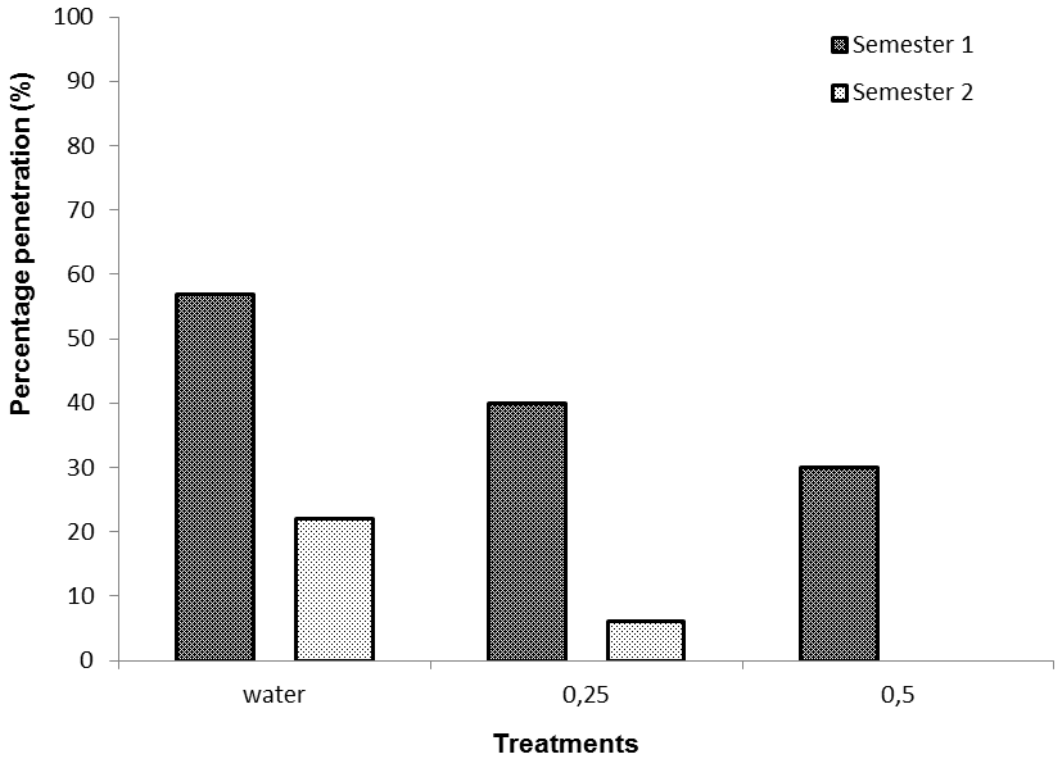

Figure 3.2.15.9. Total percentage penetration of all larvae between each treatment for experiments conducted in semester 1 vs semester 2.

A comparison was made between the results obtained from the experiment conducted in semester one vs the results obtained in semester two to determine any trends as well as to obtain a clear understanding of how the different treatments affect the number of penetration events (Fig. 3.2.15.9).

The observational results visually showed a difference in movement patterns by the larvae between each treatment. Movement on the water treated oranges was dispersed and no pattern was seen as there were indications of movement along the whole surface of the fruit. This is indicative of the larvae searching for a suitable entry point before the onset of penetration whereby the larvae would bore into the albedo and feed just below the surface leading to infestation. Movement on the 0.25% molasses treated oranges also showed fairly dispersed movement. However, there was an emergence of movement in patch-like clusters along the surface of the fruit. The larvae appeared to move along the surface in small stretches whereby they then proceeded to decrease movement remaining within a small area whilst surface feeding. Movement on the 0.5 % molasses treated oranges showed less dispersed movement that consisted of small distances being travelled between bouts of surface feeding. Many of the larvae on the 0.5% molasses treated oranges moved very small stretches as most of their time was spent surface feeding. From these visual observations it was seen that an introduction of molasses onto the surface of the fruit caused the larvae to travel less over the surface of the whole fruit but to rather move shorter distances between stretches of surface feeding.

In order to determine a scientifically accurate and quantifiable account of the effect an introduction of molasses has on FCM neonate larval behaviour, larval movement was recorded and analysed using video cameras in conjunction with imageJ software. This enabled the measurement of the distance each larva travelled over each treated fruit surface. The total mean distance travelled by the FCM neonate larvae was found to be highest for the water treatment (24±3 n=18) decreasing with the introduction of 0.25% (18±5.9 n=18) and 0.50% (11.2±6.1 n=18) molasses concentrations. Statistical analyses were performed using ANOVA whereby a significant difference ($F = 7.3$; F-crit = 3.7; df = 2; $p = 0.006116$) was detected in the total mean distance travelled by FCM neonate larvae between each treatment. This indicates that the presence of molasses significantly ($p<0.05$) influences the distance FCM neonate larvae travel over the surface of the fruit.

As the results obtained in the experiments conducted using the video cameras were scientifically more accurate a comparison was made between the results obtained using visual observations (semester 1) vs the results obtained using video footage (semester 2). Overall total mean distance (TMD) travelled

decreased in the results obtained using video footage. However, a similar trend was seen in both experiments whereby TMD travelled was highest in the water treatments from both experiments and the presence of molasses decreased TMD travelled for both the 0.25% and 0.50% molasses concentrations respectively. Although the two experiments were conducted using different observation methods, the data showed a similar decreasing trend across all three treatments.

Within the thirty minute video recording time frame, the total distance travelled by the larvae within 10 minute intervals was analysed between each treatment. The water treatment again showed higher MTD travelled within all three ten minute intervals compared with the molasses treated oranges. Although as time proceeded, the total distance travelled increased. However, between each 10 minute interval the distance travelled within intervals decreased. This decrease between each 10 minute time interval was seen across all treatments.

Penetration by larvae into the albedo is of great concern as once the larvae have penetrated the fruit, infestation takes place and the fruit is considered no longer a marketable item. In addition to quantifying the distance FCM neonate larvae travel over the treated fruit surfaces, time and total number of penetration events were also recorded and analysed. Penetration events were found to occur within an earlier time period (between 10 to 30 minutes) for the water treatment and delayed penetration was seen in the fruits treated with molasses as penetration events were more frequent within a later time period (between 30 to 60 minutes). Total penetration was the highest in the water treatment and as a percentage a decrease in total penetration events occurred with the introduction of molasses onto the fruit surfaces. The observational results displayed this trend with added confirmation obtained by analysis of the video footage data.

The data reported herein suggest that the introduction of molasses affected the behaviour of neonate FCM larvae in a number of ways. Larval movement over the molasses treated oranges was seen to be in patch-like clusters along the surface of the fruit which is indicative of two actions 1) decreased dispersed movement is due to the phagostimulatory effect of molasses whereby time is spent surface feeding and 2) the molasses coated surface acts as a sticking agent which prevents the larvae from moving freely over the fruit surface. Using video recording and imageJ analyses the total mean distance the larvae travelled was seen to decrease with an increase in molasses concentration and after statistical analyses, it was shown that the presence of molasses significantly influences the distance FCM neonate larvae travel over the fruit surface. Penetration events on the molasses treated oranges by the larvae were delayed and predominantly occurred between 30 to 60 minutes after placement onto the fruit surface. In comparison to the molasses treatments, penetration predominantly occurred within zero to 30 minutes after placement onto the fruit surface and penetration events were seen to occur throughout the observation period on the control (water) treatment. Total percentage penetration was also seen to be less with the introduction of molasses onto the fruit surfaces.

Conclusion

In analysing the effect molasses has on FCM neonate behaviour by the use of both observational and video recording aspects the implications of the data obtained are as follows: the addition of molasses as a phagostimulant prolongs surface duration by the larvae before penetration into the albedo and combined with its stickiness, the molasses can possibly increase larval exposure to potential mortality factors such as wind, dust, parasitism or predation and in conjunction with CrleGV, in the form of Crytogran or Cryptex, may aid in improving the potential for ingestion of a lethal dose of virus before penetration into the fruit thus improving viral efficacy.

Technology Transfer

This work will be presented at the Citrus Research Symposium in August 2014.

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3.2.16 PROGRESS REPORT: Classical biocontrol introduction of *Agathis bishopi* into the Western Cape

Project 1077 (2013/14 – 2014/15) by Martin Gilbert (CRI)

Summary

Collection of sanitation fruit was carried out in three citrus orchards in the Citrusdal area and one in Stellenbosch. The level of FCM infestation was very low with no presence of *A. bishopi*. FCM pressure in the Citrusdal area is extremely low so far this season. The amount of sanitation fruit has been minimal and the presence of *A. bishopi* or any other larval parasitoid has not been recorded thus far. Similarly *A. bishopi* was not recorded at Stellenbosch. FCM incidence has recently increased at a new site near Citrusdal (Bo-Bergvlei) at an increased altitude and outside the FCM SIT treatment area. Sampling of sanitation fruit will also be pursued here in further efforts to try and trace whether *A. bishopi* or any other larval parasitoids are present in the Western Cape or not. At the moment it is believed that a sufficiently large number of fruit has not yet been sampled in order to definitely say whether *A. bishopi* or other larval parasitoids are present in the Western Cape or not. This study will continue for at least another season.

Opsomming

Versamelling van sanitasie vrugte is uitgevoer in drie sitrusboorde in Citrusdal en een in Stellenbosch. Die vlak van VKM besmetting was baie laag en geen *A. bishopi* of enige andere larwe-parasiet is opgespoor nie. Die hoeveelheid sanitasie vrugte was minimal en die teenvoordigheid van *A.bishopi* of enige ander larwe-parasite was op Citrusdal nog nie vasgestel. *A.bishopi* was ook nie op Sellenbosch aangeteken nie.
Onlangs het die druk van VKM toegeneem in die BoBergvlei area naby Citrusdal, wat buite die VKM SIT area is. Die ontleding van sanitasie vrugte in die spesifieke area sal voortgesit word om verder te probeer vastel of *A. bishopi* of enige ander larwe-parasiet in die Wes Kaap teenwordig is of nie. Tot nou toe word dit geglo dat heelwat meet vrugte ontleed moet word om met sekerheid te kan sê of *A. bishopi* of enige ander larwe-parasiet wel in die Wes-Kaap teenwoordig is of nie. Hierdie studie sal vir minstens nog ŉ seisoen voortgesit word.

3.2.17 PROGRESS REPORT: Novel approaches to mating disruption of FCM

Project 1080 by Martin Gilbert (CRI)

Summary

Registered chemicals for FCM were applied in order to investigate whether increased applications of FCM mating disruption products could boost the degree of control of FCM. If this could be demonstrated, then ways of lowering production costs by, for example, mass chemical synthesis would be investigated. A suitable site was identified in the Citrusdal area for a comparison of a new River Bioscience (RB) mating disruption (MD) product, aerial applications of Checkmate and Isomate, as well as combinations thereof. A single application of the River Bioscience product was applied using special machinery supplied by RB for the trial. In the author's opinion a more even application of the material was needed. Due to the extremely low levels of FCM in the area it was decided not to apply another treatment. FCM pressure in the Citrusdal area was extremely low with no meaningful moth catches or fruit drop to date. Monitoring continues and the experiment will be repeated in the 2014-15 season, for which funds have been budgeted and approved.

Opsomming

Geregestreerde chemikalië vir VKM is toegedien om vas te stel of verhoogde toedienings van VKM paringsontwrigtingsprodukte, die beheer van VKM kan verbeter. Indien hierdie aanname bewys kan word, kan moontlik produksiekostes verlag word, deur byvoorbeeld om die massaproduksie van chemikalië te ondersoek. ŉ Geskikte proefperseel in die Citrusdal-area is geidentifiseer om vergelykings te trek tussen die nuwe Riverbioscience paringsontwrigtingsproduk, lugtoedienings van Checkmate en Isomate asook kombinasies van die produkte. ŉ Enkele toediening Riverbioscience MD produk en twee Checkmate lugbespuitings is op die proefperseel gedoen, terwyl Isomate teen die geregistreerde dosis op die res van die plaas toegedien is. Die baie lae druk van VKM in die Citrusdal area het tot geen betekenisvolle resultate van die proef gelei nie . Vondse is goedgekeur vir 2014/15 boekjaar om die proef te herhaal.

3.2.18 **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 – 2015/6) by Martin Gilbert (CRI)

Summary

A total of 13 blocks of differing fruit types are being monitored for fruit flies (using Biolure 3 component baited traps) and FCM (using Chempac Delta and lure traps) on a weekly basis. Fruit fly lures are changed at 6-weekly intervals and FCM lures at 3-monthly intervals. Fruit types being monitored include citrus, grapes, plums, nectarines, apples and pears. At Riebeek Casteel, Natal fly *Ceratitis rosa* was a significant factor in nectarines and catches were high until mid-December, post-harvest. Thereafter this species practically disappeared and did not migrate onto later-maturing fruit types such as grapes, citrus or plums. Regarding Medfly, *C. capitata*, this species was of less significance than Natal fly on early nectarines but, unlike *C. rosa*, persisted to infest later maturing fruit types in this area. During December, most flies were caught in nectarine and plum blocks. During January, numbers were generally very low but shifted to vineyards. During February and March, numbers of *C. capitata* increased considerably. During March, trap catches in (already harvested) nectarines again increased although the reason for this is unclear at present. Regarding citrus (Midknights and Clementines), numbers of flies were significantly below those in the grape and nectarine blocks but were at risk of invasion from flies originating in these blocks as the fruit colours up. At Stellenbosch, Natal fly persisted from December to April (present time), unlike at Riebeek Casteel where it all but disappeared after December. In terms of highest trap catches, during December, nectarines were the most favoured host fruit. Later in the season during February and March, plums followed by apples and then citrus were the preferred hosts. Trap catches in pears were generally low and this seems not to be a preferred host for *C. rosa*. Regarding Medfly, activity was generally very low in December being mostly confined to nectarines. During January, apples were the most favoured host. During February a well-defined peak of activity occurred on pears with apples and plums being the next favoured hosts. Fly catches on apples and plums increased considerably in March. During late March and early April, citrus began to attract large numbers of flies as Clementines coloured up. At Stellenbosch, during December, the vast majority of FCM were caught in nectarines, followed by plums. Despite being harvested in November, FCM numbers in nectarines were highest in 12 out of 19 sampling weeks. When plums and nectarines were considered together, FCM numbers in these fruit types were highest in 14 out of the 19 sample weeks. FCM numbers trapped in pear and apple orchards were lowest and these fruit types are not considered to be hosts. Regarding citrus, FCM was absent during December and only very low numbers were caught during January and February. During March numbers increased and during early April were up to 9 per trap. At Riebeek Casteel, during late November and early December, FCM numbers peaked in a plum orchard prior to a peak in citrus in mid-December. Despite the early harvesting of nectarines, during November, FCM numbers in a block of this fruit type began to increase throughout January to March such that FCM numbers were highest in 9 out of 13 sample weeks. When FCM in plum orchards and nectarines were considered together, this total increased to 10 out of 13 weeks, despite both fruit types being harvested before the end of 2013. FCM numbers caught in vineyards were comparatively low. Regarding citrus, numbers were also very low compared to those caught in nectarine and plum orchards. It is perhaps difficult to account for the tendency of FCM to persist in nectarine and plum orchards long after harvest. It was confirmed by observation that all the plum and nectarine cultivars being monitored possessed extrafloral nectaries on the leaves or nodes. It has been postulated that the presence of extrafloral nectaries may enhance the persistence and reproductive potential of Oriental Fruit Moth, *Grapholita molesta*, in peaches and nectarines by providing valuable carbohydrates. Whether this is of relevance to FCM remains to be seen but should be an avenue to be explored. Monitoring will be expanded to include where pomegranates and citrus are grown in close proximity.

Opsomming

Dertien blokke van verskeie vrugtetipes word op ŉ weeklikse basis gemonitor met VKM en vrugtevlieg valletjies (met Chempac VKM Delta valletjies) en met Biolure as lokmiddel vir vrugtevliee.Verskillende vrugte soorte sluit in sitrus, druiwe, pruime, nektariens, appels en pere. By Riebeek Kasteel, was Natalvlieg ŉ betekenisvolle faktor op nektariens en vangstes was hoog tot middel Desember (na oes). Daarna het die spesie verdwyn en het nie ander later vrugsoorte aangeval nie. Met Medvlieg was vangstes laer in Desember as dié van Natalvlieg maar Medvlieg het wel later rypwordende vrugsoorte in die area aangeval. Gedurende Desember is meeste vlieë in nektarien- en pruimboorde gevang. In Januarie het getalle oor die algemeen afgeneem maar meeste vlieë het in wingerde voorgekom. In Februarie en Maart het getalle weer oor die algemeen toegeneem. In Maart het getalle in (klaar gëoeste) nektariens toegeneem, maar die rede hiervoor is onduidelik. Wat sitrus betref, was vlieg getalle betekenisvol laer as in druiwe en nektarien blokke maar daar was ŉ risiko van immigrasie van laasgenoemde vrugtetipes. By Stellenbosch, het Natalvlieg aanhoudend van Desember tot April voorgekom terwyl by Riebeek Kasteel het die spesie na Desember

amper verdwyn. In Desember is meeste vlieë op nektariens gevang, maar gedurende Februarie en Maart was dit op pruime, gevolg deur appels en laastens sitrus. Vangstes op pere was oor die algemeen baie laag in vergelyking met die ander vrugtipes. Wat Medvlieg betref, was aktiwiteit in Desember baie laag maar meestal op nektariens. In Januarie was appels die gunsteling gasheer in terme van vangstes. Gedurende Februarie het ŉ duidelike piek op pere voorgekom gevolg deur appels en pruime. Gedurende laat Maart en April het vlieg getalle op sitrus toegeneem. Op Stellenbosch, gedurende Desember, is die groot meederheid VKM in nektarienboorde gevang, gevolg deur pruime. Teen spyte van oestyd in November, is die meeste VKM gevang in nektarienboorde in 12 uit die 19 weke van Desember tot April. As vangstes op nektariens en pruime bymekaar getel word, het hierdie syfer tot 14 uit die 19 weke gestyg. Gedurende hierdie tydperk, was VKM getalle op appels en pere oor die algemeen baie laag en kan dus nie as gashere beskou word nie. Wat sitrus betref, was VKM afwesig in Desember, met net lae vangstes in Januarie en Februarie. Gedurende Maart en April het getalle toegeneem. By Riebeek Kasteel, gedurende laat November en Desember, het pruime die meeste VKM gelok, gevolg deur sitrus. Teen spyte van ŉ vroeë oestyd (November) het VKM getalle in nektariens vanaf Januarie tot Maart toegeneem. In 9 uit 13 weke was VKM getalle in nektariens die hoogste. As vangstes in nektariens en pruime bymekaar getel word, het hierdie totaal gestyg tot 10 uit 13 weke. VKM vangstes was relatief laag in wingerde en sitrus in vergelyking met dié van nektariens en pruime. Dit mag dalk moeilik wees om te verstaan waarom VKM konstant in nektarien en pruimboorde voorkom lank nadat hulle geoes is. Dié kultivars het wel buiteblom nektarkliere op hulle blare en/of nodes. Vorige studies het al bespiegel dat die teenwoordigheid van sulke nektarkliere kan bydra tot ŉ verhoogde aanteelvermoë van Oostersevrugmot (OVM) in perske en nektarien boorde deur die verskaffing van koolhidrate. Of nektarkliere wat buite die blomme voorkom (extrafloral nectaries) van belang is vir VKM in nektarien- en pruimboorde bly ŉ ope vraag wat nagevors sal moet word. Hierdie projek sal in die toekoms ook granate insluit waar hierdie gewas naby sitrus gekweek word.

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

Project 1085 (March-October 2014) by Sean D Moore and Wayne Kirkman (CRI)

Summary

The European Plant Protection Organisation (EPPO) has completed a Pest Risk Assessment (PRA) of FCM for Europe. This may lead to more stringent regulations on importation of southern African citrus (and other fresh produce from Africa that can host FCM). The worst case scenario is that cold sterilisation (shipping at -0.6ºC for 22 days) will become mandatory for all citrus exports from South Africa to Europe. This will effectively make Europe an unviable destination, with devastating effects for the industry as a whole. Consequently, CRI has been proactive in drafting a systems approach for management of FCM as an effective alternative to cold sterilisation. The systems approach includes a series of mandatory management steps from pre-season to post-packing, including weekly inspections at orchard level up to the time of harvest, pre-packing and post-packing inspections. Infestation thresholds have been suggested which determine whether the fruit qualifies to move onto the next step in the system or if it should be rejected on the grounds of unacceptable risk. This study aims to scientifically validate these standards and to alter them if necessary in order to make the systems approach as accurately predictive and reliable as possible. Although this study is only funded from April 2014, it was initiated earlier due to its urgency. During the 2012/13 season, regular inspections of FCM infestation in fruit in eight citrus orchards throughout the season, including up to the time of harvest, inspections of fruit post-harvest (but pre-packing), post-degreening (if degreening was conducted) and post-packing. This is being repeated in 12 orchards in the 2013/14 season. Indications to date are that a) pre-harvest levels of FCM are a good indication of post-harvest risk, and b) current arbitrarily selected standards are likely to be quite accurate. However, more data are required for greater certainty.

Opsomming

Die Europese Plantbeskermingsorganisasie (EPPO) het ŉ Plaag Risiko Analise (PRA) op VKM vir Europa voltoei. Dit kan tot strenger regulasies op die invoer van suidelike Afrikaanse sitrus (en ander vars produkte van Afrika wat gashere vir VKM kan wees) lei. Die ergste moontlike scenario is dat kouesterilisasie (verskeping teen -0.6ºC vir 22 dae) verpligtend vir alle sitrusuitvoere van Suid-Afrika Europa toe sal word. Die uiteinde is dat Europa ŉ onlewensvatbare bestemming sal word, met ŉ teisterende effek op die bedryf as geheel. Daarom het CRI proaktief opgetree en ŉ stelselsbenadering vir die bestuur van VKM as ŉ doeltreffende alternatief vir koue sterilisasie opgestel. Die stelselsbenadering sluit in ŉ reeks verpligtende bestuursstappe van voorseisoen tot na verpakking. Dit sluit in weeklikse boord inspeksies tot oestyd, en voor- en na-verpakking inspeksies. Besmettings drempelwaardes is voorgestel wat sal bepaal of die vrugte kwalifiseer om aan te skyf na die volgende stap toe of as hulle afgekeur moet word as gevolg van onaanvaarbare risiko. Die doel van hierdie studie is om hierdie standaarde wetenskaplik te verifieer om die

stelselsbenadering so akuraat en betroubaar as moontlik te maak. Alhoewel hierdie studie eers van April 2014 bevonds word, is dit vroeër begin as gevolg van dringendheid. Gedurende die 2012/13 seisoen is gereelde inspeksies van VKM besmetting van vrugte in agt sitrusboorde deur die loop van die seisoen tot oestyd gedoen. Daarna is inspeksies na-oes (maar voor verpakking), na ontgroening (as ontgroening uitgevoer is) en na verpakking gedoen. Hierdie word in 12 boorde in die 2013/14 seisoen herhaal. Tans is aanduidings dat a) vooroes vlakke van VKM 'n goeie aanduiding van na-oes risiko is, en b) huidige eiemagtige standaarde lyk redelik akuraat. Nietemin word meer data benodig vir groter sekerheid.

3.2.20 PROGRESS REPORT: FCM infestation of packed lemons destined for export

Project 1087 (March-October 2014) by Sean D Moore and Wayne Kirkman (CRI)

Summary

Entry of lemons into markets which require cold-sterilisation (-0.6°C for 22 days) of fruit cannot be achieved through current protocols. Lemons cannot be cold sterilised in transit due to their susceptibility to chilling damage and it is unlikely that many lemon orchards could be declared free of false codling moth (FCM), *Thaumatotibia leucotreta*, on the basis of pheromone baited trap catches. Consequently, several years of research have been conducted in attempting to prove that lemons pose no FCM-risk for export markets. However, it has not yet been possible to conclude this, as moths have been caught in lemon orchards and lemon fruit have been shown to be susceptible to forced infestation in the laboratory. Over-ripe and very small lemons were the most susceptible. Orchard surveys of these types of lemons were conducted in 2010 and 2012, with 1.75% and 1.25% of the fruit in trial orchards being naturally infested. It is clear that although an unfavourable host, lemons cannot be proven a non-host for FCM. However, it is still possible or even probable that lemons harvested at the normal maturity level for export will not be infested with FCM. This current trial is being conducted to determine the likelihood at probit 8.7 level that no lemons packed for export will be infested with FCM. Such a finding will strengthen an argument to exclude lemons from cold-sterilisation to markets which require such a practice for FCM. In the near future, this could even apply to Europe. Up to the end of March around 9000 class 1 export lemons had already been dissected, with none of them showing any signs of FCM infestation. This will be continued until a total of at least 29956 fruit have been inspected.

Opsomming

Toegang van suurlemoene tot markte wat koue-sterilisasie (-0.6°C vir 22 dae) vereis kan nie deur gebruik van huidige protokolle bereik word nie. Suurlemoene kan nie gedurende verskeping aan koue sterilisasie blootgestel word nie omrede hulle vatbaarheid vir koue skade. Dit is ook onwaarskynlik dat veel suurlemoenboorde as valskodlingmot (VKM), *Thaumatotibia leucotreta*, vry verklaar sal kan word op die basis van feromoon-lokval vangstes. Daarom is heelwat jare se navorsing uitgevoer in 'n poging om te bewys dat suurlemoene geen VKM-risiko vir uitvoer markte inhou nie. Nietemin is dit tot dusver nie moontlik gewees om so 'n gevolgtrekking te maak nie omrede motte in suurlemoen boorde gevang is en dit gewys is dat suurlemoen vrugte vatbaar is vir geforseerde besmetting in die laboratorium. Oorryp en baie klein suurlemoene is die mees vatbaar. Boord opnames van hierdie tipe suurlemone in 2010 and 2012 het gewys dat onerskeidelik 1.75% en 1.25% vrugte in proefboorde natuurlik besmet was. Dit is duidelik dat al 'n ongunstige gasheer, kan dit nie bewys word dat suurlemoene nie vir VKM 'n gasheer is nie. Nietemin is dit nogsteeds moontlik of selfs waarskynlik dat suurlemoene, gëoes teen die regte rypheid stadium vir uitvoer, nie met VKM besmet sal wees nie. Hierdie huidige proef word tans uitgevoer om die waarskynlikheid, teen probit 8.7 vlak, te bepaal dat geen suurlemoene wat met VKM besmet is vir uitvoer gepak sal word nie. So 'n bevinding sal 'n voorstel baie versterk om suurlemoene uit te sluit van koue sterilisasie tot markte wat so 'n praktyk vir VKM vereis. In die nabye toekoms kan hierdie ook Europa insluit. Tot die einde Maart omtrent 9000 klas 1 uitvoer suurlemoene is alreeds ontleed met geen tekens van VKM besmetting nie. Hierdie opname sal voortgesit word tot 'n totaal van minstens 29956 vrute ondersoek is.

3.2.21 FINAL REPORT: A comparison of late-season FCM control options

Ad Hoc project (April 2013 – March 2014) by Sean Moore and Wayne Kirkman (CRI)

Summary

A field trial was conducted on Navel oranges in the Eastern Cape to compare the efficacy of several registered FCM control spray options applied late in the season. Runner and Delegate were the most effective treatments, followed by Cryptogran.

Opsomming

'n Veldproef is op Nawellemoene in die Oos-Kaap uitgevoer om die werking van alle geregistreerde VKM spuitmiddels laat in die seisoen te vergelyk. Runner en Delegate was die mees doeltreffende behandelinge, gevolg deur Cryptogran.

Introduction

Due to the current status of FCM as a phytosanitary threat, an ad hoc trial was conducted to test various late season control options for FCM.

Objectives

To compare the efficacy of various late season control options for FCM.

Materials and methods

A trial was conducted to compare the efficacy of most of the available control options for FCM, in a late season spray trial (Table 3.2.21.1). The trial was conducted on Far Away Farm in the Sundays River Valley, in an orchard of Witkrans Navel orange trees. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2008. The trial was laid out in a single-tree randomised block format, replicated 10 times. Treatments were applied with a Janisch hand-gun applicator on 24 April 2013, at an average rate of 11.7 L per tree for all treatments. Treatments 6, 8, 9 and 10 were applied between 10h00 and 11h30, and the remainder between 16h45 and 20h00. Treatment 11 (Broadband) received two further applications on 2 May 2013 (11.0 L/tree) and 8 May 2013 (11.5 L/tree), both in the evening after 17h00. Dropped fruit from each tree were collected weekly from three weeks after spraying, and analysed separately. FCM infestation was determined by the presence of a larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the LSD multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 2001).

Table 3.2.21.1. Various treatments applied to an orchard of Witkrans Navel orange trees on Far Away Farm in the Sundays River Valley on 24 April 2013.

Treatment no.	Treatment (all doses per 100 L water)
1	Untreated control
2	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)
3	Cryptex (3.3 ml)
4	Cryptex (3.3 ml) + molasses (500 ml)
5	GraTham (3.3 ml)
6	Runner (60 ml)
7	Cryptogran (5 ml) + Runner (30 ml) + Break-Thru (5 ml)
8	Coragen (17.5 ml)
9	Delegate (20 g)
10	Delegate (12 g)
11	Broadband (50 ml) + Break-Thru (5 ml)
12	Cryptogran (10 ml) + Runner (60 ml) + Break-Thru (5 ml)

Results and discussion

FCM infestation was low throughout the trial. Consequently, none of the treatments were significantly different from the untreated control (Table 3.2.21.2). Runner and Delegate (20 g) proved to be the most effective products in this trial, both reducing FCM infestation by 71.4%. Cryptogran was the next most effective, reducing infestation by 57.1%. Delegate was ineffective at the reduced rate of 12 g/100 L. Coragen performed surprisingly poorly (35.7% reduction), as this product has shown better efficacy in previous trials. Three applications of Broadband only reduced FCM infestation by 28.6%.

Table 3.2.21.2. FCM infestation for various treatments applied to an orchard of Witkrans Navel orange trees on Far Away Farm in the Sundays River Valley on 4 May 2012, evaluated form 16 May to 20 June 2013.

Treatment no.	Treatment (all doses per 100 L water)	Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.23±0.10a	
2	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)	0.10±0.05a	57.1
3	Cryptex (3.3 ml)	0.15±0.05a	35.7
4	Cryptex (3.3 ml) + molasses (500 ml)	0.15±0.07a	35.7
5	GraTham (3.3 ml)	0.15±0.05a	35.7
6	Runner (60 ml)	0.07±0.04a	71.4
7	Cryptogran (5 ml) + Runner (30 ml) + Break-Thru (5 ml)	0.12±0.07a	50.0
8	Coragen (17.5 ml)	0.15±0.05a	35.7
9	Delegate (20 g)	0.07±0.05a	71.4
10	Delegate (12 g)	0.22±0.16a	7.1
11	Broadband (50 ml) + Break-Thru (5 ml)	0.17±0.05a	28.6
12	Cryptogran (10 ml) + Runner (60 ml) + Break-Thru (5 ml)	0.05±0.04a	78.6

*Different letters in the same column denote significant differences between values ($P<0.05$, LSD multiple range test).

Conclusion

Runner and Delegate were the most effective products for the late-season control of FCM, the latter only at the full dose of 20 g/100 L. Cryptogran was the most effective of the biological control agents. This trial showed once again that there is no silver bullet for late-season FCM control, and highlights the importance of good FCM management throughout the season.

Acknowledgments

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Technology transfer

These results were presented by Tim Grout at a Letsitele study group meeting.

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3.3 PROGRAMME: FRUIT FLY

Programme coordinator: Aruna Manrakhan (CRI)

3.3.1 Programme summary

Fruit flies are important phytosanitary insect pests of commercial fruit. In southern Africa, the three indigenous fruit fly species of economic importance are *Ceratitis capitata*, Mediterranean fruit fly (Medfly), *Ceratitis rosa*, Natal fly, and *Ceratitis cosyra*, marula fly. In 2013, the assemblage of fruit fly pests changed in a number of areas in the northern parts of South Africa to include the invasive and exotic *Bactrocera invadens* which was declared present in the Vhembe district, Limpopo Province, and detected in areas in other districts of Limpopo, Mpumalanga, North West, Gauteng and Kwa-Zulu Natal. More recently in March 2014, the pest was also detected in five areas in the Northern Cape. The fruit fly programme in 2013-2014 consisted of a number of projects to address challenges in the management of both local and newly introduced exotic fruit fly pests. For local fruit fly pests, the focus was mainly on finding new proteinaceous baits and bait application methods for more effective control. This was addressed in two projects (Projects 915 and 1067). For *B. invadens*, a project on the dispersal capacity of the pest (Project 1075) was initiated in April 2013. Information obtained in the latter project will provide a basis in determining the effective size of area to be treated and quarantined for the pest.

For research on new baits and bait application methods, fruit fly colonies maintained at CRI were used (3.3.2). The colonies were also used for two other CRI funded research projects: cold disinfestation treatment for Medfly (Project 1054) and developmental threshold and critical thermal limits for two *C. rosa* types (Project 1067).

A new paper-based bait station developed by CRI was found to be promising in field tests (3.3.3). The new station contains a mixture of food-based attractants and spinosad The attraction range of the new station compared well with commercially available baits. On the other hand, new autolysate attractants were found to be less attractive than the commercially available attractant HymLure (3.3.7).

For research on the dispersal capacity of *B. invadens*, a colony of the pest was established at the Plant Quarantine Station in Harare, Zimbabwe (3.3.8). Experiments will soon be initiated to determine optimal marking methods for mark-release-recapture studies.

Programopsomming

Vrugtevlieë is belangrike fitosanitêre insekpeste van kommersiële vrugte. In suidelike Afrika is *Ceratitis capitata* (Mediterreense vrugtevlieg – Medvlieg), *Ceratitis rosa* (Natalse vlieg) en *Ceratitis cosyra* (Marula vlieg) die drie inheemse vrugtevliegspesies van kommersiële belang. In 2013 het die versameling vrugtevliegplae in ŉ aantal areas in the noordelike dele van Suid-Afrika verander, ten einde die indringer en uitheemse *Bactrocera invadens* in te sluit, wat teenwoordig verklaar is in die Vhembe distrik, Limpopo Provinsie, en wat in areas, in ander distrikte van Limpopo, Mpumalanga, Noordwes, Gauteng en Kwa-Zulu Natal, waargeneem is. Meer onlangs in Maart 2014, is die plaag ook in vyf areas in die Noord-Kaap waargeneem. Die vrugtevliegprojek in 2013-2014 het uit ŉ aantal projekte bestaan wat ten doel het om uitdagings in die bestuur van beide plaaslike en nuwe uitheemse vrugtevliegplae aan te spreek. Vir plaaslike vrugtevliegplae, was die fokus hoofsaaklik op die vind van nuwe proteïen-agtige lokmiddels en lokmiddel toedieningsmetodes vir meer effektiewe beheer. Dit is in twee projekte (Projek 915 en 1067) aangespreek. Vir *B. invadens*, is ŉ projek op die verspreidingsvermoë van die plaag (Projek 1075) in April 2013 geïnisieer. Inligting wat uit laasgenoemde projek verkry word, sal ŉ basis verskaf vir die bepaling van die effektiewe grootte van die area wat vir die plaag behandel en onder kwarantyn geplaas moet word.

Vir navorsing op nuwe lokmiddels en lokmiddel toedieningsmetodes, is vrugtevliegkolonies wat by CRI in stand gehou word, gebruik (3.3.2). Die kolonies is ook vir twee ander CRI-befondsde navorsingsprojekte gebruik: koue disinfestasiebehandeling vir Medvlieg (Projek 1054) en ontwikkelingsdrempelwaardes en kritiese termiese limiete vir twee *C. rosa* tipes (Projek 1067).

ŉ Nuwe papier-gebaseerde lokmiddelstasie wat deur CRI ontwikkel is, het belofte in veldproewe getoon (3.3.3). Die nuwe stasie bevat ŉ mengsel van voedingsgebaseerde aantrekkingsmiddels en spinosad. Die aantrekkingskragreeks van die nuwe stasie het gunstig met kommersieel beskikbare lokmiddels vergelyk. Aan die ander kant is gevind dat nuwe outolisaat lokmiddels minder aantreklik as die kommersieel beskikbare lokmiddel, HymLure, was (3.3.7).

Vir navorsing oor die verspreidingsvermoë van *B. invadens*, is ŉ kolonie van die plaag by die Plantkwarantynstasie in Harare, Zimbabwe, gevestig (3.3.8). Eksperimente sal spoedig begin word ten einde optimale merkmetodes vir gemerkte vrylating-hervangstudies te bepaal.

3.3.2 PROGRESS REPORT: Fruit fly rearing

Project 407 (1999/2000 – 2014/15) by A. Manrakhan, J-H. Daneel & R. Beck (CRI)

Summary

Three fruit fly species: *Ceratitis capitata* (Medfly), *C. rosa* (Natal fly) and *C. cosyra* (marula fly) are reared on artificial diets at CRI Nelspruit. Fruit fly materials were used for research on: (1) a new attract and kill fruit fly system (Project 915), (2) cold disinfestation treatment for Medfly (Project 1054), (3) development of yeast autolysate attractant for fruit fly bait (Project 1062), (4) developmental threshold and critical thermal limits for two *C. rosa* types (Project 1067) and (5) evaluation of new fruit fly bait from Arysta LifeScience (Contract research Project 1099). Fruit fly pupae were also supplied to University of Stellenbosch, University of Pretoria and University of Kwa-Zulu Natal for research and training.

Opsomming

Drie vrugtevliegspesies: *Ceratitis capitata* (Medvlieg), *C. rosa* (Natalse vlieg) en *C. cosyra* (Marula vlieg) word op kunsmatige diëte by CRI Nelspruit geteel. Vrugtevliegmateriaal is vir navorsing gebruik op: (1) ŉ nuwe aantrek-en-doodmaak vrugtevliegsisteem (Projek 915), (2) koue disinfestasiebehandeling vir die Medvlieg (Projek 1054), (3) ontwikkeling van gis outolisaat aantrekkingsmiddel vir vrugtevlieglokmiddel (Projek 1062), (4) ontwikkelingsdrempelwaardes en kritiese termiese limiete vir twee *C. rosa* tipes (Projek 1067) en (5) evaluasie van nuwe vrugtevlieglokmiddels van Arysta LifeScience (Kontraknavorsing Projek 1099). Vrugtevliegpapies is ook vir navorsing en opleiding aan die Universiteit van Stellenbosch, die Universiteit van Pretoria en die Universiteit van Kwa-Zulu Natal verskaf.

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

Project 915 (2008/9 – 2014/15) by A. Manrakhan, J-H. Daneel & R. Beck (CRI)

Summary

New paper-based bait stations containing food-based attractants combined with either spinosad or malathion were developed by CRI and evaluated in a Midknight Valencia orchard at Low's Creek, Mpumalanga between May and July 2013. The new bait stations were compared with standard fruit fly treatments: M3 bait station and 10% GF 120 weekly sprays. The bait station containing spinosad was more effective than the one containing malathion. *Ceratitis capitata* male catches were lower in blocks treated with the new bait station containing spinosad compared to the M3 treated blocks. *Ceratitis capitata* female catches on the other hand were higher in blocks treated with the new bait station containing spinosad compared to M3 treated blocks. There is a possibility that in the M3 treated blocks, the Questlure in the M3 bait stations used for monitoring female fruit flies could have caused a "shut down" of Questlure baited traps. A mark-release-recapture study was conducted in a citrus orchard in Schoemanskloof in order to compare the attraction range of the new bait station with the attraction ranges of the standard M3 bait station and Biolure 3-component (an international attractant used for mass trapping of fruit flies in other parts of the world). The new bait station, similar to M3 bait station and the Biolure 3-component, attracted flies up to a distance of 40 m (maximum distance tested). The effective attraction distances (~90% of captures) of the baits varied between 10 m and 30 m depending on species and baits.

Opsomming

Nuwe papier-gebaseerde lokstasies bevattende voedingsgebaseerde aantrekkingsmiddels, gekombineer met óf spinosad óf malathion, is deur CRI ontwikkel en in ŉ Midknight Valencia boord by Low's Creek, Mpumalanga, tussen Mei en Julie 2013, geëvalueer. Die nuwe lokstasies is met standaard vrugtevliegbehandelings vergelyk: M3 lokstasie en 10% GF 120 weeklikse toedienings. Die lokstasie bevattende spinosad was meer effektief in vergelyking met die een wat malathion bevat het. *Ceratitis capitata* mannetjie vangstes was laer in blokke wat met die nuwe lokstasie bevattende spinosad behandel is, in vergelyking met die M3 behandelde blokke. *Ceratitis capitata* wyfie vangstes was aan die ander kant hoër in blokke wat met die nuwe lokstasie bevattende spinosad behandel is, in vergelyking met M3 behandelde blokke. Daar is ŉ moontlikheid dat in die M3 behandelde blokke, die Questlure in die M3 lokstasies, wat vir die monitering van wyfie vrugtevlieë gebruik word, ŉ afsluit van Questlure lokvalle veroorsaak het. ŉ Merk-vrylating-hervangstudie is in ŉ sitrusboord in Schoemanskloof uitgevoer ten einde die aantrekkingsreeks van

die nuwe lokstasie met die aantrekkingsreekse van die standaard M3 lokstasie en Biolure 3-komponent (ʼn internasionale aantrekkingsmiddel wat vir die massa vangste van vrugtevlieë in ander dele van die wêreld gebruik word) te vergelyk. Die nuwe lokstasie, soortgelyk aan M3 lokstasie en die Biolure 3-komponent, het vlieë tot op ʼn afstand van 40 m aangetrek (maksimum afstand getoets). Die effektiewe aantrekkingsafstande (~90% van vangste) van die lokmiddels het tussen 10 m en 30 m gevarieer, afhangende van spesie en lokmiddel.

3.3.4 PROGRESS REPORT: Surveillance of *B. invadens* in commercial citrus orchards in South Africa

Project 966 (2009/10 – 2014/15) by A. Manrakhan, J-H. Daneel & R. Beck (CRI)

Summary

The status of *Bactrocera invadens* in South Africa was changed in March 2013. To date, *Bactrocera invadens* is considered to be present only in the Vhembe district in the Limpopo Province of South Africa. Between 2013 and March 2014, *B. invadens* specimens were also detected in isolated areas in other districts of Limpopo Province and in isolated areas in five other Provinces: North-West, Gauteng, Mpumalanga, KwaZulu-Natal and more recently in Northern Cape. All affected areas were placed under quarantine and the Department of Agriculture, Forestry and Fisheries (DAFF) is conducting monitoring surveys and control actions in these areas in accordance with the South African *Bactrocera invadens* Action Plan. In all affected production areas, the methyl-eugenol based male annihilation technique was included in fruit fly control programmes to effectively control *B. invadens*. Monitoring of *B. invadens* by CRI was discontinued in the Vhembe and Lephalale districts due to change of status of *B. invadens*. However, *B. invadens* monitoring by CRI is still ongoing in the Mopani district of Limpopo, in Mpumalanga and North West. Samples collected from surveys conducted by CRI, DAFF and PUCs (Production Unit Code) were identified and preserved in alcohol for future reference and studies. All CRI and PUC monitoring data were updated in the *Bactrocera invadens* national surveillance database.

Opsomming

Die status van *Bactrocera invadens* in Suid-Afrika het in Maart 2013 verander. Tot op datum word dit beskou dat *Bactrocera invadens* slegs in die Vhembe distrik, in die Limpopo Provinsie van Suid-Afrika, teenwoordig is. Tussen 2013 en Maart 2014, is *B. invadens* monsters ook in geïsoleerde areas in ander distrikte van Limpopo Provinsie en in geïsoleerde areas in vyf ander Provinsies, Noordwes, Gauteng, Mpumalanga, KwaZulu-Natal, en meer onlangs in Noord-Kaap, waargeneem. Alle geaffekteerde areas is onder kwarantyn geplaas en die Departement van Landbou, Bosbou en Visserye (DAFF) voer moniteringsopnames en beheeraksies in hierdie areas uit in ooreenstemming met die Suid-Afrikaanse *Bactrocera invadens* Aksieplan. In alle geaffekteerde produksieareas, is die metiel-eugenol-gebaseerde mannetjie uitwissingstegniek in vrugtevlieg beheerprogramme ingesluit ten einde *B. invadens* doeltreffend te beheer. Monitering van *B. invadens* deur CRI is in die Vhembe en Lephalale distrikte gestop weens die verandering in die status van *B. invadens*. Monitering van *B. invadens* deur CRI gaan egter steeds in die Mopani distrik van Limpopo, in Mpumalanga en Noordwes voort. Monsters wat in opnames wat deur CRI, DAFF en die PUCs (Produksie Eenheid Kodes) uitgevoer is, is versamel, geïdentifiseer en in alkohol opgeberg vir toekomstige verwysing en studies. Alle CRI en PUC moniteringsdata is in die *Bactrocera invadens* nasionale opname databasis opgedateer.

3.3.5 PROGRESS REPORT: Developmental threshold and critical thermal limits for two *Ceratitis rosa* types in South Africa

Project 1067 (2013/14 - 2014/15) by Aruna Manrakhan, John-Henry Daneel (CRI), Marc De Meyer (Royal Museum for Central Africa), Massimiliano Virgilio (Royal Museum for Central Africa), Christopher W. Weldon (UP) & Pia Addison (SU)

Summary

Studies on populations of the Natal fly, *Ceratitis rosa*, across its distribution range have indicated the occurrence of two genotypes and morphotypes of the pest- R1 (cold type) and R2 (hot type). The two types of *C. rosa* were found to occur in either parapatry or sympatry depending on location across its distribution range. Both genotypes and morphotypes of *C. rosa* were found to occur in South Africa. The question addressed in this project was whether the two *C. rosa* types would differ in their developmental threshold levels and thermal tolerances. Such information would be essential for assessment of risks associated with both types of *C. rosa*. This study required the establishment of colonies of the two types of *C. rosa*. While a colony of the hot type *C. rosa* (R2) was successfully established from loquat collected in Nelspruit, the establishment of a cold type *C. rosa* (R1) proved to be particularly difficult. Efforts are still ongoing to

establish a cold type *C. rosa* colony. In February 2014, Jambos fruit were sampled at the University of Pretoria. Flies emerged were identified as the *C. rosa* cold type. Despite a high mortality rate of adult flies emerging from the fruit, we were finally able to establish a colony of the *C. rosa* cold type and the colony has been maintained for approximately 6 generations. The problems experienced with the establishment of the *C. rosa* cold type colony have delayed progress in the experiments on developmental thresholds and thermal tolerances of the two *C. rosa* types. The project could not be finalised in 2013/14 and will still continue in 2014/15.

Opsomming

Studies oor populasies van die Natalse vlieg, *Ceratitis rosa*, óór sy verspreidingsreeks, het op die voorkoms van twee genotipes en morfotipes van die plaag R1 (koue tipe) en R2 (warm tipe) gedui. Daar is gevind dat die twee tipes van *C. rosa* óf in parapatrie óf in simpatrie voorkom, afhangende van ligging óór sy verspreidingsreeks. Daar is gevind dat beide genotipes en morfotipes van *C. rosa* in Suid-Afrika voorkom. Die vraag wat in die projek aangespreek is, was of die twee *C. rosa* tipes in hul ontwikkelingsdrempelwaardes en termiese toleransies sou verskil. Sulke inligting sal noodsaaklik wees vir die bepaling van risikos geassosieer met beide tipes van *C. rosa*. Hierdie studie het die vestiging van kolonies van die twee tipes van *C. rosa* vereis. Terwyl ŉ kolonie van die warm tipe *C. rosa* (R2) suksesvol vanaf lukwart wat in Nelspruit versamel is, gevestig is, het dit geblyk dat die vestiging van ŉ koue tipe *C. rosa* (R1) besonder moeilik was. Pogings is steeds onderweg om ŉ koue tipe *C. rosa* kolonie te vestig. In Februarie 2014 is Jambos vrugte by die Universiteit van Pretoria versamel. Vlieë wat uitgekom het, is as die *C. rosa* koue tipe geïdentifiseer. Ten spyte van ŉ hoë mortaliteitstempo van volwasse vlieë wat uit die vrugte gekom het, is daar uiteindelik daarin geslaag om ŉ kolonie van die *C. rosa* koue tipe te vestig, en die kolonie word al vir ongeveer 6 generasies in stand gehou. Die probleem wat met die vestiging van die *C. rosa* koue tipe kolonie ondervind is, het vooruitgang in die eksperimente op ontwikkelingsdrempelwaardes en termiese toleransies van die twee *C. rosa* tipes vertraag. Die projek kon nie in 2013/14 gefinaliseer word nie en sal steeds in 2014/15 voortgaan.

3.3.6 FINAL REPORT: Determining the sensitivity of immature *Ceratitis capitata* to a temperature of 1°C in various *Citrus* species according to a protocol received from MAFF, Japan in 2011

Project 1054 (Jul 2012 - Dec 2013) by T.G. Grout, P.R. Stephen, K.C. Stoltz & V. Hattingh (CRI)

Summary

After submitting earlier reports on research conducted by Citrus Research International with *Ceratitis capitata* in various types of citrus fruit, Japan MAFF requested that we repeat earlier research on the sensitivity of different immature life stages of *C. capitata* to cold (phase 2) in different types of citrus. In response, this research was conducted with Clementine mandarins, Marsh grapefruit, Eureka lemons and Valencia oranges. Eggs were once again found to be the most susceptible immature life stage to a cold treatment of 1°C and young larvae were generally less susceptible to cold than mature larvae in all types of citrus. These results therefore supported the prior use of young larvae in previous confirmatory treatments at a mean temperature of 1°C in Valencia oranges.

Opsomming

Nadat navorsingsverslae deur Citrus Research International ten opsigte van *Ceratitis capitata* in verskeie sitrusvrugte ingedien is, het Japan MAFF versoek dat ons die vorige navorsing op die sensitiwiteit van verskillende onvolwasse lewensstadia van *C. capitata* vir koue (fase 2) in verskillende soorte sitrus herhaal. Gevolglik is hierdie navorsing gedoen op Clementine mandaryne, Marsh pomelos, Eureka suurlemoene en Valencia lemoene. Eiers is weereens bevind om die mees vatbare lewensstadium te wees vir 1°C koue behandeling. Jong larwes was oor die algemeen minder vatbaar vir koue as meer volwasse larwes in alle soorte sitrus. Hierdie resultate ondersteun dus die gebruik van jong larwes in vorige bevestigende behandelings by ŉ gemiddelde temperatuur van 1°C in Valencia lemoene. Die finale verslag word tans geredigeer voor dit na Japan MAFF gestuur word.

Introduction

Earlier reports by Ware et al. (2006) and Stephen et al. (2008) on Phases 1 to 4 of cold treatment of Mediterranean fruit fly *Ceratitis capitata* in various *Citrus* species were submitted to MAFF Japan by DAFF South Africa. This was done in support of a request to Japan for acceptance of a 1°C fruit fly cold treatment of all citrus fruit types from South Africa to Japan. Japan MAFF requested that Phase 1 be conducted in the various fruit types. The report on this research by Grout et al. (2010) resulted in a further request from Mr T.

Fukumorita, Chief of Plant Protection Division, Japan MAFF for a repeat of the earlier Phase 2 research on the sensitivity of different life stages of *C. capitata* to cold in the various citrus fruit types, using a revised protocol. The three main requested changes to the protocol were that older eggs must be used, the young larvae must be younger to include first instars and that inoculation times must be staggered so that the cold treatment of different life stages is conducted at the same time. The larval ages recommended by MAFF for testing the susceptibility of young larvae were 5 days after inoculation for Clementine mandarins and 4 days after inoculation for the other three citrus types. Although a repeat of Phase 2 work using Clementine mandarin was not requested we did include this with the other citrus types so that they were again compared under the same conditions and with the older eggs.

Stated objective

Determine the least susceptible immature life stage of Medfly to a cold treatment of approximately 1°C in four different types of citrus

Materials and methods

The citrus types used for the experiments were "Nules" Clementine mandarins (*Citrus reticulata*) with mean diameter 67 mm and mean mass 120 g, "Eureka" lemons (*Citrus limon*) with mean diameter 69 mm and mean mass 180 g, "Marsh" grapefruit (*Citrus paradisi*) with mean diameter 92 mm and mean mass 315 g and "Valencia" orange (*Citrus sinensis*) with mean diameter 74 mm and mean mass 205 g. The quality and maturity of the fruit was compliant with commercial citrus fruit export standards.

Fruit preparation entailed the removal of the calyces and dipping the fruit in a combination of Sporekill (didecyl dimethyl ammonium chloride) (100 ml/100 ℓ water) and guazatine (480 ml/100 ℓ water) for 1 min to surface sterilize and control fungal growth. Mediterranean fruit fly reared at the CRI facility in Nelspruit, were used to produce the eggs for inoculating into the fruit. The fruit fly culture was the same as the one used in previous experimental work conducted for Japan, but was annually replenished with wild flies to preserve the genetic quality of the culture. Eggs were collected in bowls of tap water below the rearing cages over a 20 h period. For inoculation the eggs were placed in deionized water and the egg/water ratio adjusted until the number of eggs per 0.025 ml aliquot removed using an automatic pipette after stirring, was approximately 35. Prior to inoculation at least 10 x 0.025 ml aliquots of eggs were pipetted onto black cloth and counted to confirm the mean number of eggs per fruit. This test was repeated during each inoculation so that the number of eggs placed in the fruit could be accurately determined.

A 6 mm-diameter hole was bored ± 30 mm deep into the fruit beneath the calyx using a cork borer, and some Torula yeast (between 0.2 and 0.5 ml of a mixture with water in a 1:2 ratio) was injected into the inoculation hole to provide additional nourishment for the larvae. After the eggs were pipetted into each hole in the fruit the hole was plugged with cotton wool before it was sealed using molten paraffin wax. Each fruit was then placed into a brown paper bag to minimise fungal infection. The fruit were then randomly packed into plastic crates (52 by 35 by 29 cm) at 50 per crate and incubated at 26°C for egg hatching and larval development.

Three hundred and fifty fruit of each type were inoculated for each life stage in each replicate. Fifty fruit from each life stage and fruit type were used as controls in each replicate and held at 26°C for 10 d before dissection. The other 300 fruit (for each fruit type, each life stage and each replicate) went into the cold room.

In order to meet the requirement of treating all life stages simultaneously during the same cold period, inoculations were conducted for the mature larval category 9 days before the cold treatment started, 4 days before the start of the cold treatment (5 days for Clementines) for the young larval category and 1 day before the start of the cold treatment for the eggs category (Table 3.3.6.1). The fruit were held at 26°C after the inoculations to allow for the larvae to develop to the right size and eggs to mature before the cold treatment.

Thereafter, in each of the 2 replicates, 300 of these fruit from each life stage (900 fruit per fruit type) were moved into the cold room and the temperature brought down to 1°C within one day. The remaining 50 fruit of each fruit type, each life stage and each replicate were left in the 26°C room and dissected at required intervals to determine survival at the mature larval stage (Table 3.3.6.1).

Thermoprobes (thermocouples type "T" with a tolerance of 0.5°C from Temperature Controls Pty., Ltd., Randburg, South Africa) were calibrated before each disinfestation session using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reached equilibrium. A thermometer immersed in the melting ice was used to confirm the temperature. At least three

calibration runs were conducted and the mean result for each probe was used for correction purposes. Calibration was done immediately prior to any of the tests being conducted. Temperatures were recorded at the inlet and outlet of the cooling coil in the room and from 14 probes placed 30 mm inside fruit that were randomly scattered amongst the fruit in the crates. The positions of the latter probes were recorded.

When seven or more of the 14 probes within the fruit pulp of fruit in the cold room (Fig. 3.3.6.1) showed temperatures of 1.0°C or below, the cold treatment was deemed to have started. Internal fruit temperatures were logged every hour on a Grant SQ 2020 1F8 datalogger (Monitoring and Control Laboratories Pty. Ltd., Johannesburg, South Africa). Fruit were then removed from the cold room at regular intervals of 1 day, 3 days, 5 days, 7 days, 9 days and 11 days after the cold treatment started. They were placed in a room at 26°C for 2 days before the fruit were dissected and the numbers of dead and alive larvae in each fruit recorded.

Due to seasonal availability, cold treatments of Clementine mandarins and Marsh grapefruit were conducted simultaneously and the cold treatment initiation date for the first replicate was 15 August 2012 and for the second replicate 30 May 2013. The lemon and Valencia orange replicates were similarly combined and the cold treatment of the first replicate started on 31 October 2012 and the second replicate on 28 November 2012.

Table 3.3.6.1. Treatment and activity schedule used in all replicates for all fruit types. Fruit numbers are for each fruit type in one replicate.

Day	Activity 1	No. fruit	Activity 2	No. fruit	Activity 3	No. fruit
-1	Prepare fruit (remove calyx, fungicide dip etc)	350	Calibrate logger (Incubation room) on melting ice			
0	Start egg-collection (12h00)					
1	Collect last eggs (08h00)		Inoculate (Mature larvae)	350	Move infested fruit to 26°C	350
2						
3	Calibrate logger (Cold room) on melting ice					
4	Prepare fruit (de-calyx, dip etc)	350	Start egg-collection (12h00) for Clementine			
5	Collect eggs (08h00) for Clementine		Inoculate (Young larvae CLEM) and move to 26°C	350	Start egg-collection (12h00) GF, LEM, VAL	
6	Collect eggs (08h00) for GF, LEM, VAL		Inoculate (Young larvae GF, LEM, VAL)	350	Move infested fruit to 26°C	350
7	Start egg-collection (12h00)				Prepare fruit (remove calyx, dip etc)	350
8	Collect eggs (08h00)		Inoculate (Eggs)	350	Move infested fruit to 26°C	350
9	Additional day at 26°C					
10	Move all fruit for treatment to 1°	900	ALL CONTROLS stay at 26°	150	Cut CONTROL (Mature larvae)	50
11	Cold trt begins when fruit at 1°					
12	Remove 1d fruit 50X3X2	50	All to 26°	50		
13						
14	Remove 3d fruit 50X3X2	50	All to 26°	50	Cut 1d (Mature larvae)	50
15					Cut CONTROL (Young larvae)	50
16	Remove 5d fruit 50X3X2	50	All to 26°	50	Cut 3d (Mature larvae)	50
17						
18	Remove 7d fruit 50X3X2	50	All to 26°	50	Cut CONTROL (Eggs) & 1d (YL) & 5d (ML)	150
19						
20	Remove 9d fruit 50X3X2	50	All to 26°	50	Cut 1d (Eggs) & 3d (YL) & 7d (ML)	150
21						

22	Remove 11d fruit 50X3X2	50	All to 26°	50	Cut 3d (Eggs) & 5d (YL) & 9d (ML)	150
23						
24					Cut 5d (Eggs) & 7d (YL) & 11d (ML)	150
25						
26					Cut 7d (Eggs) & 9d (YL)	100
27						
28					Cut 9d (Eggs) & 11d (YL)	100
29						
30					Cut 11d (Eggs)	50

CLEM = Clementine mandarin
GF = grapefruit
LEM = lemon
VAL = Valencia orange

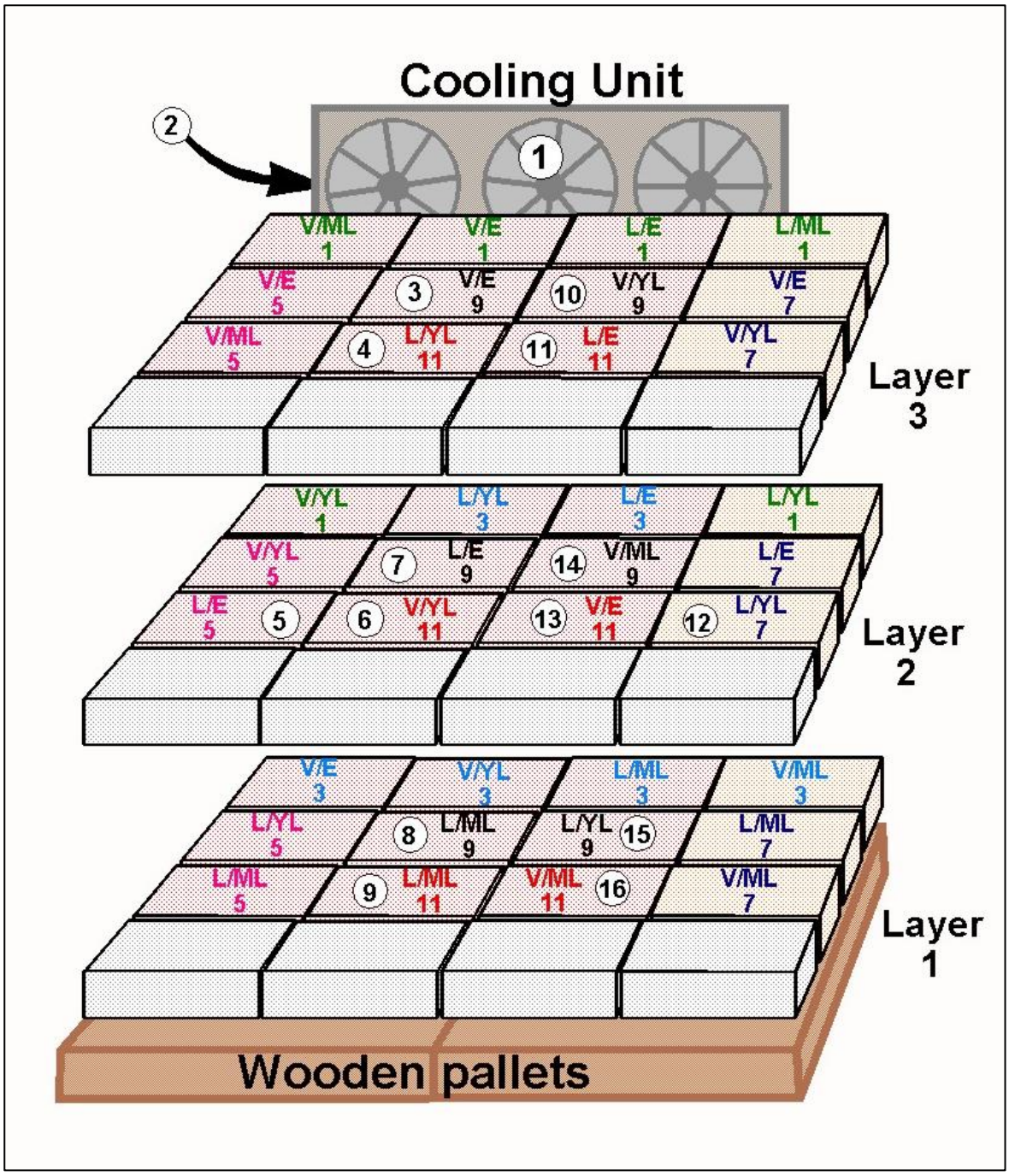

Figure 3.3.6.1. The typical layout of crates of infested fruit and thermoprobes in the cold room, in this case during the second replicate of lemons (L) and Valencia oranges (V) where the thermoprobes in the fruit are numbered 3 to 16 (inside circles), E refers to eggs, YL to young larvae and ML to mature larvae and the numbers below the letters indicate the number of days of cold treatment for that life stage.

Results and discussion

The staggering of inoculation dates to provide an uninterrupted cold period of all three life stages worked well in providing a uniform cold treatment in all replicates. The mean hourly fruit temperatures, the mean hourly maxima and mean hourly minima are illustrated and stated in Figures 3.3.6.2 to 5.

As had been found previously by Ware et al. (2006), the eggs were the most susceptible life stage to the cold treatment in all fruit types (Tables 3.3.6.2 to 5). The eggs appeared to dissolve with time and in fruit that had been cold-treated for 5 days or longer it was extremely difficult to find the dead eggs. This resulted in the total numbers of eggs counted from 7 days of cold treatment onwards being extremely low and not suitable for Probit analysis (Finney 1971).

Results from both replicates were combined for Probit analysis (using XLSTAT-Dose ver 2012.2.01, Addinsoft) on each larval life stage in each fruit type (Table 3.3.6.6). There was a trend towards young larvae being more tolerant to cold than the mature larvae. However, considering the LT99 values, there were no significant differences between the larval ages in lemons and Valencia oranges . It is concluded that although larval age may not be a critical factor, it would be preferable to use young larvae in evaluating the efficacy of cold treatments for Mediterranean fruit fly in citrus fruit. These results confirmed earlier work by Ware et al. (2000, 2006) and justified the use of young larvae in the Phase 4 verification trial previously conducted at a mean temperature of 1°C by Stephen et al. (2008), previously submitted to Japan MAFF by DAFF South Africa and published in Grout et al. (2011).

Acknowledgements

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Table 3.3.6.2. The survival of Mediterranean fruit fly in **Clementine mandarin** after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	52	1742	475	27.3
		1	52	1742	377	21.6
		3	54	1809	189	10.4
		5	54	1809	33	1.8
		7	51	1709	2	0.1
		9	50	1675	0	0.0
		11	50	1675	0	0.0
	2	0	50	1895	598	31.6
		1	49	1857	456	24.6
		3	50	1895	319	16.8
		5	50	1895	86	4.5
		7	48	1819	0	0.0
		9	50	1895	0	0.0
		11	50	1895	0	0.0
Young larvae	1	0	52	1882	611	32.5
		1	52	1882	416	22.1
		3	52	1882	410	21.8
		5	52	1882	149	7.9
		7	52	1882	49	2.6
		9	51	1846	3	0.2
		11	52	1882	0	0.0
	2	0	48	1795	530	29.5
		1	50	1870	407	21.8
		3	51	1907	288	15.1
		5	50	1870	170	9.1
		7	48	1795	21	1.2
		9	43	1608	1	0.1
		11	53	1982	0	0.0
Mature larvae	1	0	51	1760	507	28.8
		1	51	1760	729	41.4
		3	52	1794	550	30.7
		5	51	1760	306	17.4
		7	52	1794	18	1.0
		9	53	1829	2	0.1
		11	51	1760	0	0.0
	2	0	53	1919	777	40.5
		1	52	1882	697	37.0
		3	53	1919	548	28.6
		5	53	1919	207	10.8
		7	57	2063	27	1.3
		9	55	1991	2	0.1
		11	53	1919	0	0.0

Table 3.3.6.3. The survival of Mediterranean fruit fly in **Marsh grapefruit** after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	53	1776	686	38.6
		1	53	1776	504	28.4
		3	53	1776	279	15.7
		5	52	1742	26	1.5
		7	52	1742	3	0.2
		9	53	1776	0	0.0
		11	52	1742	0	0.0
	2	0	52	1940	833	42.9
		1	52	1940	813	41.9
		3	53	1977	661	33.4
		5	53	1977	424	21.4
		7	50	1865	86	4.6
		9	52	1940	0	0.0
		11	50	1865	0	0.0
Young larvae	1	0	52	1882	589	31.3
		1	52	1882	677	36.0
		3	52	1882	589	31.3
		5	52	1882	233	12.4
		7	53	1919	45	2.3
		9	50	1810	1	0.1
		11	52	1882	1	0.1
	2	0	50	1790	1032	57.7
		1	51	1826	965	52.9
		3	52	1862	706	37.9
		5	51	1826	234	12.8
		7	52	1862	24	1.3
		9	50	1790	0	0.0
		11	53	1897	0	0.0
Mature larvae	1	0	54	1863	990	53.1
		1	52	1794	837	46.7
		3	52	1794	678	37.8
		5	52	1794	394	22.0
		7	52	1794	52	2.9
		9	51	1760	3	0.2
		11	53	1829	0	0.0
	2	0	50	1655	795	48.0
		1	53	1754	677	38.6
		3	50	1655	689	41.6
		5	52	1721	227	13.2
		7	49	1622	14	0.9
		9	50	1655	1	0.1
		11	51	1688	0	0.0

Table 3.3.6.4. The survival of Mediterranean fruit fly in **Eureka lemon** after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	52	1898	1310	69.0
		1	52	1898	901	47.5
		3	49	1789	590	33.0
		5	52	1898	337	17.8
		7	51	1862	16	0.9
		9	51	1862	1	0.1
		11	52	1898	0	0.0
	2	0	52	1742	877	50.3
		1	53	1776	572	32.2
		3	52	1742	425	24.4
		5	53	1776	87	4.9
		7	54	1809	1	0.1
		9	50	1675	0	0.0
		11	51	1709	0	0.0
Young larvae	1	0	52	2038	1035	50.8
		1	52	2038	851	41.7
		3	52	2038	518	25.4
		5	52	2038	129	6.3
		7	52	2038	4	0.2
		9	51	1999	0	0.0
		11	52	2038	0	0.0
	2	0	52	1882	847	45.0
		1	53	1919	771	40.2
		3	52	1882	312	16.6
		5	53	1919	80	4.2
		7	54	1955	7	0.4
		9	50	1810	0	0.0
		11	50	1810	0	0.0
Mature larvae	1	0	52	1955	1260	64.4
		1	53	1993	1167	58.6
		3	51	1918	946	49.3
		5	52	1955	186	9.5
		7	52	1955	27	1.4
		9	50	1880	6	0.3
		11	54	2030	0	0.0
	2	0	52	1794	337	18.8
		1	52	1794	345	19.2
		3	52	1794	142	7.9
		5	52	1794	41	2.3
		7	51	1760	5	0.3
		9	50	1725	1	0.1
		11	51	1760	0	0.0

Table 3.3.6.5. The survival of Mediterranean fruit fly in **Valencia orange** after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	50	1935	823	42.5
		1	50	1935	648	33.5
		3	49	1896	637	33.6
		5	49	1896	392	20.7
		7	49	1896	24	1.3
		9	49	1896	2	0.1
		11	52	2012	0	0.0
	2	0	50	1710	746	43.6
		1	49	1676	438	26.1
		3	51	1744	360	20.6
		5	50	1710	73	4.3
		7	49	1676	2	0.1
		9	50	1710	0	0.0
		11	53	1813	0	0.0
Young larvae	1	0	51	1877	984	52.4
		1	54	1987	910	45.8
		3	50	1840	681	37.0
		5	53	1950	167	8.6
		7	51	1877	16	0.9
		9	52	1914	0	0.0
		11	58	2134	3	0.1
	2	0	52	1804	762	42.2
		1	50	1735	700	40.3
		3	50	1735	303	17.5
		5	52	1804	276	15.3
		7	53	1839	21	1.1
		9	51	1770	2	0.1
		11	51	1770	0	0.0
Mature larvae	1	0	52	1986	792	39.9
		1	52	1986	819	41.2
		3	51	1948	570	29.3
		5	52	1986	202	10.2
		7	53	2025	8	0.4
		9	51	1948	1	0.1
		11	53	2025	0	0.0
	2	0	52	1773	336	18.9
		1	51	1739	321	18.5
		3	53	1807	319	17.7
		5	52	1773	85	4.8
		7	51	1739	7	0.4
		9	52	1773	5	0.3
		11	51	1739	1	0.1

Table 3.3.6.6. LT50, 90 and 99 (95% confidence limits) in days for young and mature fruit fly larvae in Clementine mandarin, Marsh grapefruit, Eureka lemon and Valencia orange, when exposed to a mean temperature of 1°C.

Citrus type	Larval age	LT_{50}	LT_{90}	LT_{99}
Clementine mandarin	Young	5.28 (5.13 – 5.43)	8.33 (8.03 – 8.68)	12.06 (11.39 – 12.86)
	Mature	4.37 (4.30 – 4.44)	6.42 (6.30 – 6.55)	8.76 (8.50 – 9.05)
Marsh grapefruit	Young	5.09 (4.99 – 5.18)	7.40 (7.22 – 7.58)	10.02 (9.67 – 10.42)
	Mature	4.41 (4.35 – 4.48)	6.49 (6.38 – 6.61)	8.86 (8.62 – 9.13)
Eureka lemon	Young	5.29 (5.19 – 5.38)	6.25 (6.13 – 6.39)	7.14 (6.94 – 7.39)
	Mature	3.54 (3.48 – 3.60)	5.40 (5.30 – 5.51)	7.59 (7.36 – 7.85)
Valencia orange	Young	5.64 (5.55 – 5.73)	6.88 (6.77 – 7.02)	8.05 (7.85 – 8.29)
	Mature	3.46 (3.38 – 3.54)	5.46 (5.35 – 5.57)	7.75 (7.51 – 8.01)

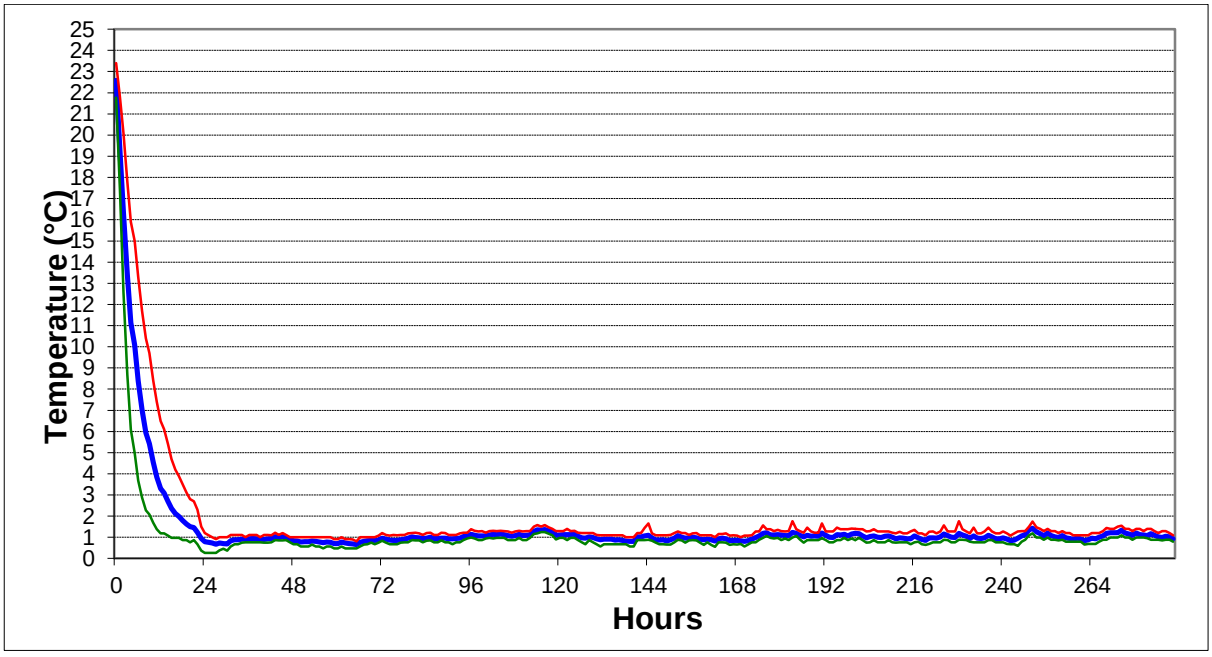


Figure 3.3.6.2. Highest (red line), lowest (green line) and mean (blue line) temperatures of Clementine mandarins and Marsh grapefruit in replicate 1. **Mean temperatures from commencement of the cold treatment period: mean of hourly averages 0.991, mean of hourly minima 0.791 and mean of hourly maxima 1.219°C.**

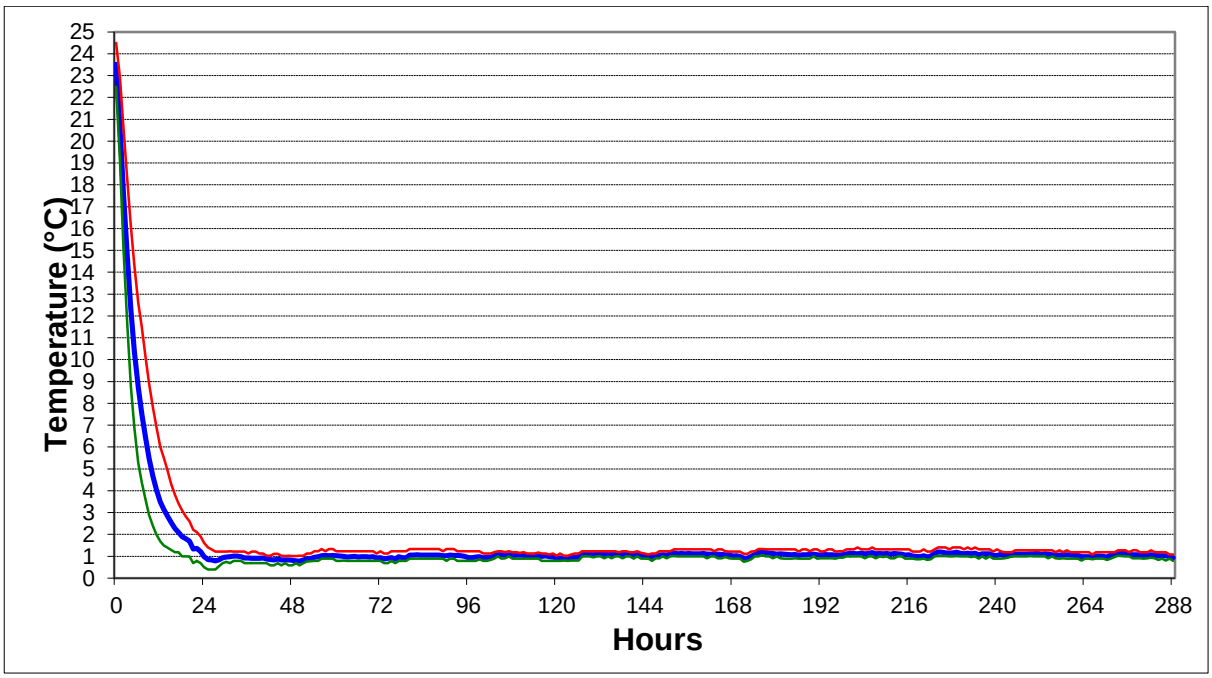


Figure 3.3.6.3. Highest (red line), lowest (green line) and mean (blue line) temperatures of Clementine mandarins and Marsh grapefruit in replicate 2. **Mean temperatures from commencement of the cold treatment period: mean of hourly averages 1.032, mean of hourly minima 0.876 and mean of hourly maxima 1.232°C.**

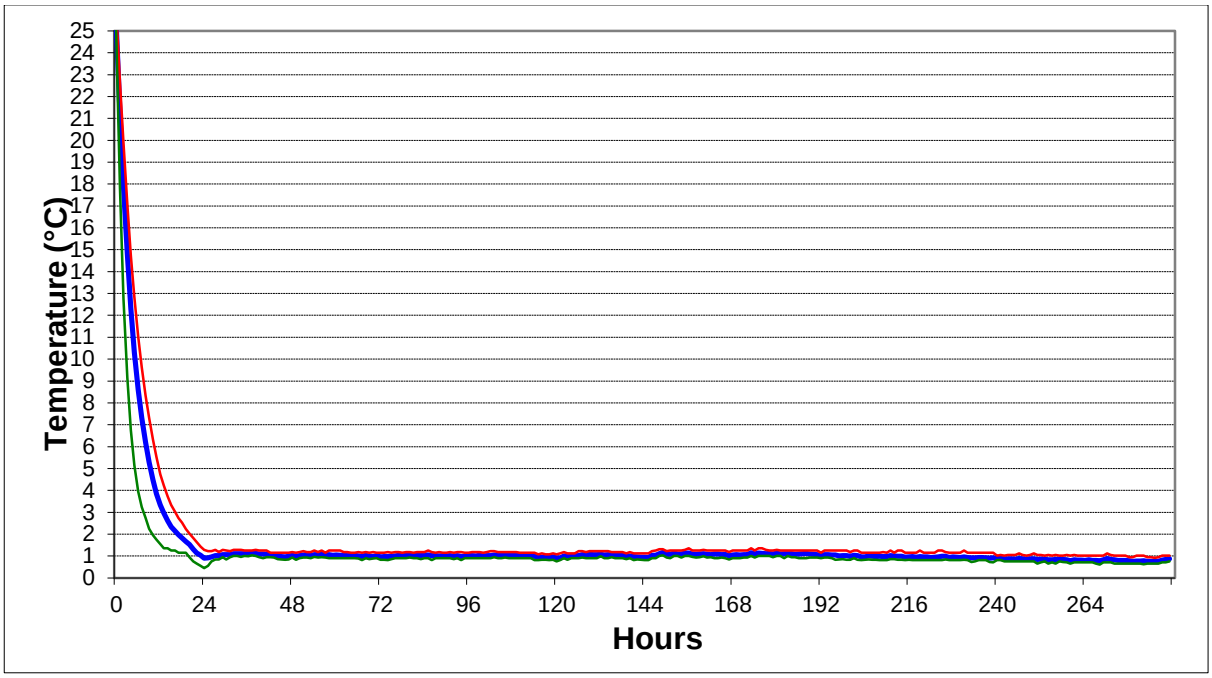


Figure 3.3.6.4. Highest (red line), lowest (green line) and mean (blue line) temperatures of lemons and Valencia oranges in replicate 1. **Mean temperatures from commencement of the cold treatment period: mean of hourly averages 0.991, mean of hourly minima 0.854 and mean of hourly maxima 1.169°C.**

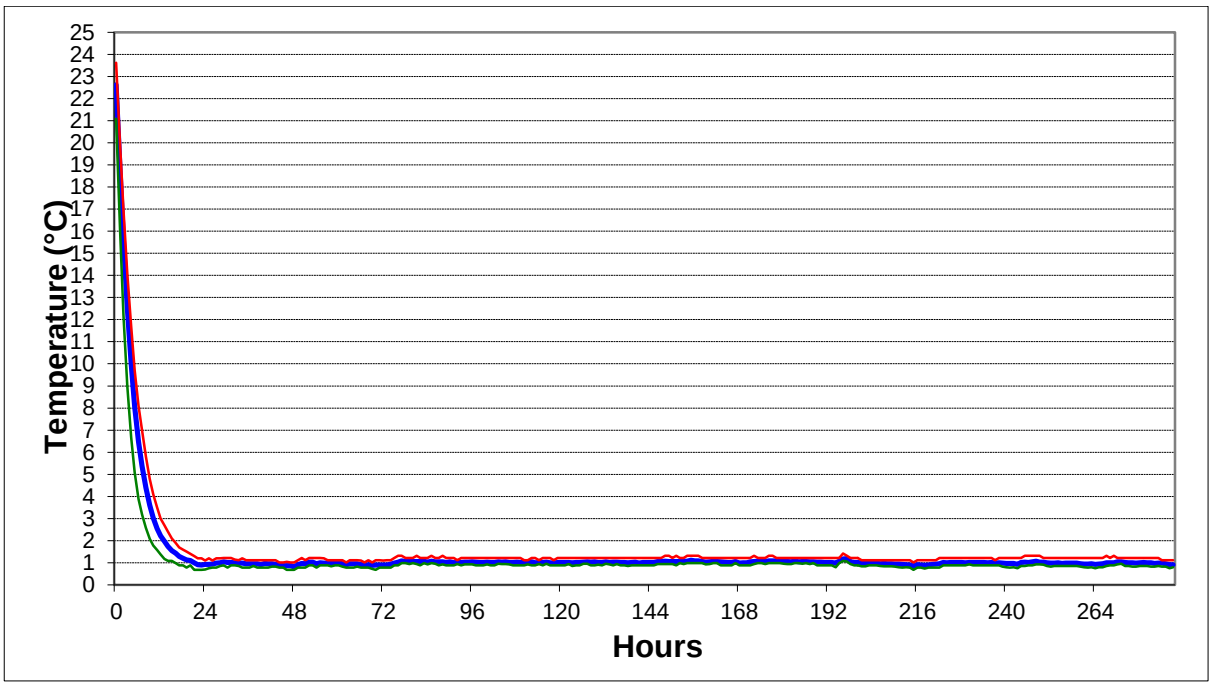


Figure 3.3.6.5. Highest (red line), lowest (green line) and mean (blue line) temperatures of lemons and Valencia oranges in replicate 2. **Mean temperatures from commencement of the cold treatment period: mean of hourly averages 0.999, mean of hourly minima 0.869 and mean of hourly maxima 1.199°C.**

3.3.7 PROGRESS REPORT: Develop a yeast autolysate attractant for fruit fly bait that is safe with copper and more palatable than hydrolysate

Project 1062 (2013/14 - 2014/15) by Tim Grout & Peter Stephen (CRI)

Summary

This research was conducted to counter the threat of losing the use of mancozeb and having to rely more on copper sprays to control citrus black spot. When protein hydrolysate fruit fly bait is applied to fruit with copper residues, permanent dark marks often result, particularly when exposed to the direct sun. This makes the use of copper late in the season hazardous if hydrolysate bait sprays are being used for fruit fly control. Yeast autolysate is used in Queensland, Australia and elsewhere for the control of fruit flies without concern about copper-related phytotoxicity. We therefore evaluated the product used in Queensland (B4BAY50) and locally-manufactured attractants with varying amounts of autolysate. When evaluated for attractiveness in McPhail traps in a field cage against both sexes of protein-starved Medfly, Natal fly and Marula fly released simultaneously, B4BAY50 was significantly inferior to local mixtures of 100% autolysate and 80:20% autolysate:hydrolysate which were both inferior to Hym-Lure. Results also showed that females were more attracted to these products than males and confirmed that these attractants were not very effective for Marula fly. Evaluations of palatability or consumption that quantified the amount of bait consumed by flies in J-tubes gave similar or inferior results for B4BAY50 compared to Hym-Lure for all fly species. As the autolysate baits were inferior to Hym-Lure and because continued used of mancozeb was now assured, no further research was conducted on the autolysates. However, an alternative Mauritian attractant formulation will still be evaluated before this project is terminated.

Opsomming

Hierdie navorsing is gedoen in reaksie op die moontlike verlies van mancozeb omdat daar dan meer op koperbehandelings staatgemaak sal moet word om sitrus swartvlek te beheer. Wanneer proteïen hidrolisaat vrugtevlieg-lokaas toegedien word op vrugte met koperresidue, vorm permanente donker vlekke, veral as die vrugte aan direkte sonlig blootgestel is. Dit maak dit gevaarlik om koper laat in die seisoen toe te dien indien hidrolisaat lokaas blaarbespuitings gebruik word vir vrugtevliegbeheer. Gis outolisaat word gebruik in Queensland, Australië en op ander plekke om vrugtevlieg te beheer sonder die gevaar van koperverwante fitotoksisiteit. Ons het die produk wat in Queensland gebruik word en ander plaaslikvervaardigde produkte met verskillende hoeveelhede outolisaat geëvalueer. Tydens evaluasie is albei geslagte van protein-uitgehongerde Medvlieg, Natalvlieg en Maroelavlieg gelyktydig vrygelaat in ŉ veldhok met ŉ McPhail lokval. Onder hierdie omstandighede het B4BAY50 aansienlik slegter gevaar as plaaslike mengsels van 100%

outolisaat en 80:20% outolisaat:hidrolisaat wat albei slegter gevaar het as Hym-Lure. Resultate wys ook dat wyfie vlieë meer aangetrokke was tot hierdie produkte as die mannetjie vlieë en die resultate het bevestig dat hierdie lokaas nie baie doeltreffend teen Maroelavlieg is nie. Evaluasies van die smaaklikheid of inname van die hoeveelheid lokaas wat deur die vlieë in J-buisies geëet is, het soortgelyke of swakker resultate vir B4BAY50 in vergelyking met Hym-Lure vir al die vliegspesies opgelewer. Omdat die outolisaat swakker gevaar het as Hym-Lure en omdat voortgesette gebruik van mancozeb nou bevestig is, is geen verdere navorsing gedoen op outolisaats nie. Alhoewel, 'n alternatiewe Mauritiaanse lokaasformule sal nog geëvalueer word voordat hierdie profek beëindig word.

3.3.8 PROGRESS REPORT: Dispersal capacity of *Bactrocera invadens*

Project 1075 (2013/14 – 2015/16) by C.W. Weldon, R Anguelov (UP) & A Manrakhan (CRI)

Summary

The key outcome of this project is to establish the dispersal capacity of the invasive fruit fly, *Bactrocera invadens*, with regard to environmental and physiological variables. Progress has been delayed substantially by the very long timeframe required to negotiate memoranda of agreement between the University of Pretoria and industry partners associated with the project. While an agreement has been made with and funding received from CRI (with matching funds from THRIP), agreements are yet to be signed by Hortgro and the South African Table Grape Industry. Despite these delays, we have done our best to work towards reaching the objectives of the project. A PhD student, Mrs Louisa Makumbe, was appointed to the project in April 2013. Mrs Makumbe is based at the Plant Quarantine Station, Harare, Zimbabwe, where she has progressively worked to establish a *B. invadens* culture from locally-collected infested fruit with larval rearing medium supplied by CRI. To permit work on the best pigment dose and colours for marking *B. invadens* in dispersal experiments, a microscope (with digital camera), UV light source, insect rearing cages, and six fluorescent pigments have been purchased and received by Mrs Makumbe, who will commence this work as soon as sufficient fly numbers are available. Preliminary modelling has been done by Prof. Anguelov on the effectiveness of various trap arrangements to optimise fly recaptures. This modelling has been published in an open access, peer-reviewed journal and will benefit planned dispersal experiments. Related research is being conducted by an Honours student, Mr Sandiso Mnguni, who is supervised by Dr. Weldon. This research will determine how the diet of fruit fly larvae affects their tolerance of water stress, and data produced will aid in the interpretation of trap captures from planned dispersal experiments.

Opsomming

Die hoofdoel van die projek is om die verspreidingsvermoë van die indringer vrugtevlieg, *Bactrocera invadens*, ten opsigte van die omgewings- en fisiologiese veranderlikes te bepaal. Vordering is wesentlik vertraag deur die baie lang tyd wat die onderhandeling van die memoranda van ooreenkoms, tussen die Universiteit van Pretoria en medewerkers in die industrie op hierdie projek geneem het. Hoewel 'n ooreenkoms bereik is met, en fondse reeds ontvang is van CRI (met gelykstaande fondse van THRIP), moet ooreenkomstes nog met Hortgro en die Suid-Afrikaanse Tafeldruif Industrie onderteken word. Ten spyte van hierdie vertragings, het ons ons bes gedoen om die doelwitte van die projek te bereik. Mev. Louisa Makumbe, 'n doktorale student, is in April 2013 by die projek betrek. Mev. Makumbe is aan die Plantkwarantynstasie in Harare, Zimbabwe, verbonde, waar sy toenemend gewerk het om 'n *B. invadens* kultuur, met plaaslik besmette vrugte en 'n medium (vir die kweek van larwes) wat deur CRI verskaf is, te vestig. Om werk oor die beste pigmentdosisse en kleure vir die merk van *B. invadens* in verpreidingseksperimente te kan doen, is 'n mikroskoop (toegerus met 'n digitale kamera), 'n UV-ligbron, insekteelhokke en ses fluoresserende pigmente deur mev. Makumbe aangekoop. Hierdie toerusting is reeds ontvang en mev. Makumbe sal met hierdie werk begin sodra voldoende vlieë beskikbaar is. Voorlopige modellering om die doeltreffendheid van verskilende tipes lokval-opsette te ondersoek sodat die hervangs van vlieë geoptimaliseer kan word, is deur prof. Anguleov gedoen. Hierdie modellering is in 'n vrye-toegang, eweknie-hersiende joernaal gepubliseer, en sal tot voordeel wees van verspreidingseksperimente wat beplan word. Verwante navorsing word tans deur 'n mnr. Sandiso Mnguni ,'n honneursstudent, onder toesig van Dr. Weldon, gedoen. Hierdie navorsing sal vasstel hoe die dieet van vrugtevlieglarwes hul vermoë om waterstres te weerstaan, beïnvloed. Die data wat uit laasgenoemde navorsing verkry word, sal help om die lokvalvangstes van beplande verspreidingseksperimente te interpreteer.

3.4 PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS

Programme coordinator: Sean D Moore (CRI)

3.4.1 Programme summary

Three of the seven mealybug species known to occur on citrus in South Africa are considered as phytosanitary threats for certain export markets. Two of the five projects reported within this programme address different aspects of mealybug management. However, one of them also incorporated citrus thrips. Two projects focussed on carob moth, which is often closely associated with mealybug. Carob moth is not a phytosanitary pest for most markets. However, there are exceptions, such as China. Carob moth has always been considered as a secondary and sporadic pest with negligible pest status. However, its occurrence on citrus now appears to be slightly higher than originally observed. Additionally, the morphological similarity of its larvae to those of false codling (FCM) moth have made its recognition and management more important. The last project in the programme focussed on a post-harvest treatment for phytosanitary pests in general.

In the first of the mealybug projects, which was funded by the International Atomic Energy Agency (IAEA), gamma irradiation at 150 Gy was evaluated on a probit-8.7 scale to confirm this dose's value as a potential phytosanitary treatment (3.5.2). All reproductive females were fully sterilized by the treatment. In the second project on mealybug, locally isolated entomopathogenic fungi (EPF) were screened in laboratory bioassays against citrus mealybug (3.5.3). A *Beauveria bassiana* isolate and a *Metharizium anisopliae* isolate both resulted in 67.5% mealybug crawler mortality and a second *B. bassiana* isolate caused 64% crawler mortality. Dose-response relationships were subsequently determined for the three isolates against both adult and crawler mealybug. These EPF isolates were also tested against citrus thrips adults, resulting in between 60 and 70% mortality.

The first of the carob moth projects looked at the morphology and ecology of carob moth in citrus orchards (3.5.4). No results are available yet. The other carob moth project was a field study in the Vaalharts region, where citrus and pecan nuts are grown in close proximity (3.5.6). The aim was to determine the composure of the lepidopteran borer complex on the two crops and any movement of the pests between crops. Indications thus far are that carob moth infestation of both pecans and citrus is higher than for FCM, and carob moth appears to migrate from pecans into citrus.

The final project tested a GRAS post-harvest fumigant and CO_2 against phytosanitary pests (3.5.5). Total mortality of grain chinch bug was achieved. However, the main research focus during the report period was to determine the maximum mortality that could be obtained of internal pests such as fruit fly and FCM without being detrimental to the fruit. Fruit fly appeared to be slightly more susceptible to both Grasfum and CO_2 than FCM. Further work focused on CO_2 and its use as a shock treatment before a shortened cold treatment for FCM.

Programopsomming

Drie van die sewe witluis spesies wat op sitrus in Suid-Afrika bekend is word as ŉ fitosanitêre bedreiging vir sekere uitvoer markte beskou. Twee van die vyf projekte in hierdie program wat hier gerapporteer word, spreek verskillende aspekte van witluis bestuur toe. Alhoewel een van hulle het ook blaaspootjie behels. Twee projekte het op karobmot gefokus wat gereeld met witluis geassosieer word. Vir meeste markte is karobmot nie ŉ fitosanitêe plaag nie, maar daar bestaan sekere uitsonderings soos China. Karobmot is altyd as ŉ sekondêre en sporadiese plaag beskou met ŉ besonderse lae plaagstatus. Sy voorkoms op sitrus blyk nou egter hoër as wat voorheen waargeneem is. Nog ŉ faktor wat sy herkenning en bestuur meer belangrik maak is die morfologiese gelykheid van sy larwes met dié van die valskoldingmot (VKM). Die laaste projek in die program het gefokus op na-oes behandeling vir fitosanitêre plae oor die algemeen.

In die eerste van die witluis projekte, wat deur die Internasionale Kernenergie Agentskap (IAEA) bevonds is, is gammabestraling van 150 Gy op ŉ probit 8.7 skaal geëevalueer om die dosis se waarde as ŉ moontlike fitosanitêre behandeling te bepaal (3.5.2). Alle reproduktiewe wyfies is ten volle deur die behandeling gesteriliseer. In die twede projek op witluis, plaaslik geïsoleerde entomopatogeniese swamme (EPS) is in labortorium biotoetse teen witluis getoets (3.5.3). ŉ *Beauveria bassiana* isolaat en ŉ *Metharizium anisopliae* isolaat het albei ŉ mortaliteit van 67.5% van witluis kruipers veroorsaak en ŉ tweede *B. bassiana* isolaat het 64% kruiper mortaliteit veroorsaak. Dosis-respons verhoudings vir die drie isolate is daarna teen albei volwasse en kruiper witluis bepaal. Hierdie EPS isolate is ook teen sitrusblaaspootjie volwassenes getoets en het tussen 60 and 70% mortaliteit veroorsaak.

Die eerste van die karobmot projekte het die morfologie en ekologie van karobmot in sitrusboorde ondersoek (3.5.4). Op hierdie stadium is resultate nog nie beskikbaar nie. Die ander karobmot projek is ŉ veldstudie in die Vaalharts streek, waar sitrus en pekanneute langs mekaar gekweek word (3.5.6). Die doel was om die saamestelling van die Lepidoptera boorder kompleks op die twee gewasse te bepaal en ook enige beweging van die plase tussen die gewasse. Aandudings op hierdie stadium is dat karobmot besmetting op albei pekanneute en sitrus hoër is as vir VKM en dat dit blyk dat karobmot van pekans tot sitruus migreer.

Die finale projek het ŉ GRAS na-oes berokings middel en CO_2 teen fitosanitêre plae getoets (3.5.5). Een honderd persent mortaliteit van graan stinkbesie is bereik, alhoewel die hoof navorsings fokus geduurende die verslag tydperk was om te bepaal wat die maksimum mortaliteit was wat bereik kon word vir VKM en vrugtevleig, sonder om vrugte te beskadig. Dit het geblyk dat vrugtevleig effens meer vatbaar as VKM was vir albei Grasfum en CO_2. Verdere werk het gefkous op CO_2 en sy gebruik as ŉ skok behandeling voor ŉ verkorte koue behandeling vir VKM.

3.4.2 FINAL REPORT: Evaluation of gamma irradiation as a post-harvest control measure for citrus mealybug, *Planococcus citri* (Risso)

Project IAEA 15634/RO (2009-2014) by J.H. & M. Hofmeyr (Citrus Research International)

Summary

An experiment was conducted on small scale to assess the radiotolerance of reproductive mealybugs to 150 Gy of ionizing radiation. All females were fully sterilized and similar to non-ovipositing females, no progeny developed. The efficacy of 150 Gy was consequently evaluated on probit-8.7 scale to confirm this dose's value as a potential phytosanitary treatment. Two experiments were conducted with respectively 70 440 and 3 150 reproductive females respectively. In the control treatments of both experiments females oviposited normally on control butternuts. The F1 progeny from these females developed into non-ovipositing females before the control treatments were terminated. In the ionizing radiation treatments a small number of 1st instar F1 nymphs were produced before treatment. Subsequent to treatment these nymphs either died *in situ*, or migrated to fresh butternuts where they also died without further development. All reproductive females were fully sterilized by the treatment and continued production of a new generation was prevented. The results are regarded as adequate to meet the requirements of Research Contract 15634/RO and the study is concluded.

Opsomming

ŉ Proef is op klein skaal uitgevoer om die radiovatbaarheid van reproduktiewe witluiswyfies vir 150 Gy ioniserende straling vas te stel. Alle wyfies is ten volle gesteriliseer en soortgelyk aan volwasse nie-reproduktiewe wyfies, het geen nageslag ontwikkel nie. Die doeltreffendheid van 150 Gy ioniserende straling is vervolgens op probit-8.7 vlak geëvalueer om dié dosis se waarde as ŉ potensiële fitosanitêre behandeling te bevestig. Twee proewe is met onderskeidelik 70 440 en 3 150 reproduktiewe wyfies uitgevoer. In die kontrolebehandelings van beide proewe het die onbehandelde wyfies normaal eiers op botterskorsies gelê. Die F1-nageslag van dié wyfies het tot volwasse wyfies ontwikkel voordat die kontrole-behandelings tot niet gemaak is. In die ioniserende stralingbehandeling is ŉ klein aantal 1ste instar F1-nimfe alreeds voor behandeling geproduseer. Ná behandeling is hulle almal óf *in situ* dood óf het na vars botterskorsies migreer waar hulle sonder verdere ontwikkeling gevrek het. Alle reproduktiewe wyfies is ten volle deur die behandeling gesteriliseer en voortgesette voortplanting is voorkom. Die resultate is as voldoende vir die vereistes van navorsingskontrak 15634/RO beskou en die studie is afgesluit.

Introduction

The effects of 50-100 Gy and 50-400 Gy ionizing radiation (IoR) respectively on eggs and pre-ovipositing females of citrus mealybug, *Planococcus citri*, were presented in the CRI annual reports for 2009-10, 2010-11 and 2011-12. It was demonstrated that 100 Gy IoR would prevent the development of 1st instar nymphs hatching from treated eggs. At 150 Gy, pre-ovipositing females were prevented from ovipositing. These studies were regarded as sufficient supportive evidence that 150 Gy gamma irradiation would prove to be an effective phytosanitary treatment for *P. citri*. The studies in this report were conducted to confirm that opinion.

In 2012 the *P. citri* study was expanded to include a second mealybug species, *P. ficus*. This species has proved difficult to mass rear and at the time of writing the first of several planned treatment repetitions had failed. Results of this study will be discussed in the progress report for 2013-14.

Objectives

The objective of the project was to establish the Lowest Effective Radiation Dose (LERD) capable of preventing reproduction of the most radiotolerant developmental stage of the citrus mealybug, *Planococcus citri* (Risso). The research was aimed at establishing an irradiation dose capable of producing results at a phytosanitary level, i.e. complete mortality or sterility. The ability of treated insects to reach maturity and produce fertile eggs was used as the measure of efficacy. A treatment producing one or more F1 generation females was therefore regarded as inadequate.

1 **Experiment 1: Reproductive females**

Introduction

Subsequent to earlier studies already reported on in CRI annual reports (see main Introduction), the opinion was expressed that reproductive females may possibly be more radiotolerant than pre-ovipositing females (Hallman, pers. com.). This aspect was therefore investigated on a small scale before a final decision with regard to the suitability of 150 Gy as the LERD for a conclusive large scale assessment was made.

Materials and methods

The availability of test material in the rearing off-season restricted the number of replicates to 2 per treatment. Each replicate consisted of a single butternut infested with a mean of 435 and 1 359 reproductive females in the control and IoR treatments respectively. A single dose of 150 Gy was applied. The butternuts were incubated for 21 days and then assessed destructively for numbers and developmental stages of mealybugs.

Results and discussion

Mealybug proliferation on the untreated control butternuts progressed normally and the F1 progeny had developed into 3rd instar nymphs and young females at the time of assessment (Table 3.5.2.1).

Table 3.4.2.1. The effect of 150 Gy gamma irradiation on reproductive mealybug females.

Attribute	0 Gy		150 Gy	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Pre-treatment: No. of females with ovisacs	562	307	847	1871
Post-treatment: No. of F1 progeny (3rd instar nymphs plus young females)	1481	1286	0	0

Most of the females on the treated butternuts were dead – a few still showed weak, spasmodic leg movements. Apart from the adults only the dried-out remains of dead 1st instar nymphs were observed. No live nymphs were observed in the ovisacs. No evidence was found that any 1st instar nymph had maintained its development. Although the sample size was relatively small, it was considered worth the risk to continue with a larger scale study.

2 **Experiment 2: Probit-8.7**

Introduction

From the positive results in all studies so far, including the preceding experiment, it was decided to confirm the efficacy of 150 Gy IoR to reproductive females on a large scale. Project consultants at the IAEA recommended that the study be conducted at probit-8.7 scale, *viz*. 30 000 insects.

Materials and methods

The study was divided into a primary and a smaller secondary experiment conducted consecutively. Infested butternuts were collected at random from a rearing culture specifically maintained for the project. The instar distribution was relatively harmonized and consisted mainly of reproductive females. A single treatment dose of 150 Gy was used.

1 **Primary experiment**

Thirty butternuts infested with reproductive females were divided at random between the various treatments:

- *Untreated control*: Ten replicates, each consisting of one infested butternut, were used. They were divided at random into 2 groups (A and B) of 5 butternuts each. All mealybugs were counted destructively in group A. Group B was used to verify the infestation capability of the untreated P1 females. This was accomplished by placing fresh, uninfested butternuts on the butternuts of group B for 24 hours to subject them to infestation by 1st instar F1 nymphs. They were then incubated to allow nymphal development.

- *Ionizing radiation treatment*: Twenty replicates, each consisting of one infested butternut, were used. After treatment with 150 Gy IoR they were divided at random into 2 groups (C and D) of 10 butternuts each. The butternuts of group C were incubated for a period of 2 months and examined for nymphal development at monthly intervals. The butternuts of group D were used to determine the infestation potential of the treated P1 females. This was accomplished by placing 8 fresh, uninfested butternuts on the treated butternuts and subjecting them to infestation by 1st instar F1 nymphs. They were removed after 7 days and then incubated for 45 days – under normal circumstances long enough for the development of 1,5 generations. Assessments were conducted at weekly intervals to monitor potential nymphal migration and development.

- *Confirmation of insect age*: On the day of treatment 60 insects were collected at random from a representative butternut in the rearing culture. They were placed into 75% ethyl alcohol to be examined for confirmation of developmental stage.

2 **Secondary experiment**

It was initially planned to separate the probit-8.7 study into three consecutive replicates of approximately 10 000 insects each for mainly logistical reasons. Due to the number of required mealybugs already treated in the primary experiment, it was decided to omit the third replicate and treat a second replicate only, but on a much smaller scale to confirm of the primary results. The methodology was similar to that used in the primary experiment.

The mealybugs used for control and treatment purposes were obtained by exposing fresh butternuts to those infested with the adult F1 progeny of the P1 females used in the primary experiment control (see Primary experiment – *Untreated control*).

➢ *Untreated control*: The methodology was similar to the primary experiment, except that 8 replicates, divided into 2 groups of 4 butternuts each, were used.

➢ *Ionizing radiation treatment*: The methodology was similar to the primary experiment, except that 10 replicates divided into 2 groups of 5 butternuts each, were used.

Results and discussion

1 Primary experiment

➢ *Untreated control*: The butternuts of group A were infested with more mealybugs than anticipated and a mean of 4 361 individuals was established per butternut. Of these, 95% (4 143) were confirmed to be adult females of which 3 522 (84%) were ovipositing.

The number of F1 progeny from P1 control females in group B was not recorded. They were reared to adulthood and their progeny (F2) on butternuts was used as test material for the secondary experiment (see section 2 below). There can consequently be no doubt about the reproductive potential of the insects used in this study (Fig. 3.4.2.1).

Fig. 3.4.2.1. F2 progeny from the F1 generation produced by P1 females in the untreated control.

➢ *Ionizing radiation treatment*: The mean number of mealybugs recorded per butternut in the untreated control (4 361) was used to determine the number treated, *viz*. 87 220 insects. Of these, 70 440 were calculated to be reproductive females.

Although the population was well-synchronized, it was inevitable that all females would not commence ovipositing simultaneously. Treatment consequently had to be postponed for 48 hours until the bulk of females started ovipositing. By the time of treatment this delay had resulted in a population consisting of reproductive females, eggs and 1st instar nymphs. It is known from a previous study that eggs treated with 100 Gy can hatch into 1st instar nymphs that will die without developing further (CRI annual report for 2010-11). The effect of 150 Gy on eggs was not studied. However, when pre-ovipositing females were treated with 150 Gy in a later experiment no 1st instar nymphs were subsequently recorded (CRI annual report for 2011-12). The relatively small numbers of 1st instar nymphs present on the treated butternuts of group C in this study were therefore those that had already been present at the time of treatment. These nymphs died without developing further. A small number of these nymphs produced on the butternuts of group D migrated to fresh butternuts. They also died without developing further (Fig. 3.4.2.2).

Fig. 3.4.2.2. Butternuts incubated for 45 days after 7 days exposure to radiotreated P1 generation female mealybugs. Dead 1st instar F1 nymphs are evident on the butternuts.

2 Secondary experiment

➢ *Untreated control*: A mean of 354 mealybugs was counted on each butternut. Of these, 96% were adult females, of which 92% were ovipositing. As before, the reproductive potential of the P1 females was demonstrated in large numbers of 1st instar nymphs that infested fresh butternuts to continue their natural development.

➢ *Ionizing treatment*: A calculated total of 3 150 reproductive females was radiotreated. Similar to the primary experiment a small number of 1st instar F1 nymphs (already present at the time of treatment) succeeded in migrating to fresh butternuts. All died without further development.

Conclusion

In the research project gamma doses ranging from 50 Gy to 400 Gy were studied on all possible developmental stages of *P. citri*. Doses capable of preventing either (i) further development of the treated stage or (ii) a viable off-spring, ranged from 100 Gy (eggs, 1st-3rd instar nymphs) to 150 Gy (non-ovipositing and reproductive females). The primary experiment involving the stage regarded to be the most tolerant to gamma radiation, *viz*. reproductive females, was conducted successfully on a scale 2.3 times larger than required. These results were confirmed in a secondary experiment and are therefore regarded to be adequate to meet the requirements of Research Contract 15634/RO.

Technology Transfer

This work was presented at an IAEA workshop in Vienna in 2014.

3.4.3 FINAL REPORT: Screening of entomopathogenic fungi against citrus mealybug (*Planococcus citri* (Risso)) and citrus thrips (*Scirtothrips aurantii* (Faure))

Project 1048 (2012 – 2014) by V Chartier-Fitzgerald, J Dames, MP Hill (RU) and SD Moore (CRI)

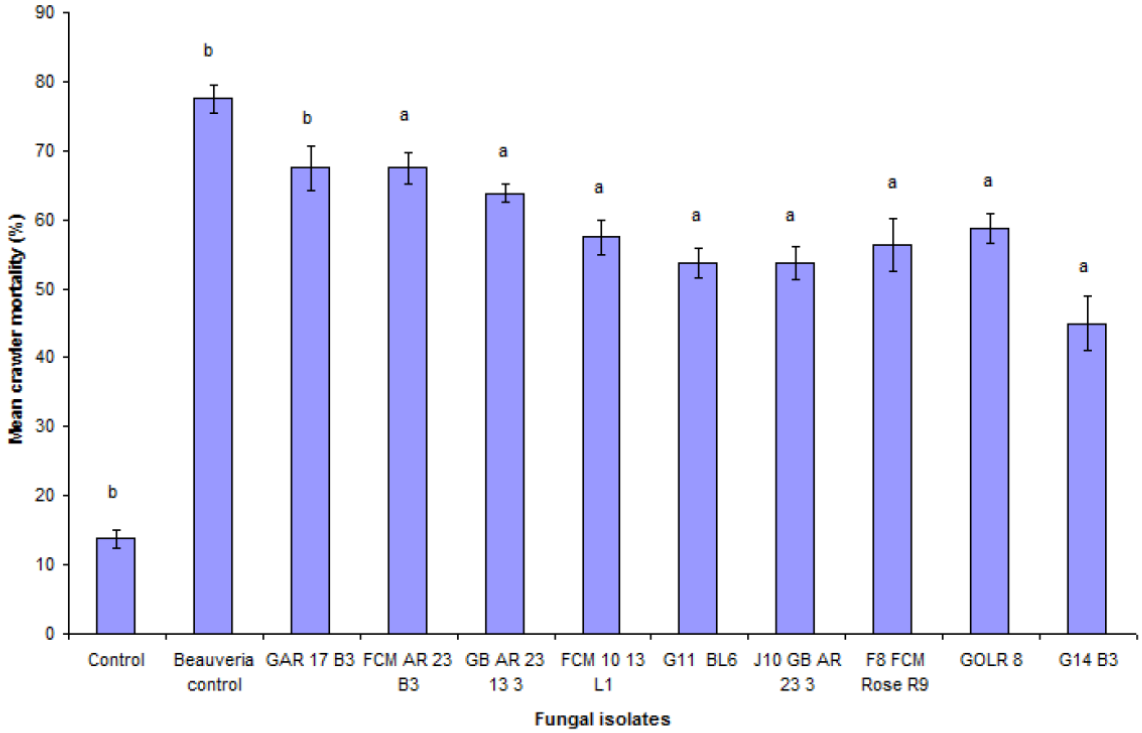
Summary

Mealybugs (*Planococcus citri*) and thrips (*Scirtothrips aurantii*) are common and extremely damaging citrus crop pests which have proven difficult to control via conventional methods, such as chemical pesticides and insect growth regulators. The objective of this study was to determine the efficacy of entomopathogenic fungi against these pests in laboratory bioassays. Isolates of *Metarhizium anisopliae* and *Beauveria bassiana* from citrus orchards in the Eastern Cape, South Africa were maintained on Sabouraud Dextrose 4% Agar supplemented with Dodine, chloramphenicol and rifampicin at 25°C. Infectivity of the fungal isolates was initially assessed using 5th instar false codling moth, *Thaumatotibia leucotreta*, larvae. Mealybug bioassays were performed in 24 well plates using 1 x 10^7 ml^{-1} conidial suspensions and kept at 26°C for 5 days with a photoperiod of 12 L:12 D. A *Beauveria* commercial product and an un-inoculated control were also screened for comparison. Isolates GAR 17 B3 (*B. bassiana*) and FCM AR 23 B3 (*M. anisopliae*) both resulted in 67.5% mealybug crawler mortality and GB AR 23 13 3 (*B. bassiana*) resulted in 64% crawler mortality (Fig. 3.4.3.1). These 3 isolates were further tested in dose-dependent assays. Probit analyses were conducted on the dose-dependent assays data using PROBAN to determine LC_{50} values (Fig. 3.4.3.2). For both the mealybug adult and crawlers FCM AR 23 B3 required the lowest concentration to achieve LC_{50} at 4.96 x 10^6 conidia ml^{-1} and 5.29 x 10^5 conidia ml^{-1}, respectively (Table 3.4.3.1). Bioassays on adult thrips were conducted in Munger cells with leaf buds inoculated with the conidial suspensions. Isolate GAR 17 B3 had the highest mortality rate at 70% on thrips while FCM AR 23 B3 resulted in 60% mortality (Table 3.4.3.2). Identification of the isolates, FCM AR 23 B3, GAR 17 B3 and GB AR 23 13 3, were confirmed to be correct using both microscopic and molecular techniques. ITS sequences were compared to other sequences from GenBank and confirmed phylogenetically using MEGA6. Mealybug infection was investigated using scanning electron microscopy, mycosis was confirmed but the infection process could not be followed due to the extensive waxy cuticle. These results indicate that there is potential for the isolates FCM AR 23 B3 and GAR 17 B3 to be developed as biological control agents for the control of citrus mealybug and thrips. Further research would be required to determine their ability to perform under field conditions.
This study was completed for an MSc and the thesis is available from CRI or Rhodes University.

Opsomming

Witluis (*Planococcus citri*) en blaaspootjie (*Scirtothrips aurantii*) is algemene en hoogs beskadigende sitrusplae wat soms moeilik beheerbaar is deur konvensionele metodes, soos chemiese bestryding en insek-groeireguleerders. Die doel van hierdie studie was om die werking van entomopatogeniese swamme teen hierdie plae in laboratorium biotoetse te bepaal. Isolate van *Metarhizium anisopliae* en *Beauveria bassiana* van sitrusboorde in die Oos-Kaap, Suid-Afrika, is op Sabouraud Dekstrose 4% Agar (gesupplementeer met dodien, chlooramfenikol en rifampisien) teen 25°C onderhou. Infektiwiteit van die swam isolate is oorspronklik bepaal met die gebruik van 5de instar valskodlingmot, *Thaumatotibia leucotreta*, larwes. Witluis biotoetse is in 24-put plate uitgevoer met 1 x 10^7 ml^{-1} konidiële suspensies en is gehou vir 5 dae teen 26°C met ŉ lig-donker verhouding van 12 L:12 D. ŉ *Beauveria* kommersiele produk en ŉ onbehandelde kontrole is ook vir vergelykings doeleindes ontleed. Isolate GAR 17 B3 (*B. bassiana*) en FCM AR 23 B3 (*M. anisopliae*) het albei 67.5% mortaliteit van witluis kruipers veroorsaak en GB AR 23 13 3 (*B. bassiana*) het 64% kruiper

mortaliteit veroorsaak (Fig. 3.4.3.1). Hierdie drie isolate is in dosis-respons biotoetse verder getoets. Probit analises is op die dosis-respons biotoets data uitgevoer met die gebruik van PROBAN en LC_{50} waardes is dus geskat (Fig. 3.4.3.2). Vir albei witluis volwassenes en kruipers het FCM AR 23 B3 die laagste konsentrasie benodig vir die LC_{50}, 4.96 x 10^6 conidia ml^{-1} en 5.29 x 10^5 conidia ml^{-1}, onderskeidelik (Tabel 3.4.3.1). Biotoetse op volwasse blaaspootjie is in Munger-selle uitgevoer op jong blare wat met konidiele suspensies ingeënt is. Isolaat GAR 17 B3 het die hoogste mortaliteit van blaaspootjie van 70% veroorsaak terwyl FCM AR 23 B3 60% mortaliteit veroorsaak het (Tabel 3.4.3.2). Identifikasies van die isolate, FCM AR 23 B3, GAR 17 B3 en GB AR 23 13 3, is bevestig met gebruik van albei mikroskopiese en molekulêre tegnieke. ITS basispaar volgorde is vergelyk met ander basispaar volgordes van GenBank en filogeneties bevestig met gebruik van MEGA6. Witluis besmetting is met gebruik van skandeer-elektronmikroskopie ondersoek. Mikose is bevestig maar die besmettings proses kon nie gevolg word nie as gevolg van die wasserige kutikula van die insek. Hierdie resultate dui aan dat daar potensiaal is om die isolate FCM AR 23 B3 en GAR 17 B3 te ontwikkel as biologiese beheer agente vir beheer van sitruswitluis en blaaspootjie. Verdere navorsing word benodig om hulle vermoë onder veld toestande te bepaal.
Hierdie studie is uitgevoer vir ŉ MSc graad. Die volle tesis kan dus of van CRI of Rhodes Universiteit gekry word.



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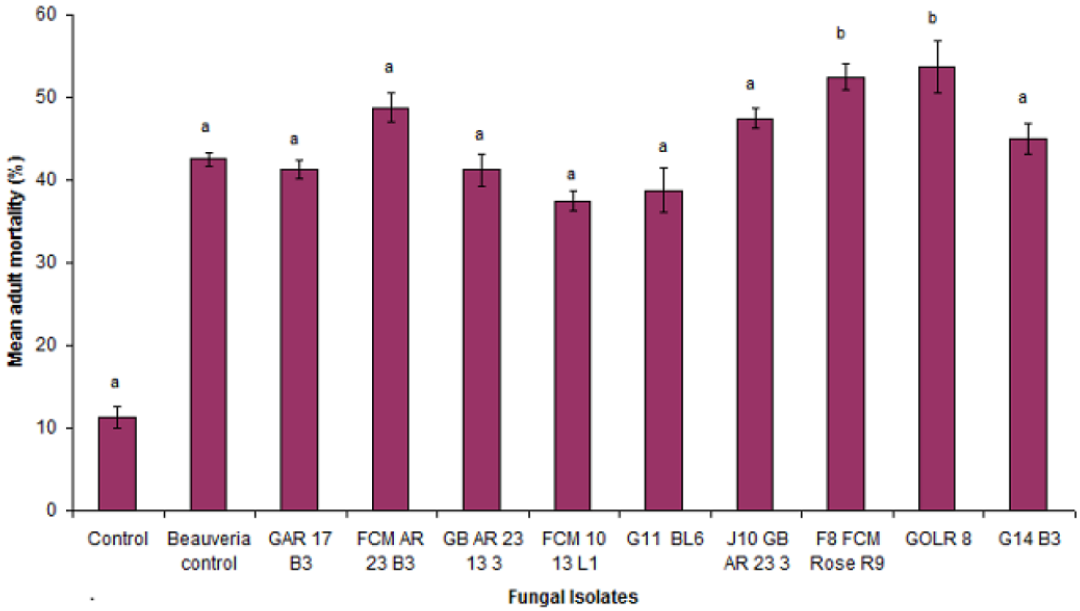
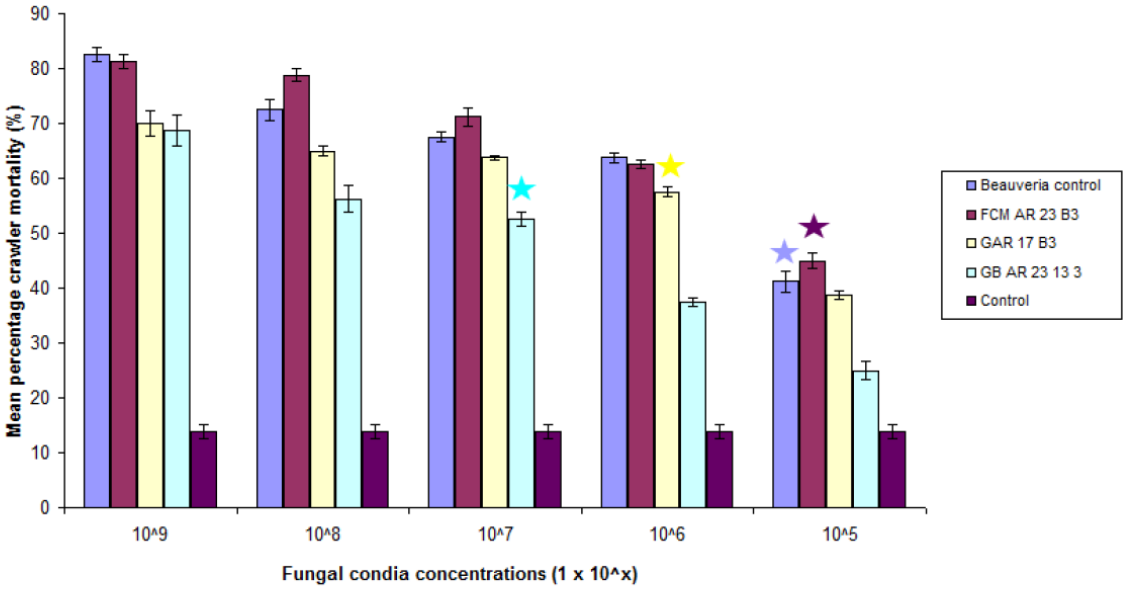


(b)

Figure 3.4.3.1. Mean mortality percentages using selected entomopathogenic fungal isolates at a concentration of 1 x 10^7conidia ml-1: (a) crawler mortality (Kruskal-Wallis test H (10, N=55) = 22.78476 p=0.0116), (b) adult mortality (Kruskal-Wallis test; H (10,N=55) =21.42390 p=0.0183). Columns with the same letters are not significantly different from each other. Bars represent standard deviation.



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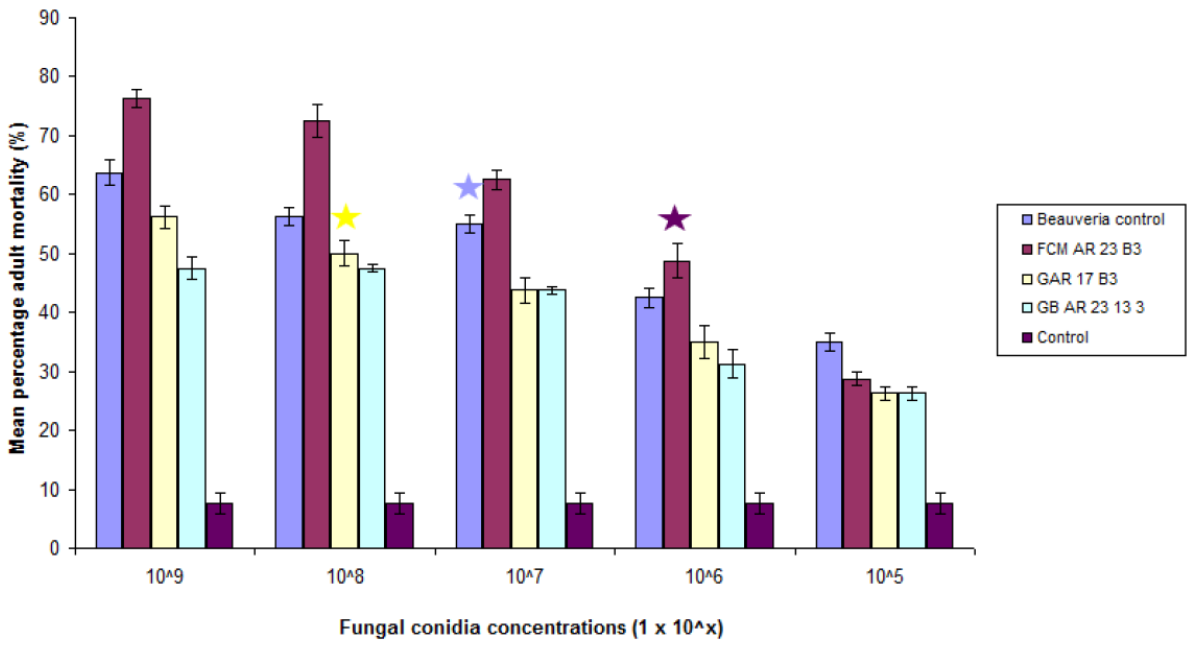


(b)

Figure 3.4.3.2. Mortality of the mealybug dose-dependent bioassay at different fungal concentrations (a) crawler mortality (Kruskal-Wallis test: H (20, N=105) = 82.46991 p=0.00001), (b) adult mortality (Kruskal-Wallis test; H (20, N=105) =72.29002 p=0.00001). Stars indicate approximate 50% mortality.

Table 3.4.3.1. Summarised PROBAN data for crawler and adult mortality data for each of the fungal isolates tested.

Life stage	Isolate	Significant concentration (Conidia/ml)	Lethal concentration				Fiducial limits		
			LC_{50}	LC_{50} (SE)	LC_{90}	LC_{90} (SE)	G	Upper (LC_{50})	Lower(LC_{50})
Crawlers	*Beauveria* Control	$10^9$and 10^7	9.94 x 10^5	7.27 x 10^5	3.50 x 10^{10}	5.86 x 10^{10}	0.13	3.40 x 10^6	1.43 x 10^5
	FCM AR 23 B3	$10^9$and 10^8	5.29 x 10^5	4.27 x 10^6	2.14 x 10^{10}	3.49 x 10^{10}	0.14	1.97 x 10^6	5.68 x 10^4
	GAR 17 B3	10^7	4.25 x 10^6	3.63 x 10^6	8.75 x 10^{12}	3.11 x 10^{13}	0.25	2.20 x 10^7	4.36 x 10^5
	GB AR 23 13 3	-	6.65 x 10^7	3.89 x 10^7	4.98 x 10^{11}	9.35 x 10^{11}	0.12	2.70 x 10^8	2.28 x 10^7
Adults	*Beauveria* Control	10^9	1.67 x 10^7	1.29 x 10^7	5.47 x 10^{12}	1.77 x 10^{13}	0.22	1.04 x 10^8	3.27 x 10^6
	FCM AR 23 B3	10^9, $10^8$and 10^7	4.96 x 10^6	2.38 x 10^6	2.27 x 10^{10}	2.82 x 10^{10}	0.08	1.24 x 10^7	1.71 x 10^6
	GAR 17 B3	-	3.50 x 10^8	3.70 x 10^8	2.20 x 10^{14}	8.75 x 10^{14}	0.21	1.00 x 10^{10}	6.86 x 10^7
	GB AR 23 13 3	-	-	-	-	-	-	-	-

Table 3.5.3.2. Thrips bioassay percentage mortality Chi-squared test; X2 (df=3, n=4)= 0.0062, p=0.033.

EP fungal isolate	Mortality (%)
Control	10
Beauveria control	60
FCM AR 23 B3	60
GAR 17 B3	70

Technology Transfer

This work will be presented at the Citrus Research Symposium in August 2014.

3.4.4 PROGRESS REPORT: The morphology and ecology of the Carob moth in citrus orchards

US/ENT-11-A3 (2012/7-2014/12) by P. Addison, G. Morland and H. Geertsema (SU)

Summary

The carob moth *Ectomyelois ceratoniae* is a field pest that occurs on growing fruits and carob tree pods. It is a species that originated from the Mediterranean region. Carob moth is a stored product pest that has caused up to 40% damage in both the date and pomegranate industry. It is difficult to tell how much damage the carob moth has caused in the South African citrus industry because in its larval stage it can be easily confused with the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). The aims of this study are to produce a valid morphological description of each life stage of the carob moth, as well as to determine its seasonal cycle within citrus orchards of the Western Cape. Other objectives include a life table study and a wing morphometrics study. To achieve these aims a series of field tests and laboratory tests will be done. The field tests include the hanging of yellow delta traps baited with chemically synthesized pheromone within orchards, as well as a damage assessment. The laboratory tests will be done by rearing a colony, which will then be tested at temperature gradients to determine intrinsic rates of increase and other relevant developmental parameters. The predicted outcome of the study is to establish the threat status of carob moth on citrus and other hosts associated with citrus in order to limit crop losses.

Opsomming

Die karobmot, *Ectomyelois ceratoniae*, is ʼn plaag wat op vrugte en karobpeule voorkom. Die mot het ʼn Mediterreense oorsprong. Karobmot is ook ʼn plaag van gestoorde produkte wat tot 40% skade in die dadel en granaat bedrywe al aangetoon het. Dit is moeilik om karobmot skade op sitrus in Suid Afrika te skat, omrede sy larwe stadium dikwels met valsekodling mot, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), verwar kan word. Die doel van hierdie studie is om ʼn geldige morfologiese beskrywing van elke lewensstadium van die karobmot op te lewer, asook om sy seisoenale siklus in sitrusboorde te bepaal. Lewenstabel en vlerk morfometriese studies sal ook uitgevoer word. Om hierdie doele te bereik, sal ʼn reeks veld en laboratorium studies uitgevoer word. Die veld toetse behels die uitplasing van geel delta lokvalle asook n' skade beraming. Laboratorium toetse sal uitgevoer word deur om ʼn kolonie aan te teel en aan temperatuur gradiënte blood te stel om ingebore vermeerderingskoerse en ander relevante ontwikkelings parameters te bepaal. Die voorspelde uitkoms van die studie is om die bedreigingstatus van karobmot op sitrus en ander verwante gashere te bepaal om oes verliese te beperk.

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

Project 913 (2011/2 – 2015/6) by T G Grout, K C Stoltz and P R Stephen (CRI)

Summary

Further evaluation of the fumigant Grasfum has shown that grain chinch bug is very susceptible to the fumigant and 100% mortality of 3 623 insects was obtained after 6 h at 170 g/m^3. This could prove a valuable treatment for Western Cape growers. However, the main research focus during the report period was to determine the maximum mortality that could be obtained of internal pests such as fruit fly and false codling moth (FCM) without being detrimental to the fruit. An off-taste was once found in Shamoutis with high acid that were treated with Grasfum at 225 g/m^3 and this led to a range of taste tests being conducted with different cultivars but no significant effects were found with Grasfum or CO_2 treatments. Penetration of fruit with Grasfum appears to vary more with fruit condition and cultivar than CO_2. In the case of Turkey Valencia this was extreme with Grasfum at 170 g/m^3 causing less than 10% mortality in FCM and fruit fly but 60% CO_2 caused 90%+ mortality of the same pests using the same batch of fruit. Fruit fly appeared to be slightly more susceptible to both fumigants than FCM. Grasfum is probably unaffordable at 420 g/m^3 so 315 g/m^3 will most probably be the highest dosage used in South Africa. Further work has therefore focused on CO_2 and its use as a shock treatment before a shortened cold treatment for FCM. Four trials have now shown that if the fruit is left at ambient temperature for 24 h after being fumigated with CO_2 and before starting a short cold treatment, mortality is lower than if the fruit goes into the cold room shortly after fumigation. We will now use higher numbers of FCM larvae to determine how much shorter the cold treatment can be when preceded with 24 h of CO_2 at 60%.

Opsomming

Verdere evaluasie van die berokingsmiddel Grasfum het gewys dat graanstinkbesie baie vatbaar vir die berokingsmiddel is en 100% mortaliteit van 3 623 insekte is na 6 h teen 170 g/m^3 bereik. Hierdie kan moontlik ʼn waardevolle behandeling vir Wes-Kaapse boere wees. Nietemin is die hoof navorsings fokus geduurende die verslag tydperk om die maksimum moontlike mortaliteit van interne plae soos vrugtevlieg en

valskodlingmot (VKM) te bepaal sonder om nadelig vir die vrugte te wees. ʼn Af-smaak met hoë suur is eenmalig in Shamoutis verkry wat met Grasfum teen 225 g/m^3 behandel is. Hierdie het gelei na die uitvoer van ʼn reeks smaak-toetse met verskillende kultivars, maar geen beduidende effekte is met of Grasfum of CO_2 behandelings gekry nie. Dit blyk dat penetrasie van vrugte met Grasfum meer varieer met vrug toestand en kultivar as wat die geval met CO_2 is. In die geval van Turkey Valencia was dit uiters so gewees. Grasfum teen 170 g/m^3 het minder as 10% mortaliteit van VKM en vrugtevlieg veroorsaak maar 60% CO_2 het 90%+ mortaliteit van dieselfde twee plae in dieselfde besending vrugte veroorsaak. Vrugtevlieg blyk effens meer vatbaar vir albei berokingsmiddels as VKM. Grasfum is waarskynlik bekostigbaar teen 420 g/m^3, dus sal 315 g/m^3 mees waarskynlik die hoogste dosis wees wat in Suid-Afrika gebruik sal word. Verdere werk het daarom op CO_2 gefokus en sy gebruik as a skok behandeling voor ʼn verkorte koue behandeling vir VKM. Vier proewe het nou gewys dat as die vrugte teen kamertemperatuur vir 24 h na beroking met CO_2 gelos word voor die begin van ʼn kort koue behandeling, is mortaliteit laer as wanneer die vrugte onmiddelik na beroking aan die koue behandeling blootgestel word. Ons sal nou hoër getalle VKM larwes gebruik om te bepaal hoeveel korter die koue behandeling kan wees wanneer dit voorafgegaan word met 24 h van CO_2 teen 60%.

3.4.6 PROGRESS REPORT: The association of a lepidopteran borer complex between pecan nuts (*Carya illinoensis*) and citrus (*Citrus sinensis*) in the Vaalharts region

Project 1051-UFS (2013-14) by Andre van Rooyen, Vaughn Swart (UFS) and Sean Moore (CRI)

Summary

Carob moth, *Ectomyelois ceratoniae*, is generally a secondary pest of citrus i.e. it will normally only infest fruit which is already infested with mealybug or has residues of honey dew and sooty mould, associated with mealybug and other sucking insects or fruit which is already damaged (eg navel-end splitting). However, it has been noted that fruit which is in close proximity to more favoured hosts (eg oak trees, pomegranates, pecan nuts) can also become infested. In the Vaalharts region, citrus and pecan nuts are grown in very close proximity. This provides the ideal opportunity to a) determine what the lepidopteran borer complex on citrus and pecans is composed of (it is suspected that it is chiefly false codling moth (*Thaumatotibia leucotreta*) (FCM) and carob moth); b) determine relative proportions of the different species on the two crops; c) determine seasonal fluctuations in specific pest levels; and d) therefore determine the role of shuttling of lepidopteran pests between the two crops. It will thus be possible to determine a) the potential severity of carob moth as a pest for citrus, and b) the threat which a favoured alternative can pose for citrus in both carob moth and FCM risk. A snap survey conducted on pecan nuts in winter 2010 revealed that 29% of nuts were infested. All identified moths were carob moth. Subsequently, further small surveys conducted by the University of the Free State and Stellenbosch University, revealed FCM and *Ephestia* sp. as pests of pecans. Another potential advantage of determining the pest status of FCM on pecans is that if it can be shown that a significant portion of this complex is indeed FCM, then an SIT programme could be initiated in the Vaalharts irrigation scheme. This study was initiated in 2012, although only funded by CRI since 2013. It will continue until July 2014. Approximately 100 pecan nuts and 50 citrus fruit (from the trees and ground) were collected each month from adjacent orchards, 10 and 50 m from the edge of pecan and citrus orchards. Pheromone traps for both FCM and carob moth were also deployed at the study sites, in order to establish a relationship between trap captures and infestation from collected material. Processor reports will be collected and incorporated in the results. Seven sites that met these conditions were selected as study sites. Pecan nut samples were placed into emergence boxes to allow larvae to develop to adulthood. Citrus fruit were internally examined for larvae; recovered larvae were placed on feeding medium and allowed to pupate and emerge. Specimens are sent to Dr M Krüger (Transvaal Museum) for identification. Indications thus far are that carob moth infestation of both pecans and citrus is higher than for FCM; and carob moth appears to migrate from pecans into citrus.

Opsomming

Karob mot, *Ectomyelois ceratoniae*, word gewoonlik as ʼn sekondêre plaag van sitrus beskou dus besmet dit gewoonlik slegs vrugte wat reeds besmet is met witluis of wat nalaatsels van heuning dou en roetskimmel bevat, hierdie hou verband met witluis en ander suigende insekte of vrugte wat reeds beskadig is (bv. nawelentbars). Dit is egter ook opgemerk dat die vrugte in die nabyheid van meer voordelige gashere (bv. eikebome, granate, pekanneute) ook besmet kan raak. In die Vaalharts streek, word sitrus en pekanneute naby aan mekaar verbou. Dit bied die ideale geleentheid om die voglende te bepaal a) die samestelling van die Lepidoptera stronkboorder kompleks op sitrus en pekanneute (dit word vermoed dat dit hoofsaakliks valskodlingmot (*Thaumatotibia leucotreta*) (VKM) en karobmot is); b) die relatiewe proporsies van die verskillende spesies op die twee gewasse; c) seisoenale fluktuasies in spesifieke plaag vlakke en d) die impak van Lepidoptera pes migrasie tussen die twee gewasse. Dit sal dus moontlik wees om a) die potensiële erns van karobmot as ʼn plaag vir sitrus, en b) die bedreiging wat ʼn alternatiewe voedselbron kan

inhou vir sitrus aangaande karobmot en VKM risiko. ŉ Opname van pekanneute in die winter 2010 het getoon dat 29% van die neute was besmet. Alle geïdentifiseerde motte was karobmot gewees. Daarna, het verdere klein opnames deur die Universiteite van die Vrystaat en Stellenbosch dit tot lig gebring dat beide VKM en *Ephestia* sp. as plae van pekanneute optree. Nog ŉ potensiële voordeel van die bepaling van die plaagstatus van VKM op pekanneute is indien dit bewys kan word dat ŉ beduidende gedeelte van die kompleks inderdaad VKM is, kan ŉ SIT program in die Vaalharts-besproeiingskema opgestel word. Hierdie studie het begin in 2012 alhoewel slegs deur CRI befonds sedert 2013, en sal voortgaan tot Julie 2014. Ongeveer 100 pekanneute en 50 sitrus vrugte (van die bome en grond) is elke maand van aangrensende boorde, 10 en 50 m van die rand af ingesamel van beide pekan- en sitrusboorde. Feromoonlokvalle vir beide VKM en karobmot is ontplooi by alle studie areas, ten einde om ŉ verhouding tussen lokval data en besmetting van versamelde materiaal te bepaal. Verwerker verslae sal verkry word en by die resultate bygevoeg word. Sewe persele met hierdie omstandighede is as studie persele gekies. Pekanneut monsters is in uitbroei bokse geplaas en toegelaat om tot volwassenheid te ontwikkel. Sitrusvrugte is intern ondersoek vir larwes; versamelde larwes is op ŉ voedings medium geplaas en toegelaat om papies te vorm en tot volwassenheid te ontwikkel. Monsters is na Dr M Krüger (Transvaalse Museum) gestuur vir identifikasie. Aanduidings tot dusver is dat karobmot besmetting van beide pekanneute en sitrus is hoër as vir VKM; en karob mot blyk te migreer van pekanneute tot in sitrus.

3.5 PROGRAMME: MINOR PESTS AND MITES

Programme coordinator: Tim G Grout (CRI)

3.5.1 Programme summary

Only one project received attention in this report period and that was on the development of an attractant for fruit-piercing moths, which though a very sporadic pest, can cause serious economic losses. Natural banana was shown to be an effective attractant for fruit-piercing moths but synthetic alternatives do not work. A much expanded research effort in three production regions in 2014 included Tshipise where fruit-piercing moths in the genus *Eudocima* were found to damage less than 1% of the fruit. Research to improve the banana formulation will continue (3.5.2).

Programopsomming

Slegs een projek het tydens hierdie verslagperiode aandag geniet en dit was oor die ontwikkeling van ŉ lokmiddel vir vrugte suigmotte, wat alhoewel ŉ baie sporadiese plaag is, ernstige ekonomiese verliese kan veroorsaak. Natuurlike piesangs het getoon om ŉ effektiewe lokmiddel vir vrugte suigmotte te wees, maar kunsmatige alternatiewe het nie gewerk nie. ŉ Uitgebreide navorsingspoging in drie produksie streke in 2014 het Tshipise ingesluit waar vrugte suigmotte in die genus *Eudocima* minder as 1% van die vrugte beskadig het. Navorsing om die piesang formulasie te verbeter sal voortgaan (3.5.2).

3.5.2 PROGRESS REPORT: Using banana odour as an attractant for monitoring fruit piercing moth in citrus orchards

Project RU 1058 by Mathew Goddard, Martin Hill (RU) & Sean Moore (CRI)

Summary

Serrodes partita (Fabricius, 1775) (Lepidoptera: Noctuidae), *Achaea lienardi* (Boiduval, 1833) (Lepidoptera: Noctuidae) and other fruit piercing and fruit sucking moths are injurious in citrus orchards. However, there is no method to monitor their populations. The aim of this study was to quantify the attractiveness of banana and a banana essence, isopentyl acetate, to fruit piercing and fruit sucking moths in six Satsuma mandarin orchards in the Kat River Valley and Grahamstown growing regions in 2013. A further goal was to establish a relationship between trap catches of fruit-feeding moths in the orchards and the level of damage they caused to fruit. Traps were deployed and emptied weekly over a three month period, from February 2013. Isopentyl acetate was not attractive. The average numbers of moths caught per trap per week for all the sites was 7.7 moths using banana and 0 moths using isopentyl acetate. Five to 10% of moths caught weekly were *S. partita*. *Achaea* species had a far greater abundance of 40-80 % of the weekly catch. Two to three weeks after a peak in *S. partita* catches, damage was observed on the fruit, but this relationship was not significant. Fruit-piercing moth damage to fruit was higher than any other cause, both within the orchard and on the northern edge of the orchard next to the windbreak (where moths would enter the orchard). The study continued into the 2014 growing season where five new banana odour baits were compared with both fresh and frozen banana. The trials continued in 18 Satsuma mandarin orchards in the Kat River Valley and the Sundays River Valley growing regions. The trial ran from the end of January 2014 for three months, where 106 traps were deployed and emptied weekly. Ten traps were used to establish a relationship between trap catches of fruit-feeding moths in the orchards and the level of damage they caused to fruit. A trial was also

conducted on Alicedale Packers Farm in Tshipise, Limpopo, using frozen banana. The trial ran from the end of January 2014 for 7 weeks, where 10 traps were deployed and emptied weekly. The only fruit-piercing moths caught in the traps were of the genus *Eudocima* and damage to fruit was low, with less than 1% of the fruit being damaged from this cause. Banana has shown to still be the most attractive bait and therefore further research is underway to improve the banana formulation.

Opsomming

Serrodes partita (Fabricius, 1775) (Lepidoptera: Noctuidae), *Achaea lienardi* (Boiduval, 1833) (Lepidoptera: Noctuidae) en ander vrugtesteek motte en vrugtesuig motte kan skade in sitrusboorde veroorsaak, maar daar bestaan egter geen metode om hul populasies te moniteer. Die doel van hierdie studie was om die aantreklikheid te bepaal van piesang en ŉ piesang reukgoed, isopentiel-asetaat, vir vrugtesteek en vrugtesuig motte in ses Satsuma mandaryn boorde in die Katriviervallei en Grahamstad produksie streke in 2013. ŉ Verdere doel is om ŉ verhouding te bepaal tussen lokvalvangstes van hierdie motte en die vlak van vrugskade wat hulle in boorde veroorsaak. Lokvalle is gehang en weekliks leegemaak oor ŉ drie-maande tydperk van Februarie 2013. Isopentiel-asetaat was nie aantreklik nie. Die gemiddelde getal motte gevang per lokval per week vir al die persele is 7.7 motte met piesang en geen motte met isopentiel-asetaat. Vyf tot 10% van motte wat gevang is was *S. partita*. Meer *Achaea* spesies is gekry: van 40-80% van die weeklikse vangstes. Twee tot drie weke na ŉ piek in *S. partita* vangstes is skade op vrugte opgelet maar dié verhouding is nie betekenisvol nie. Vrugtesteekmot skade op vrugte is hoër as enige ander tipe skade, albei binne boorde en op die noordelike rand van die boord langs die windbreek (waar motte die boord sou intree). Die studie is in die 2014 produksieseisoen voortgesit waar vyf nuwe piesang geur lokmiddels met vars piesang en gevriesde piesang vergelyk is. Die proewe is in 18 Satsuma mandaryn boorde in die Katriviervallei en Sondagsriviervallei produksie streke uitgevoer. Die proef het geloop van einde Januarie 2014 vir drie maande, waar 106 lokvalle gehang is en weekliks leegemaak is. Tien lokvalle is gebruik om ŉ verhouding tussen lokvalvangstes van vrugtevoedingsmotte in die boorde en die vlak van vrugskade te bepaal. ŉ Proef is ook op Alicedale Packers Plaas in Tshipise, Limpopo, met gevriesde piesang gedoen. Die proef het geloop van einde Januarie 2014 vir 7 weke, waar 10 lokval weekliks gemoniteer is. Die enigste vrugtesteek motte wat in die lokvalle gevang is is van die genus *Eudocima*. Vrugskade is laag, met minder as 1% met vrugtesteekmot skade. Piesang bly die mees aantreklike lokmiddel en verdere navorsing is onderweg om die piesang formulasie te verbeter.

3.6 PROGRAMME: NON-PHYTOSANITARY KEY PESTS

Programme Coordinator: Tim G Grout (CRI)

3.6.1 Programme summary

Thirty years ago, the priorities in citrus pest management were to prevent organophsphate-resistant red scale from killing your trees by using poor-quality mineral oils that often reduced the crop, and developing long-residual thripicides that could protect fruitlets for at least six weeks. Now the emphasis is on controlling internal phytosanitary pests that can threaten market access; there are several long-residual thripicides available and several plant protection products have been developed that are extremely effective against red scale. The focus in this programme has now turned to the management of the non-phytosanitary key pests such as citrus thrips, citrus psylla and red scale when their populations are at maintenance levels or late in the season when Maximum Residue Limits restrict the number of chemical options available. Two entomopathogenic fungal isolates were evaluated against thrips and mealybug that had previously looked promising in the laboratory but gave disappointing results in the field (3.6.2). Entomopathogenic fungi were evaluated by Rhodes University against red scale and although they caused some mortality to crawlers they had no effect on second instar males or adult females (3.6.3). Some new chemistry was evaluated in a field trial against the green citrus leafhopper but results were not as good as Phosdrin at the bollworm dosage (3.6.4). A bioassay technique was developed for woolly whitefly and several botanical or organic products gave similar results to mineral oil at 0.5% under these ideal conditions, but these must be evaluated further in the field.

Opsomming

Dertig jaar gelede was die prioriteite van sitrus plaagbestuur om te verhoed dat rooidopluis, wat bestand is teen organosfosfate, jong bome doodmaak deur minerale olies van swak gehalte te gebruik, wat dikwels die oes verminder het en die ontwikkeling van lang nawerkende blaaspootjiedoders wat vruggies vir ten minste ses weke kon beskerm. Nou is die klem op die beheer van interne fitosanitêre plae wat marktoegang kan bedreig; daar is verskeie lang residuele blaaspootjiedoders beskikbaar en verskeie plantbeskermende produkte is ontwikkel wat uiters doeltreffend teen rooidopluis is. Die fokus in hierdie program het nou beweeg na die bestuur van die nie-fitosanitêrre sleutelplae soos sitrusblaaspootjie, sitrusbladvloei en

rooidopluis wanneer hul populasies op beheerbare vlakke is of laat in die seisoen wanneer maksimum residu vlakke die aantal chemiese opsies wat beskikbaar is, beperk. Twee entomopatogeniese swamkulture wat voorheen in die laboratorium belowend gelyk het is teen blaaspootjie en witluis geëvalueer, maar dit het teleurstellende resultate in die veld gegee (3.6.2). Entomopatogeniese swamme is by Rhodes Universiteit teen rooidopluis geëvalueer en alhoewel hulle ŉ mate van kruiper mortaliteit veroorsaak het, het hulle geen effek op die tweede instar mannetjies of volwasse wyfies gehad nie (3.6.3). Nuwe chemie is in ŉ veldproef teen die groensitrusblaarspringer geëvalueer maar resultate is nie so goed soos Phosdrin nie teen die bolwurm dosis (3.6.4). ŉ Biotoets tegniek is vir wollerige witvlieg ontwikkel en verskeie botaniese of organiese produkte het soortgelyke resultate as mineralie olie teen 0.5% onder hierdie ideale toestande gegee, maar dit moet verder in die veld geëvalueer word.

3.6.2 PROGRESS REPORT: Evaluation of entomopathogenic fungi against thrips and mealybug

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

Summary

There is an urgent need for thripicides that are IPM-compatible and can be used late in the season without disrupting natural enemies of key pests such as false codling moth. The situation is similar for mealybug control. 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. In the north, two trial sites had to be scrapped for lack of infestation or fruit. In a third trial site in Limpopo the larval and adult thrips infestion levels of fruit sprayed one week earlier with both rates and isolates of EPFs, were not significantly different from the untreated control. Thrips scarring showed similar results. The registered abamectin plus oil treatment was significantly better than any of the EPF treatments and gave similar efficacy to sulfoxaflor at 12 ml/hl water. There was no mealybug at this site. In the south, thrips was suppressed by the higher concentrations of two EPF isolates at two trial sites, but for only one week. Consequently, thrips scarring was reduced at one of the sites. Mealybug suppression was noted for all treatments at only one of the sites. A third trial, applied correctively in January against mealybug, provided no suppression at all with any of the three EPFs used. However, buprofezin also did not perform very well in this trial.

Similar trials will be conducted in the 2014/5 season if promising EPF isolates are available or other plant protection products that promise to be IPM compatible.

Opsomming

Daar bestaan ŉ dringende behoefte vir blaaspootjiedoders wat IPM-verenigbaar is en wat laat in die seisoen gebruik kan word sonder dat hulle natuurlike vyande van sleutel plae soos valskodlingmot versteur nie. Die situasie met witluis beheer is gelyksoortig. In 2011/2 het die evaluasie van twee dosise van ŉ kommersieel geformuleerde entomopatogeniese swam (EPS) in verskillende streke van die land, teleurstellende resultate gegee. Daarom in die 2013/4 seisoen is twee eksperimentele EPS isolate teen twee dosise gëevalueer in albei die noordelike en suidelike sitrus streke. In die noorde moes twee proewe gekanseleer word as gevolg van ŉ tekort aan besmetting of vrugte. By ŉ derde proef perseel in Limpopo het besmettingsvlakke van blaaspootjie larwes en volwassenes een week na bespuiting van vrugte met albei EPS konsenstrasies, nie betekenisvol van die onbehandelde kontrole verskil nie. Blaaspootjie skade het soortgelyke resultate getoon. Die geregistreerde abamektien plus olie behandeling was betekenisvol beter as enige van die EPS behandelings en sy werking was soortgelyk aan dié van sulfoxaflor teen 12 ml/hl water. Daar was geen witluis by hierdie perseel nie. In die suide is blaaspootjie deur die hoër konsentrasie van albei EPS isolate by twee persele vir net een week onderdruk. Gevolglik is blaaspootjie skade by een van die persele ook verminder. Witluis onderdrukking is vir alle behandelings opgelet, maar net by een van die persele. ŉ Derde proef wat in Januarie korrektief teen witluis toegedien is, het geen onderdrukking met enige van drie verskillende EPS isolate gegee, alhoewel, buprofezin ook nie in hierdie proef te imponerend presteer het nie.

Soortgelyke proewe sal in die 2014/15 seisoen uitgevoer word as belowende EPS isolate of ander moontlik IPM-verenigbare plantbeskermings produkte beskikbaar is.

3.6.3 FINAL REPORT: The potential of several isolates of entomopathogenic fungi for the control of California red scale (*Aonidiella aurantii* Maskell (Hemiptera: Diapsidae)), a pest of citrus in South Africa

Project (2013/4) by Danielle Wiblin, Martin Hill (RU) & Sean Moore (CRI)

Summary

The pathogenicity of one isolate of *Metarhizium anisopliae* (Metschnikoff) Sorokin, one local isolate of *Beauveria bassiana* (Balsamo) Vuillemin, and one commercially produced isolate of *B. bassiana* (Broadband®) (Biological Control Products, South Africa) were tested under laboratory conditions against three life stages of the citrus pest, California red scale, *Aonidiella aurantii* (Maskell) (Diapsidae). These insects were treated with concentrations of 1 x 10^4, 1 x 10^5, 1 x 10^6 and 1 x10^7 conidia/ml and checked for mortality twice a week. The crawlers treated with *M. anisopliae* showed around 50-60% mortality while the 2nd instar male and adult female scale insects were unaffected by the fungus. The 2nd instar males and adult females which were treated with *B. bassiana* and Broadband® were also unaffected.

Opsomming

Die patogenisiteit van een *Metarhizium anisopliae* (Metschnikoff) Sorokin isolaat, een plaaslike *Beauveria bassiana* (Balsamo) Vuillemin isolaat, en een kommersiële *B. bassiana* (Broadband®) (Biological Control Products, South Africa) isolaat, is in laboratorium biotoetse teen drie lewensstadiums van die sitrusplaag, rooidopluis, *Aonidiella aurantii* (Maskell) (Diapsidae), getoets. Hierdie insekte is met konsentrasies van 1 x 10^4, 1 x 10^5, 1 x 10^6 en 1 x10^7 konidia/ml behandel en mortaliteit is twee keer ŉ week gemoniteer. Die kruipers wat met *M. anisopliae* behandel is het mortaliteit van om en by 50-60% getoon, terwyl die swam geen effek op 2de instar mannetjies en volwasse wyfie dopluise gehad het nie. Die 2de instar mannetjies en volwasse wyfies wat met *B. bassiana* en Broadband® behandel is het ook geen reaksie getoon nie.

3.6.4 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 & Peter R Stephen (CRI)

Summary

There is a shortage of registered control options that can be used for late season control of thrips, psylla, leafhoppers and woolly whitefly. The objective of this research is to evaluate treatments with short preharvest intervals against these pests that have recently been registered against other pests on citrus in addition to evaluating unregistered short residual treatments. A field trial against the green citrus leafhopper gave disappointing results for sulfoxaflor and DPX8723 relative to Phosdrin SL at the bollworm dosage (30 ml/hl) which was significantly better than the control ($P<0.05$). After establishing a culture of woolly whitefly, a bioassay technique was developed to screen woolly whitefly nymphs against pesticides based on honeydew production by immature life stages. The first results have shown that Medium grade horticultural mineral oil at 0.5% can cause 100% mortality and relative to this, results from Requiem, Bio-Cure, and Pygar-Super were promising and require further evaluation. Sulfoxaflor had very little effect but DPX8723 and spinetoram may provide some benefit. Promising products will be evaluated further in field trials.

Opsomming

Daar is ŉ tekort aan geregistreerde beheermaatreëls om blaaspootjie, sitrusbladvlooi, blaarspringer en wollerige-witvlieg laat in die seisoen te beheer. Die doel van hierdie navorsing is om behandelings met kort voor-oes intervalle teen hierdie plae te evalueer met produkte wat reeds teen ander sitrusplae geregistreerd is. Ongeregistreerde behandelings met ŉ kort nawerking word ook geëvalueer. ŉ Veldproef teen die groensitrusblaarspringer het teleurstellende resultate met sulfoxaflor en DPX8723 opgelewer, daarenteen het Phosdrin SL met ŉ bolwurmdosering (30 ml/hl) heelwat beter as die kontrole ($P<0.05$) gevaar. Nadat ŉ kultuur van wollerige-witvlieg gevestig is, is ŉ biotoets-tegniek ontwikkel om wollerige-witvlieg nimfe teen insekdoders te evalueer. Die evaluasie is gegrond op heuningdouproduksie tydens die onvolwasse lewensstadia. Aanvanklike resultate het aangedui dat 0.5% mediumgraad hortologiese mineraalolie 100% mortaliteit kan veroorsaak. Terselfdertyd was die resultate van Requiem, Bio-Cure en Pygar-Super belowend en vereis verdere evaluasie. Sulfoxaflor het nie ŉ groot effek gehad nie, maar DPX8723 en spinetoram mag voordelig wees. Belowende produkte sal in veldproewe verder geëvalueer word.

4 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 during the 2013-14 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. 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). 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, 1056), while applied research projects evaluate the suitability of candidate cross-protection sources for various climate regions, citrus types and cultivars (679, 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). Evaluation of three embryo-rescue clones that proved to be greening tolerant in pre-screening is ongoing (815).

The Soilborne Diseases programme researchers sustainable options (alternatives to harsh chemicals) for root rot and citrus nematode control, and certain promising options have been identified (762, 1030). A new project was also initiated on the etiology and control of Armillaria root rot (1068). On a more fundamental level, rootstock resistance against *Phytophthora* was studied; specifically with the aim of identifying marker substances that can be used to screen rootstocks for resistance in selection or breeding programmes (UP_CRR1-09). 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 (910). In January 2014, the Soilborne Diseases programme experienced some personnel changes with MC Pretorius's move to CRI Extension Services. Dr Jan van Niekerk, who had been the co-ordinator for Plant Pathology and later Development Manager at Westfalia Technological Services in Tzaneen, after obtaining his PhD(Agric) at Stellenbosch University in March 2008, is now the programme coordinator and researcher in soilborne diseases.

In the Citrus Black Spot programme, market access support is an ongoing service. In 2013/14 this has been highlighted by CRI's CBS researchers' involvement in South African and international CBS expert panels formulating responses to the European Food Safety Authority's CBS Pest Risk Assessment. These researchers are also involved in 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 (1026). CBS epidemiology is being studied in this project, as well as in the Eastern Cape, Limpopo and Mpumalanga provinces through spore trapping and weather monitoring (919, 1026). The global population structure of the CBS pathogen is being studied, which will further elucidate CBS epidemiology and global movement of the pathogen (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, 1044).

In the Fruit and Foliar Diseases programme, new control options for Alternaria brown spot (ABS) are continuously being studied (750). Research also focuses on improving spray application through optimal use of spray machines or adjuvants (891). Control of Botrytis blossom blight and fruit drop in lemons are also being studied. Suitable fungicides were identified, but the optimal timing of application needs to be determined (1015).

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 residue loading following application in drench, fungicide bath, wax (936), as well as the JBT heated flooder application (1050) and subsequent bio-efficacy against sensitive and resistant *Penicillium* strains, were studied, giving valuable insight into the optimal use of these postharvest fungicides. Study of the practical impact of imazalil resistance clearly showed that this very effective fungicide was

rendered ineffective when applied against resistant isolates (1034). Resistance management is therefore vitally important and methods to determine resistance frequencies in packhouses are presently being evaluated (936). Integration of preharvest silicon, and postharvest heat and biocontrol against green mould are also being studied (UKZN1). Potential pre-harvest risk indicators for postharvest decay were studied in an attempt to identify those indicators that will enable growers or packhouses to classify fruit consignments in risk categories (1073).

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 project. The DC also continued providing quality control analyses for River Bioscience. In total, a staggering 9 571 samples were analysed by one diagnostician, a technician and assistant.

In general, good progress was made in Disease Management. Apart from excellent 'non-research', such as support for biosecurity, improvement scheme, market access, and formal and *ad hoc* extension activities, the quality and quantity of tangible research outputs has improved through consolidated and focused research. In 2013-14, 8 scientific papers were published.

PORTEFEULJEOPSOMMING

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 2013-14 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. 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 ŉ projek begin om die hortologiese prestasie van voor-Skema materiaal, met SVS materiaal, te evalueer (1074). Navorsingsdoelwitte fokus grootliks op volhoubare beheer van *Sitrus Tristeza virus* (CTV), wat op kruisbeskerming gebaseer is. Op ŉ meer basiese vlak, word die meganismes betrokke in kruisbeskerming nagevors (885B, 1056), terwyl toegepaste navorsingsprojekte die volhoubaarheid van potensiële kruisbeskermingsbronne vir verskeie klimaatstreke, sitrustipes en kultivars evalueer (679, 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). Evaluasie van drie kultivars wat in vooraf-evaluasies toon om vergroeningsbestand te wees, gaan voort (815).

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). ŉ Nuwe projek is ook op die etiologie en beheer van Armillaria wortelvrot geïnisieer (1068). Op ŉ meer basiese vlak, is onderstamweerstand teen *Phytophthora* bestudeer; spesifiek met die doel om merkerstowwe te identifiseer wat gebruik kan word om onderstamme in seleksie- of teelprogramme vir weerstand te evalueer (UP_CRR1-09). 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 (910). In Januarie 2014, het die Grondgedraagde Siekteprogram sekere personeel veranderinge ondergaan met MC Pretorius wat na die CRI se Voorligtingsdienste geskuif het. Dr. Jan van Niekerk, wat die koördineerder vir Plantpatologie en later Ontwikkelingsbestuurder by Westfalia Tegnologiese Dienste in Tzaneen was, nadat hy sy PhD(Agric) by Stellenbosch Universiteit in Maart 2008 behaal het, is nou die program-koördineerder en navorser in grondgedraagde siektes.

In die Sitrus Swartvlek (SSV) program is marktoegang-ondersteuning ŉ voortgesette diens. Hoogtepunte in 2013/14 was die betrokkenheid van CRI se SSV navorsers in Suid-Afrikaanse en internasionale SSV kundigheidspanele wat terugvoer aan die Europese Voedselveiligheid Beheerliggaam se SSV Pesrisikobepaling moes formuleer. Hierdie navorsers is ook in ŉ gesamentlike projek met die VSA, Brasiliaanse en Argentynse navorsers betrokke ten einde ŉ waarskynlikheidsmodel te ontwikkel om kwantitatief die risiko van vrugte as ŉ verspreidingsweg vir SSV te voorspel (1026). SSV-epidemiologie word in hierdie projek deur spoorvangstudies en weermonitering in die Oos-Kaap, Limpopo en Mpumalanga provinsies bestudeer (919, 1026). Die globale populasie-struktuur van die SSV-patogeen word bestudeer, wat verder SSV-epidemiologie en globale beweging van die patogeen sal uitlê (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, 1044).

In die vrug- en blaarsiekte program, word nuwe beheer-opsies vir Alternaria bruinvlek (ABV) voortdurend bestudeer (750). Navorsing fokus ook op die verbetering van spuittoediening deur optimale gebruik van spuitmasjiene of byvoegmiddels (891). Beheer van Botrytis bloeiselversenging en vrugval in suurlemoene word ook bestudeer. Geskikte fungisiedes is geïdentifiseer, maar die optimale tyd vir toediening moet egter nog bepaal word (1015).

Die na-oes siekte program bly ŉ 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 residu-lading, volgende op doop, fungisiedbad- en wakstoedienings (936), asook die JBT vloed-toediening (1050), en gevolglike bio-effektiwiteit teen sensitiewe en weerstandbiedende *Penicillium* isolate, is bestudeer, en het waardevolle insig in die gebruik van ons belangrikste na-oes fungisiede gegee. Studies oor die praktiese impak van imazalil-weerstand het duidelik getoon dat hierdie baie effektiewe fungisied oneffektief raak wanneer teen weerstandbiedende isolate toegedien word (1034). Weerstandsbestuur is gevolglik van kardinale belang, en metodes om die weerstandsfrekwensies in pakhuise te bepaal, word tans geëvalueer (936). Integrasie van voor-oes silikon, en na-oes hitte en biobeheer teen groenskimmel, word ook tans bestudeer (UKZN1). Potensiële voor-oes risiko-indikators vir na-oes verval is bestudeer in ŉ poging om daardie indikators te identifiseer wat produsente of pakhuise in staat sal stel om vrugbesendings in risiko-kategorieë te klassifiseer (1073).

Die Diagnostiese Sentrum (DS) lewer steeds ŉ uitstekende diens aan die Sitrusverbeteringskema deur roetine grond- en waterontledings vir *Phytophthora* en aalwurms, asook deur ontledings vir navorsers in die Grondgedraagde Siekte projek. Die DC doen ook gehalte-beheer vir River Bioscience. In totaal het die DS 9571 monsters geanaliseer, en dit met slegs een diagnostikus, een tegnikus en een assistent.

Goeie vordering is oor die algemeen in Siektebestuur gemaak. Afgesien van uitstekende 'nie-navorsing', soos ondersteuning vir biosekuriteit, verbeteringskema, marktoegang, en formele en *ad hoc* voorligtingsaktiwiteite, het die kwaliteit en kwantiteit van tasbare navorsingsuitsette deur gekonsolideerde en gefokusde navorsing verbeter. In 2013-14 is 8 wetenskaplike artikels gepubliseer.

4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: G. Cook (CRI)

4.2.1 Programme summary

Management strategies developed over the years have dramatically reduced the negative impact that *Citrus tristeza virus* (CTV) and African Greening had on production and tree lifespan. To minimize losses caused by CTV on grapefruit, the Southern African Citrus Improvement Scheme (CIS) implemented cross-protection. All other citrus types except lemons are also pre-immunised with "mild" sources of CTV. The initial philosophy behind the broader cross-protection was that other citrus types were planted adjacent to grapefruit and by pre-immunising all citrus with a "mild" source it would reduce the establishment of "severe" CTV strains and the field inoculum pressure would be lowered. These initial philosophies were possibly incorrect considering the recent understanding that the citrus host plays a role in both selection and symptom expression of specific strains. The effect of CTV on citrus types apart from grapefruit and limes is difficult to assess as no apparent decline is observed and minimal deleterious effects have been observed on sweet oranges and mandarins. Nonetheless field testing of various CTV sources has shown that production is influenced by various CTV sources and this has led to the use of sources that improve production. Research is ongoing to elucidate the cross-protection mechanism, to establish which strains play a role in tree decline (Projects 885B & 1056) and to determine which CTV pre-immunisation sources yield the best production (Projects 679, 738, 739, 742, 789 & 968). Long term field testing of CTV sources in grapefruit in varying climatic regions are ongoing and pre-immunisation sources applied in grapefruit trials show consistency between trials in similar climatic conditions with regard to tree health (Project 742, 679), but differences are seen in symptom expression with the sources under different climatic conditions (Project 738). Ultimate selection for the preferred CTV pre-immunisation sources will also be based on their effect on fruit production. Field trials evaluating CTV pre-immunisation sources in three Valencia and four soft citrus types are also ongoing (Projects, 739, 789 and 968).

Claims of better fruit quality and yields using old clone (pre-scheme) material containing viroids and CTV compared to those obtained using the viroid-free and CTV pre-immunised material supplied by the Citrus

Improvement Scheme (CIS), is being investigated in a comparative study. Trial trees have been prepared and will be planted in the spring (Project 1074).

Field evaluation of sweet orange, embryo-rescued clones, selected for resistance/tolerance to greening is ongoing. No greening was detected in these trees after the sixth year in the field. The fourth crop was harvested and fruit quality and production compare favourably to a standard commercial variety (Project 815). Resistant varieties are seen as an important disease control mechanism and these clones hold much promise.

Indigenous plants of the citrus family (Rutaceae) are evaluated for their ability to host "*Candidatus* Liberibacter africanus" (Laf) the African greening pathogen (Project 886B). Laf has not been detected in any indigenous host yet and findings to date indicate they do not play a role in the epidemiology of citrus greening. Some Rutaceous genera have now been shown to harbour Liberibacters similar, but differing from Laf and include *Zanthoxylum*, *Vepris*, *Clausena* and *Calodendrum*. These genera were shown to be graft compatible with sweet orange and this will enable transmission studies of the Liberibacters found in these genera to *Citrus*. Liberibacters differing from Laf were also detected in *Teclea spp* and *Oricia bachmannii* samples.

Programopsomming

Bestuurspraktyke ontwikkel oor jare, het drasties die newe effekte op produksie en boomleeftyd weens *Citrus Tristeza virus* (CTV) en Sitrusvergroening verminder. CTV kruis-beskerming is toegepas deur die Sitrus Verbeteringskema (SVS) om verliese hoofsaaklik op pomelo produksie te beperk. Alle ander sitrus tipes, behalwe suurlemoene word vooraf gepreimmuniseer met matige bronne van CTV. Die aanvanklike filosofie agter die breër kruisbeskerming is dat ander sitrus tipes langs pomelos aangeplant word en deur hulle ook te preimmuniseer sou dit die vestiging van "skadelike" CTV rasse verminder en die veld inokulum druk ook sodoende verlaag. Hierdie aanvanklike filosofie is moontlik verkeerd weens die onlangse bevinding dat die sitrus gasheer ŉ rol speel in beide seleksie en simptoom uitdrukking van spesifieke CTV rasse. Die effek van CTV op sitrus tipes afgesien van pomelo en lemmetjie is moeilik om te bepaal aangesien daar geen oënskynlike nadelige uitwerking op sitrus soos soetlemoene en naartjies waargeneem word nie. Nietemin het veldproewe getoon dat produksie wel beïnvloed word deur verskeie CTV bronne en dit het gelei tot die gebruik van CTV bronne wat produksie verbeter. Navorsing is gemik om die kruisbeskermingsmeganisme te verklaar en om vas te stel watter rasse ŉ rol speel in boom terugsterwing (Projekte 885B & 1056) asook om te bepaal watter CTV preimmunisering bronne die beste beskerming bied en produksie lewer (Projekte 679 , 738, 739, 742 , 789 & 968). Langtermyn veld proewe word gedoen om CTV bronne in pomelo's in verskillende klimaatstreke te vergelyk. Pre-immuniseringsbronne aangewend in pomelo proewe toon konsekwentheid in soortgelyke klimaatstoestande met betrekking tot simptoom uitdrukking (Projek 742, 679), maar verskille in simptoom uitdrukking word verkry met die bronne onder verskillende klimaatstoestande (Projek 738). Die uiteindelike keuse vir die beste CTV pre-immuniseringsbron sal ook gegrond word op die uitwerking daarvan op vrugproduksie. Veldproef evaluasies van CTV pre-immuniseringsbronne in drie soetlemoen en vier sagtesitrus tipes is voortgesit (Projects, 739, 789 en 968).

Stellings wat gemaak word dat beter gehalte vrugte en beter opbrengste verkry word met die gebruik van ou kloon (voor-skema) materiaal, wat viroïede en CTV bevat, as dié verkry met viroïed-vrye materiaal van die SVS, word ondersoek in ŉ vergelykende studie. Proefbome is voorberei en sal in die lente geplant word (Projek 1074).

Veld evaluering van embrio-herwinningsklone vanaf soetlemoen wat potensiële weerstand / toleransie teen vergroening toon, is voortgesit. Geen vergroening is in hierdie bome na die sesde jaar in die veld opgespoor nie. Die vierde oes vanaf hierdie bome dui dat vruggehalte vergelykbaar met ŉ standaard kommersiële kultivaar is (Projek 815). Bestandheid teen vergroeningsiekte word beskou as die uiteindelike beheer meganisme en die potensiaal van hierdie klone hou groot belofte in.

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 (Projek 886B). Laf is nog nie in enige inheemse gasheer gevind nie, en die huidige resultate dui daarop dat hulle nie ŉ rol in die epidemiologie van sitrusvergroening speel nie. Sommige genera in die *Rutaceae* familie, *Zanthoxylum*, *Vepris*, *Clausena* en *Calodendrum*, hawe wel soortgelyke Liberibacters wat van Laf verskil. Dit is bepaal dat hierdie genera entversoenbaar met soet lemoen is en dit sal oordrag studies van die Liberibacters gevind in hierdie genera vergemaklik. Liberibacters verskil van Laf is ook waargeneem in *Teclea* spp. en *Oricia bachmannii* monsters.

4.2.2 FINAL REPORT: CTV Cross-protection of Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain

Project 679 (2003 - 2013) by J.H.J. Breytenbach, G. Cook & S.P. van Vuuren (CRI)

Summary

The Nartia source (GFMS 12) was replaced with GFMS 35 as a cross protecting source for red grapefruit in 1998 and for all grapefruit in 2007. In the search for optimal cross protection sources, 20 sub-isolates were derived from single aphid transfers (SATs) of two Nartia sources (A=GFMS 12, C=GFMS 14) and Mouton sub-isolates obtained from SATs done in Beltsville MD, USA, and imported back to South Africa. Four of these sub-isolates showed potential as cross-protecting agents in glasshouse trials and their cross-protection abilities were assessed for field performance. Virus-free Star Ruby and Marsh grapefruit trees were pre-immunised with the four Beltsville sub-isolates, two sub-isolates from the ITSC (GFMS 12/7, GFMS 12/9) and the cross-protection sources, GFMS 12 and GFMS 35. Control trees were left virus-free. Pre-immunisation was confirmed by means of ELISA. The Marsh trees were planted at Riversbend in the Nkwaleni valley and the Star Ruby trees at Tambuti Estates in Swaziland during 2003. The citrus orchards at Riversbend, including the trial trees, were removed in 2009 and replaced with sugarcane. Limited yield data was obtained from the Marsh trial. Star Ruby trees were evaluated for yield and stem pitting for 9 years. As a final analysis of this trial, windows were cut in the bark of the trunk of each trial tree to do an accurate stem pitting assessment. General observations of the trial are: i) GFMS12 suppressed growth and was associated with severe stem pitting; ii) sub-isolate12/7 inoculated trees also developed unacceptable stem pitting; iii) the current cross protector, GFMS35, in addition to sources B389/1 and B389/4 showed no stem pitting and trees pre-immunised with B390/3 and B390/5 showed mild stem pitting. These five sources are therefore considered superior to GFMS12 with regard to tree health performance. Unfortunately the Star Ruby trees were hedged as part of the normal management practices of the farm, and smaller trees, due to the CTV infection, were not hedged. Yield data was thereafter not reliable for comparative yield assessment, but indications are that sources B389/4 and B390/3 might be promising candidates for cross protection. Virus-free trees started to show stem pitting 6 years after planting, which indicated that new CTV infections following aphid vectoring did occur, but field challenge was low and further trials are required to ensure the cross protection abilities of these sources. The trial was terminated as per the proposed schedule. Data of this trial will be evaluated in combination with similar grapefruit trials (Projects 738 and 742) on Marsh and Star Ruby when completed.

Opsomming

Die Nartia CTV preïmmuniseringsbron (GFMS 12) is gedurende 1998 met GFMS 35 as ʼn CTV-kruisbeskermingsbron vir rooi pomelos vervang en gedurende 2007 vir wit pomelos en pompelmoese. In die soeke na meer geskikte kruisbeskermingsbronne, is 20 sub-isolate deur middel van enkel plantluis oordragings vanaf twee afsonderlike Nartia bronne (A=GFMS 12, C=GFMS 14) en die Mouton bron bekom. Laasgenoemde is in Beltsville MD, VSA voorberei. Vier uit 20 sub-isolate, het potensiaal as kruisbeskermingsagente in glashuisproewe getoon en is gebruik om hul kruisbeskermingsvermoëns in die boord te evalueer. Virus-vrye Star Ruby en Marsh pomelo boompies is met die vier Beltsville sub-isolate gepreïmmuniseer, twee sub-isolate van die LNR-ITSG (12/7, 12/9) en die GFMS12 en GFMS35 bronne. Virusvrye bome is as kontroles gebruik. Preïmmunisasie is deur middel van ELISA bevestig. Die Marsh proef is gedurende 2003 by Riversbend in die Nkwaleni vallei geplant en die Star Ruby proef by Tambuti landgoed in Swaziland. Die sitrusboorde, insluitend die Marsh proef, is by Riversbend in 2009 verwyder en met suikerriet vervang. Beperkte opbrengsdata is van die Marsh proef verkry. Star Ruby bome is vir opbrengs en stamgleuf ontwikkeling vir 9 jaar geëvalueer. As deel van die proef se finale evaluasie is vensters in die stamme van elke proefboom gesny om ʼn akkurate stamgleuf evaluasie te doen. Die volgende waarnemings is gemaak, wat betref boom gesondheid: i) GFMS 12 onderdruk groei en veroorsaak strawwe stamgleuf; ii) sub-isolaat 12/7 het ook onaanvaarbare stamgleuf veroorsaak; iii) die huidige kruisbeskermer GFMS35 asook B389/1 en B3894 het geen stamgeluf getoon nie en bome wat gepre-immuniseer was met B390/3 en B390/5 het ligte stamgleuf getoon. Die laasgenoemde vyf bronne het beter presteer as GFMS12 in terme van boom gesondheid. Ongelukkig is die Star Ruby bome meganies gesnoei as deel van die boordbestuur van die plaas, en kleiner bome, as gevolg van CTV-infeksie, is nie gesnoei nie. Produksie data was dus daarna nie vergelykbaar in die analise nie, maar bronne B389/4 en B390/3 toon belowende kruisbeskermings eienskappe en produksie potensiaal. Virus-vry geplante bome het stamgleuf ses jaar na plant begin ontwikkel wat aandui dat nuwe CTV besmetting deur middel van plantluis oordraging plaasgevind het. Dit wys dat daar wel infeksiedruk in die omgewing was, maar die druk was laag en meer kruisbeskermingsproewe is nodig om die beskermingsvermoëns van die bronne te bevestig. Die proef is binne die voorgestelde skedule beëindig. Data sal in kombinasie met soortgelyke pomelo proewe (projekte 738 en742) op beide Marsh en Star Ruby geëvalueer word.

Introduction

Citrus tristeza virus (CTV) is the causal agent of one of the most destructive diseases of citrus. The disease is spread by propagation material and various aphid species of which *Toxoptera citricida* is the most efficient. Many strains of CTV occur and co-exist as mixtures in individual field trees. Symptoms induced by CTV, range from mild with no noticeable effect on the host to severe stem pitting and decline, resulting in uneconomic production (Marais *et al.,* 1996). The only practical means of controlling CTV at present is by mild strain cross-protection (van Vuuren *et al.*, 1993). A breakdown in the protection offered by the Nartia A (GFMS 12) source owing to the probable presence of a severe component within the complex (van Vuuren *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained from two Nartia sources [(Nartia A = GFMS 12 and Nartia C = GFMS 14) van Vuuren *et al.*, 1993] and the Mouton source that was collected by L.J. Marais (Outspan). These sub-isolates have undergone biological indexing to evaluate their severity. Some sub-isolates showed no potential as cross-protecting sources as titre and movement of the virus in the plant were poor and/or severe stem pitting was associated with them. Four of these sub-isolates were evaluated in field trials as potential cross-protecting sources. Promising SAT sub-isolates of Nartia obtaining from the ARC-ITSC (van Vuuren, *et al.*, 2000) were also included in this experiment. As CTV exhibits host and climatic specificity, it is imperative that mild protective isolates be tested in the different production areas.

Objectives

To compare the cross-protecting ability of CTV sub-isolates to existing pre-immunising sources in grapefruit under field conditions.

Performance evaluation is based on:

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

Materials and methods

The 20 SAT sub-isolates of the Nartia A and C CTV cross-protecting sources (A=GFMS 12 and C=GFMS 14) as well as those from the Mouton source were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. In a greenhouse experiment, they were bud-inoculated separately onto CTV sensitive Mexican lime indicator plants to differentiate the sub-isolates according to their effects on the host. Growth and stem pitting ratings were determined and the virus titer was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate 6 months after inoculation. The four sub-isolates with the best potential (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) were used to pre-immunise virus-free Star Ruby grapefruit on MxT rootstocks that were prepared under insect-free conditions in the greenhouse. Their potential as cross-protectors are being compared to GFMS 12 (previous standard for white grapefruit), GFMS 35 (present standard for grapefruit), GFMS 12/7 and GFMS 12/9 (ARC-ITSC single aphid transfer sub-isolates from GFMS 12) and un-inoculated control trees planted virus-free. Pre-immunisation has been confirmed by ELISA 6 months after inoculation. In 2003 the Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates. Each treatment was planted with 5 replications. Tree sizes were measured and the canopy volumes calculated according to Burger *et al.* (1970), who use a formula assuming the trees' form is a half sphere *viz.* volume = R^2(PIH-1.046R), where R = the radius of the tree and H = the height of the fruit bearing part. Production and tree health were monitored annually.

Results and discussion

Objective / Milestone	Achievement
A. Evaluate horticultural performances over 10 years:	
1. Canopy volume measurement	Assessed
2. Stem pitting development and decline assessment	Achieved, results presented
3. Yield assessment	Achieved, results presented

Tree size:
The trial trees were planted in a commercial block of Star Ruby and were hedged along with the commercial orchard by the grower since 2009 at 6 years. Canopy volume measurements were not recorded thereafter. Canopy volume measurements 4 and 5 years after planting (2007 and 2008 respectively) are presented in Table 4.2.2.1. Trees pre-immunised with GFMS 12 were significantly smaller than trees of all other treatments. Visually, the trees pre-immunised with GFMS 12 remained smaller than the trees of other treatments and because of their smaller size were not hedged along with the other treatments. This discrepancy in the management of the trial trees had an influence on the production as discussed later.

Table 4.2.2.1. Tree canopy volume (m^3) of Star Ruby grapefruit trees pre-immunised with various CTV sources, 4 and 5 years after planting.

Treatment	2007*	2008*
B389/1	29.7 a	48.3 a
B389/4	33.0 a	49.7 a
B390/3	29.8 a	47.2 a
B390/5	26.2 a	41.7 a
GFMS 12/7	27.6 a	41.8 a
GFMS 12/9	24.8 a	43.3 a
GFMS 12	23.7 b	30.9 b
GFMS 35	29.0 a	36.2 a
Virus-free	32.5 a	46.1 a

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

Tree health:
Trial trees were inspected annually for the occurrence of stem pitting which was assessed externally and rated on a severity scale of 0 to 3, where 0 is a smooth trunk with no visible pits and 3 is severe stem pitting accompanied by twig decline. The results for the last external stem pitting rating are presented in Table 4.2.2.2. As a final evaluation, bark flaps were removed to better assess the stem pitting. The results of the external assessments were confirmed with the exception of mild stem pitting also seen on trees inoculated with the B390/5 source. No stem pitting was observed on trees pre-immunised with sources B389/1, B389/4 and GFMS 35, whereas severe stem pitting was evident on all 5 trees pre-immunised with GFMS 12.

The onset and progression of stem pitting of individual trial trees from 2005 to 2012 for treatments; B390/3, GFMS12/7, GFMS12/9, GFMS12 and the un-inoculated controls is presented in Figures 4.2.2.1A to E. A single tree of the un-inoculated controls showed severe stem pitting 6 years after planting. This indicated that new CTV infections by aphid vectoring did occur, but that the field challenge was low. Trees inoculated with GFMS 35, B389/1 and B389/4 showed no external signs of stem pitting in contrast to GFMS12 where all trees pre-immunised with GFMS12 started to show moderate to severe stem pitting three years after planting. As all the trees with this treatment showed the symptoms simultaneously and with a very early onset, it is most probable that the source itself is the cause for the stem pitting decline observed with GFMS12. These yearly ratings also suggest that in cases of milder stem pitting the trees can recover from the stem pitting and that pits are no longer externally visible the next season. This was noted with treatments of B390/3 and GFMS12/9.

Scrapings of the cambium and phloem layers of the bark flaps were tested for the various CTV strains or genotypes using RT-PCR so that the strains impacting at the assessment site could be determined. This information is extensive and falls outside the scope of this report, but will be used within a larger PhD study. Results obtained however, suggest that segregation of strains from the original source is likely the cause for breakdown of GFMS12 field performance and not challenge infections by other strains.

Table 4.2.2.2. Average external stem pitting rating of Star Ruby grapefruit trees pre-immunised with the various CTV cross protecting sources and sub-isolates, 9 years after planting*.

Treatment	Stem pitting rating**
B389/1	0.0 a*
B389/4	0.0 a
B390/3	0.1 a

B390/5	0.0	a
GFMS 12/7	1.6	b
GFMS 12/9	0.7	a
GFMS 12	3.0	c
GFMS 35	0.0	a
Virus-free	0.6	a

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

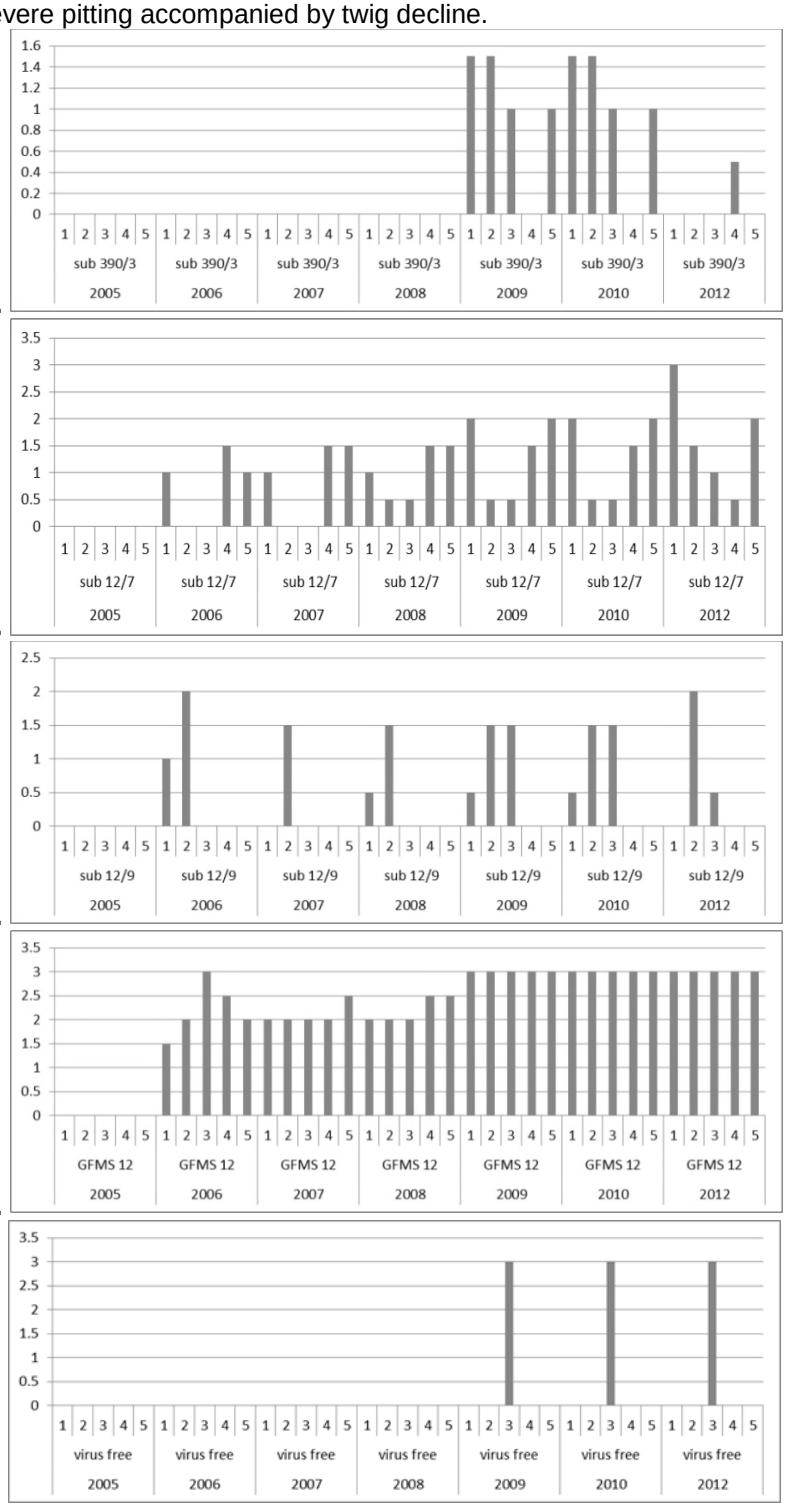
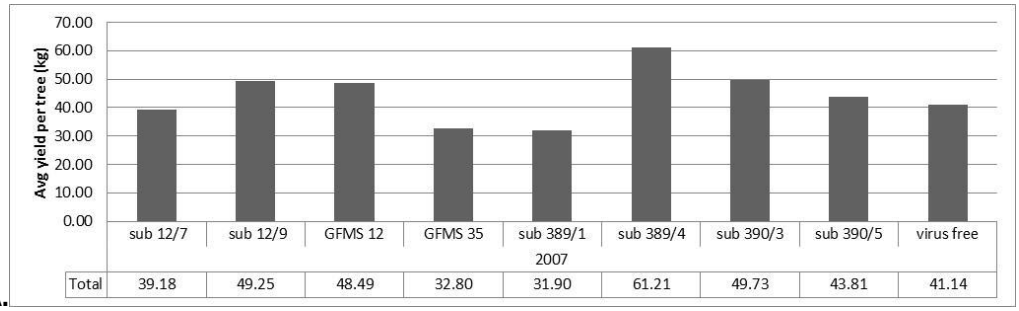


Figure 4.2.2.1. Stem pitting ratings of individual trees from 2005 to 2012 of treatments: **A,** B390/3; **B,** GFMS 12/7; **C,** GFMS 12/9; **D,** GFMS12; **E,** un-inoculated control.

Production:
The trees were planted in a commercial block of Star Ruby and were hedged along with the commercial orchard by the grower since 2009 when the trees were 6 years old. Trees pre-immunised with GFMS 12 were significantly smaller than trees of all other treatments (Table 4.2.2.1) and remained smaller than those of other treatments throughout the trial. Due to their smaller size they were not hedged along with the other treatments. This difference in tree management had an influence on the production and data is not comparable between the other treatments and GFMS 12. It is our view that drastic hedging reduces production as the flower/fruit bearing branches/twigs are removed. Vegetative regrowth forms a dense matt preventing proper light penetration to the inside canopy of the tree and results in lower yields. Graphs presented in Figures 4.2.2.2A to **E** show average yield per tree of each treatment for 2007, 2008, 2010, 2011 and 2012. Trees were harvested by the grower in 2009 and no data was collected for that production year. Yield per tree across the treatments were similar for the 2007 and 2008 harvests. The trees were hedged in 2009 and no yield data was obtained that year, but the 2010 harvest showed a drastic yield decline over most treatments apart from the GFMS12 treatment where trees were not hedged. The 2011 and 2012 harvests demonstrate a recovery in the production for most treatments. Excluding GFMS12, for above mentioned reasons, treatments B389/4 and B390/3 had the highest cumulative yield per tree and the virus-free trees the lowest over the trial period (Table 4.2.2.3).

Table 4.2.2.3. Average yield (kg/tree) for five production seasons and the cumulative yield per tree for each treatment of Star Ruby grapefruit trees pre-immunised with the various CTV sources.

	Yield (kg/tree)					
Treatment	**2007**	**2008**	**2010**	**2011**	**2012**	**Cumulative yield per tree**
B389/1	32	79	28	13	47	200
B389/4	61	64	60	48	84	318
B390/3	50	90	48	60	65	313
B390/5	44	58	23	74	41	240
GFMS 12/7	39	45	17	59	41	201
GFMS 12/9	49	38	42	73	47	250
GFMS 12	48	59	87	101	73	367
GFMS 35	33	77	21	23	64	217
Virus-free	41	75	14	24	27	180



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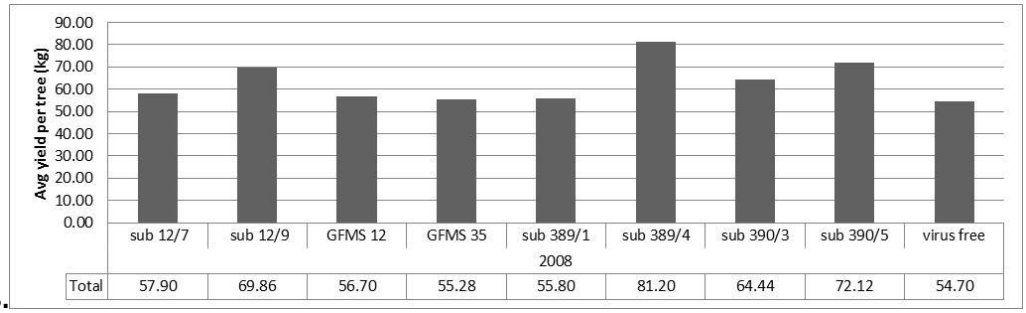

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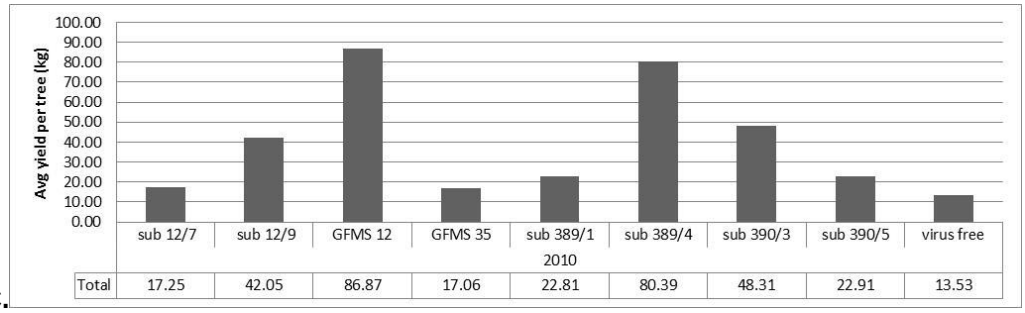

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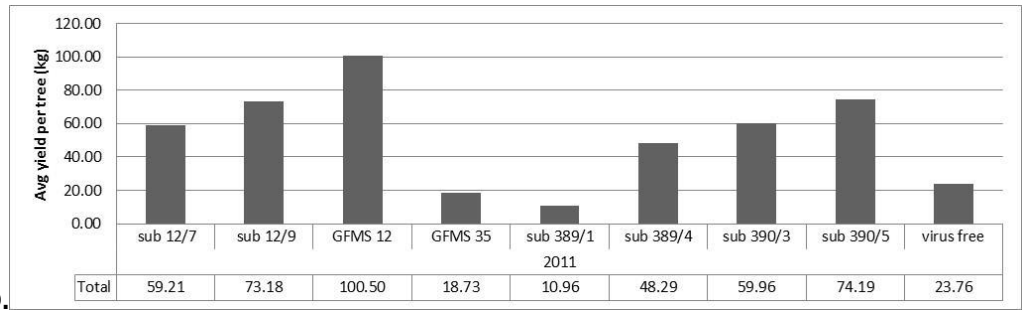

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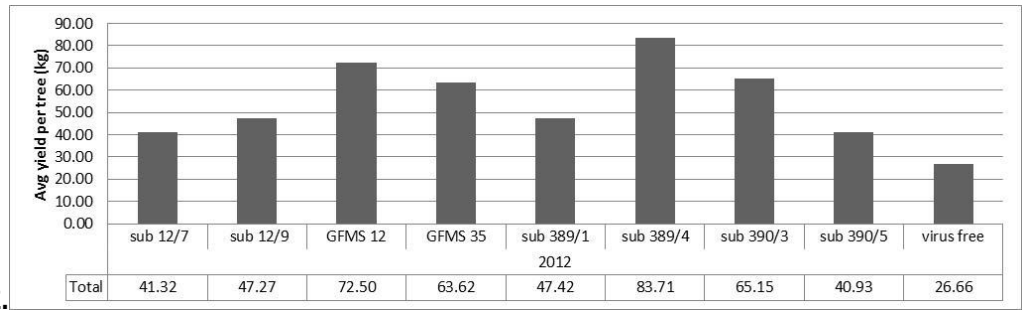

2E.

Figure 4.2.2.2. Average yield (kg) per tree for each treatment for five years: **A,** 2007; **B,** 2008; **C,** 2010; **D,** 2011; **E,** 2012.

Conclusion

Severe stem pitting was associated with GFMS 12 and growth was suppressed. Since all the trees with this treatment showed the symptoms simultaneously and at a very early onset, it is most probable that the source itself is the cause for the stem pitting decline observed with GFMS 12. Trees inoculated with sub isolate GFMS 12/7 developed unacceptable stem pitting. No stem pitting was observed where GFMS 35, B389/1 and B389/4 were used as cross protecting sources and these sources performed better than the previous

cross-protector, GFMS12, with regard to tree health. Sub isolate B389/4 and B390/3 gave the best average yield per tree cumulatively. Data of this trial will be evaluated in combination with similar grapefruit trials on Marsh and Star Ruby.

Technology transfer

1. Presentation at the 5th Citrus Symposium at the Champagne Sports resort, Drakensberg, 2010: The effect of single-aphid transferred *Citrus tristeza virus* sub-isolates on the growth of young Marsh and Star Ruby grapefruit trees. J.H.J. Breytenbach and S.P. van Vuuren (CRI).
2. Presentation at the 6th Citrus Symposium at the Champagne Sports resort, Drakensberg, 2011: *Citrus tristeza virus* (CTV) cross-protection of Marsh and Star Ruby grapefruit trees. J.H.J. Breytenbach and S.P. van Vuuren (CRI).
3. Presentation at 7th Citrus Research Symposium at the Champagne Sports resort, Drakensberg, 2012; Citris Tristeza Virus cross-protection of Star Ruby grapefruit: field trial results. 2012. J.H.J. Breytenbach, S.P. van Vuuren and G.Cook (CRI).
4. Presentation at the 19th Conference of the International Organisation of Citrus Virologists.Skukuza, South Africa, 2013. South African CTV Cross-Protection and Source Characterisation. G.Cook, V.Z. Maqutu, J.H.J. Breytenbach and S.P. van Vuuren (CRI).

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4.2.3 PROGRESS REPORT: CTV Cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Project 738 (2004 - 2015) by J.H.J. Breytenbach, G. Cook & S.P. van Vuuren (CRI)

Summary

Indications of a possible severe *Citrus tristeza virus* component in the Nartia (GFMS 12) cross-protecting source necessitated the separation of the strain populations into sub-isolates by single aphid transmissions. These sub-isolates were derived from two Nartia sources (A=GFMS 12, C=GFMS 14) and a Mouton source derived from sweet orange. The GFMS 14 and Mouton sub-isolates were done at Beltsville, USA, and imported back to South Africa. After biological indexing, four sub-isolates showed potential for further evaluation (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). Two sub-isolates from the ARC-ITSC sources (GFMS 12/7, GFMS 12/9) were included in the trial as well as GFMS12 (previous standard cross-protector for white grapefruit) and GFMS 35 (standard cross-protector for red grapefruit). Virus-free Star Ruby trees were prepared in a glasshouse and were pre-immunised with the various sources. A virus-free treatment was included as a control and field-infection indicator. After confirming pre-immunisation by ELISA, trees were planted in the Kakamas area in September 2004. This is a duplicate experiment of Project 679, planted in 2003 in Swaziland, and the two experiments are aimed at assessing the CTV expression in different climatic conditions. In 2007 similar trials were also planted in the Malelane and Letsitele areas (Project 742). During this report period, tree sizes were measured at the Kakamas trial, 9 years after planting. Trees grew much slower than in other grapefruit production areas. Stem pitting evaluations were done and trees containing sub-isolate B389/1 developed moderate stem pitting and those with GFMS 12, 12/7 and B390/5, mild pitting. Fruit were also harvested and graded into export sizes. The average yield per tree of all the treatments did not differ significantly. Trees with B389/1 yielded the least fruit and had significantly more small fruit than all the other treatments while trees with GFMS 12/9 yielded the most fruit as well as the highest percentage large fruit. The virus-free trees still remain uninfected after 9 years indicating low natural pressure by aphids and/or CTV strains in the area.

Opsomming

Weens aanduidings van ŉ strawwe *Citrus tristeza virus (*CTV) komponent in die Nartia (GFMS 12) kruisbeskemingsbron 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 ge-evalueer is, is gevind dat slegs vier potensiaal gewys het 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 GFMS35 (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 herbesmettings sal aandui. Hierdie proef is ʼn herhaling van Projek 679 wat in Swaziland aangeplant is, asook gedeetelike 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 preïmunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing gedurende September 2004 uitgeplant en word jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte ge-evalueer. Die bome se groottes is 9 jaar na uitplant gemeet. Die bome groei heelwat stadiger as bome in die ander pomelo produserende streke. Stamgleuf evaluasies is gedoen en sub-isolaat B389/1 het matige stamgleuf ontwikkel en GFMS12, 12/7 en B390/5 ligte stamgleuf. Vrugte is ge-oes en gegradeer volgens uitvoer groottes. Die gemidddelde opbrengs per boom van al die behandelings het nie betekenisvol verskil nie. Bome met B389/1 het die minste vrugte geproduseer en betekenisvol meer klein vrugte as die ander behandelings, terwyl bome met GFMS 12/9 die meeste vrugte geproduseer het met die meeste groot aantal vrugte. Die virusvrye bome is na 9 jaar nog steeds nie besmet nie, wat aandui dat daar ʼn lae druk van plantluise of CTV in die omgewing is.

4.2.4 PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations in the Orange River Valley

Project 739 (2004 - 2015) by J.H.J. Breytenbach, G. Cook & S.P. van Vuuren (CRI)

Summary

Disease expression of *Citrus tristeza virus* (CTV) is influenced by host cultivar and climatic conditions. It is therefore necessary to evaluate the various cross-protecting CTV sources in various citrus production areas. Mild CTV sources derived from sweet orange trees (SM 46, SM 47, SM 48, SM 49) were used to pre-immunise virus-free Delta -, Midknight -, and Turkey Valencia on C35 citrange rootstocks. These sources will be compared to LMS 6 (standard pre-immunisation source for sweet oranges) and virus-free controls. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Karsten Boerdery in the Kakamas area in September 2007. Tree size was measured 6 years after planting. Trees with SM 48 were significantly smaller in all three cultivars. Although there are significant differences in growth between trees with the different CTV treatments, the results should be seen only as trends at this stage. Trees will be evaluated annually for growth, production, fruit size distribution and tree health. The influence of pre-immunisation sources will only be apparent after evaluation over a number of seasons.

Opsomming

Siekte uitdrukking van C*itrus tristeza virus* (CTV) verskill tussen sitrusgashere en onder verskillende klimaatstoestande. Dit is dus nodig om verskillende CTV bronne in verskillende sitrus produserende streke te evalueer. Potensiële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemoenbome versamel is (SM 46, SM 47, SM 48, SM 49), is gebruik om virusvrye Delta -, Midknight -, en Turkey Valencia op C35 citrange onderstam te preïmmuniseer. Hierdie bronne word met LMS 6 (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 by Karsten Boerdery in die Kakamas omgewing geplant is. Die boomgroottes is 6 jaar na uitplant gemeet. Bome met SM 48 was in al drie kultivars betekenisvol die kleinste. Alhoewel daar statistiese verskille tussen die verskillende behandelings by elke kultivar was, is dit nodig om data van produksie en boomgesondheid oor ʼn aantal jare te versamel vir ʼn volledige evaluasie.

4.2.5 PROGRESS REPORT: Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa

Project 742 (2004 - 2015) by J.H.J. Breytenbach, G. Cook & S.P. van Vuuren (CRI)

Summary

Budwood was obtained from the different grapefruit production areas of southern Africa from 108 superior grapefruit trees that harbour possible mild CTV sources. After the CTV sources were established in the glasshouse, material was inoculated to virus-free Mexican lime indicator plants to evaluate the severity of the CTV sources. After the first biological test, 19 were selected for further evaluation. These 19 sources were inoculated again to virus-free Mexican lime plants and compared to GFMS 12, GFMS 35, GFMS 12/7, GFMS

12/9, and the four best Beltsville sub-isolates (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). The Mexican lime plants were evaluated for growth and stem pitting. Virus titre was determined by ELISA. The most promising of 19 field sources (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwaleni Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), indexed free for citrus viroids and are being used as pre-immunising agents for Marsh and Star Ruby trees. These sources are compared to GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), as well as the four best Beltsville sub-isolates (B389-1, B389-4, B390-3, B390-5) and the ITSC sub-isolates (GFMS 12/7, GFMS 12/9). Pre-immunisation was confirmed by means of ELISA and the Star Ruby trees were planted at Bosveld Sitrus Farm in the Letsitele area in February 2007, while the Marsh trees were planted at Riverside in the Malelane area in March 2007. The trees were evaluated for growth and health 6 years after planting. Both the Marsh and Star Ruby trees containing GFMS12 had developed unacceptably high stem pitting and resulted in suppressed tree growth. Although differences were observed, conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons.

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 (biologiese indeksering) om te bepaal of die bome moontlik ligte rasse van C*itrus tristeza virus* (CTV) huisves wat as kruisbeskermingsbronne kan dien. Na die eerste biologiese indeksering van 6 maande het slegs 19 bronne potensiaal getoon en is vir verdere evaluering gebruik. Hierdie 19 bronne is ʼn tweede keer op Meksikaanse lemmetjie geïnokuleer en met bekende bronne GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5) vergelyk. Na ʼn tydperk van 6 maande is die geïnokuleerde plante vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA ge-evalueer. Die 4 mees belowende bronne, wat vry is van viroiede, is Tabankulu 1 (versamel vanaf Star Ruby in Swaziland), New Venture 41/2 (versamel vanaf Star Ruby in die Nkwaleni Vallei), ORE 8 (versamel vanaf Marsh in die Hoedspruit gebied) en Tshipise 19/5 (versamel vanaf Marsh in Tshipise). Hierdie bronne is verder gebruik om virus-vrye Marsh en Star Ruby boompies vir boord evaluasie te preïmmuniseer. Die bronne word met GFMS 12 (vorige standaard vir pomelos), GFMS 35 (huidige standaard vir pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR-ITSG sub-isolate (GFMS 12/7, GFMS 12/9) vergelyk. Preïmmunisering is deur middel van ELISA bevestig voordat bome geplant is. Die Star Ruby boompies is gedurende Februarie 2007 op Bosveld Sitrus Plaas in die Letsitele omgewing geplant en die Marsh boompies is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is die sesde jaar na uitplant vir groei en stamgleuf ge-evalueer. In die stadium het bome met GFMS12 in beide Marsh en Star Ruby onaanvaarbare hoë stamgleuf ontwikkeling getoon wat ook sodoende die groei belemmer het. Alhoewel daar verskille voorkom, tussen die veskillende bronne ten opsigte van vrug en boom groottes, kan gevolgtrekkings eers gemaak word na ʼn aantal jare se oes en boomgesondheid opnames.

4.2.6 PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for cross-protecting Turkey Valencia

Project 789 (2005 - 2015) by J.H.J. Breytenbach, G. Cook & S.P. van Vuuren (CRI)

Summary

Turkey Valencia appears to be more sensitive to CTV than other Valencia types. Since Turkey Valencia is an early Valencia type, it is an important component of the local citrus export portfolio and it is therefore important to identify a suitable CTV pre-immunising source for this cultivar. Virus-free Turkey Valencia on Troyer citrange rootstocks were prepared in the glasshouse and inoculated with different CTV sources; LMS 6 (standard), SM 46, SM 47, SM 48, SM 49 (all obtained from sweet orange) to identify the best source for cross-protection purposes. Trees inoculated with GFMS12 and virus-free trees will serve as positive and negative controls, respectively. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Riverside in the Malelane area in March 2007. Tree growth and yield were measured 6 years after planting and differences were observed although the yields were very low; it is suspected that fruit might have been stolen prior to harvest. At this stage the virus free trees, performed significantly better overall while trees with SM48 the poorest. Conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons.

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is (CRI Groep Navorsingsjaarverlag, 2003). Aangesien Turkey Valencia ŉ vroeë Valencia is, is dit ŉ belangrike kultivar in die bedryf en daarom is dit ŉ hoë prioriteit om ŉ geskikte CTV preïmmunisasie bron vir Turkey Valencia te vind. Virusvrye Turkey Valencia op Troyer citrange onderstam is in ŉ glashuis voorberei en met verskeie CTV bronne, LMS 6 (standaard), SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel), geïnokuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS 12 bron geïnokuleer is en bome wat virusvry gelaat is, dien as positiewe en negatiewe kontroles onderskeidelik. Preïmmunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is 6 jaar na uitplant, vir groei en produksie ge-evalueer. Produksie was baie laag en dit is vermoed dat daar moontlik vrug diefstal plaasgevind het. Op die stadium het die virusvrye bome betekenisvol die beste gevaar en SM48 die swakste tussen die verskillende behandelings. Alhoewel daar verskille voorkom tussen die veskeie bronne ten opsigte van vrug en boom groottes, kan gevolgtrekkings eers gemaak word na ŉ aantal jaar se oes en boomgesondheid opnames.

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

Project 968 (2009 - 2019) by J.H.J. Breytenbach, G. Cook & 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 prior pre-immunisation. This caused concern as the bud wood 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 will be used 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 cultivars. This current trial is an extension of the glasshouse trial. Two clementine selections (Clemenluz, Esbal) and two mandarin selections (Valley Gold, Morr 22) on Troyer citrange rootstock have been grown and pre-immunized with different CTV sources; *i.e* .EM, SM 47, SM 48 and SM 49. 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 within different climatic regions suitable for the production of soft citrus, i.e. Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Due to frost and drainage problems one year after planting at both sites, new trees had to be prepared and the trial re-planted. New trees were prepared and the trial replanted during December 2012 in the Citrusdal area. The heights and diameters of the 1-year-old trees were measured and the canopy volumes (m^3) calculated. Although there were significant differences between the sources in Clemenluz and Morr22, no conclusion can be made currently, as it is only one year after planting and no production data is available yet. Trees for the Groblersdal site have been prepared and will be planted in spring, 2014.

Opsomming

Tydens die her-indeksering van die Grondvesblok se moederbome gedurende 2003 is gevind dat ŉ groot aantal clementine en mandaryn bome geen CTV bevat het nie. Dit het kommer gewek as gevolg van die feit dat okuleerhout wat aan die kommersiële kwekerye verskaf word, virusvry is en nie bekerming bied teen natuurlike CTV rasse wat in die veld deur plantluise oorgedra word nie. Die CTV bekermingsbron is noodgedwonge na GFMS12 verander totdat ŉ geskikte bron vir sagte sitrus gevind is. ŉ Glashuis proef is gedurende 2006 gedoen om CTV bronne in vier verskillende kultivars te evalueer. Hierdie veldproef is dus ŉ uitbreiding van die glashuis proef. Twee clementine seleksies (Clemenluz, Esbal) en twee mandaryn seleksies (Valley Gold, Morr 22) is op Troyer citrange onderstamme ge-okuleer en gepreïmmuniseer met verkillende CTV bronne, nl. EM, SM 47, SM 48 en SM 49. Bome met hierdie bronne sal met GFMS12 (standaard) en bome wat virus vry geplant is, vergelyk word. Nadat preïmmunisering deur middel van ELISA bevestig is, is die bome gedurende 2010/11 in twee verskillende klimaatstreke geskik vir sagte sitrus geplant (Groblersdal in Mpumalanga en Citrusdal in die Wes Kaap). As gevolg van dreinerings probleme en koue skade is nuwe bome voorberei. Die nuwe boompies is geplant gedurende Desember 2012 in die Citrusdal omgewing. Die boomgroottes is gemeet een jaar na uitplant Alhoewel daar beteknisvolle verskille was tussen die verskillende bronne in Clemenluz en Morr22 kan geen gevolgtrekking gemaak word nie, want dit was slegs een jaar na uitplant. Bome vir die Groblersdal omgewing sal gedurende die lente van 2014 geplant word.

4.2.8 PROGRESS REPORT: Dynamics of *Citrus tristeza virus* mild and severe strains in mild strain cross-protection strategies

Project 885B (2013 - 2015) by G. Pietersen, D. Read, J. Lubbe & K. Snyders (ARC-PPRI and UP)

A PCR targeting the p33 and RNA-dependent RNA polymerase (RdRp) gene of CTV was developed. The test is unlikely to suffer from bias of the previously used A-fragment PCR. The p33 gene is a relevant gene to use in the characterisation of CTV populations in plants because of its association with the super-infection exclusion of CTV strains. 647 samples of GFMS12 pre-immunised Star Ruby were collected from widely separated grapefruit production areas and the nucleotide sequences of the p33/RdRp amplicon of 192 of these determined. At least 8 p33/RdRp sequences were shown to occur in CTV populations of the individual samples. Cloning of amplicon from a subset of 6 these plants confirm the direct sequencing results regarding presence of various genotypes, but also show the presence of other genotypes including a novel one. Illumina platform based sequencing on 48 samples using the same amplicon was conducted, the analysis of which is still underway. Large numbers of replicates of single aphid transmissions and dilution end point inoculations using bark slash and bark flap methods were conducted to isolate CTV genotypes. We await substantial viral replication in the plants before testing the inoculated plants. The potential use of overlapping amplicons as templates for the whole genome sequencing of CTV was demonstrated using five overlapping amplicons which amplify the 3' half of the genome of the CTV sources 12-7, 12-8 and 12-9. This confirmed the dominance in these sources of CTV-ZA.

One hundred Rio-Red grapefruit samples collected by systematic sampling from a site in Swaziland in which a T36-like source was thought to occur were tested to detect this genotype. Of these 95 tested positive in T36-specific primer based test. The amplicon of a subset of 25 of these were sequenced. In 24 of the samples Taiwan/PUM/Sp/T1-like sequences were identified while in one sample NZRB-TH28/NZRB-M12-like sequences were identified. No T36-like sequences were obtained.

The B390/3 source was inoculated to ten citrus hosts in order to confirm the mild nature of the CTV source and to confirm the CTV genotype purity of the sample in various hosts. No severe symptoms were observed and most inoculated plants yielded amplicons using the generic CTV, T36 and NZRB2, suggestive of the presence of only a NZRB2-like genotype as demonstrated using overlapping 3' half amplicons in Illumina tests. Two plants however yielded weak bands in the B165 PCR. This will be investigated further.

Pathogenicity of the *A. tumefaciens* strain used thus far on *Nicotiana benthamiana* plants has hampered progress in setting up the transformation pipeline required to be able to prepare and test hybrid infectious clones in order to extend the general genotype specificity principle of the p33 gene of CTV beyond those tested by Foliminova to include it to genotypes commonly found in South Africa on grapefruit.

Opsomming

Tydens die huidige verslag periode het ons ŉ PKR toets ontwikkel, gerig teen die p33 en RNA-afhanklike RNA polimerase (RdRp) gene van sitrus tristesa virus (CTV) wat waarkynlik nie ly aan dieselfde vooroordeel van die A-fragment sisteem wat voorheen gebruik is nie. Die p33 geen is ŉ relevante geen om te gebruik in die karakterisering van CTV populasies aangesien die' geen geassosieer is met super-infeksie uitsluiting van virus rasse. 647 monsters van GFMS12 ge-immuniseerde Star Ruby pomelos is versamel vanuit wyduiteenlopende pomelo produksie areas, en die nukleotied volgorde van die p33/RdRp amplikon van 192 monsters is bepaal. Ten minste 8 p33/RdRp nukleotied volgordes was gevind in die CTV bevolkings van individuele monsters. Klonering van die amplikon van ŉ kleiner groep van 6 van die plante bevestig die direkte sekwensresultate betreffend die teenwoordigheid van die genotipes, maar wys ook die teenwoordigheid van ander genotipes insluitend die van ŉ nuwe een aan. Illumina platform gebaseerde nukleotied-volgordebepaling op 48 monsters se amplikoon is gedoen maar verg nog verdere analiese. Groot getalle enkel plantluis oordragings (SAT's) sowel as verdunnings-eindpunt meganiese oordragings met bas vlap en bas sny tegnieke is gedoen. In vroeë toetse is almal negatief. Ons wag vir virus replikasie in die ontvanger plante voor die toets van hulle vir CTV. Die potensiaal vir die gebruik van oorvleuende amplikone as templaat vir heelgenoom nukleotied-volgordebepaling met Illumina is gewys met 5 oorvleulende fragmente wat die hele 3' helfte van die genoom van CTV bronne 12-7, 12-8 and 12-9 se nukleotiede kon bepaal. Hierdie het die dominansie in die CTV populasie van hierdie bronne van CTV-ZA bevestig.
Een-honderd Rio-Red pomelo monsters is sistematies versamel in ŉ boord in Swaziland waarin ons vroeër vermoed het ŉ T36-agtige bron voorkom. Van hierdie het 95 plante positief in ŉ T36-voorvoorder PKR getoets. Die amplikoon van ŉ onderafdeling van 25 van hierdie is aan nukleotied volgordebepaling blootgestel. In 24 van die monsters het Taiwan/PUM/Sp/T1-agtige nukleotied volgordes voorgekom en in een monster ŉ NZRB-TH28/NZRB-M12-agtige nukleotied volgorde. Geen T36-agtige volgordes is gevind nie.

Die B390/3 kandidaat bron is ge-inokuleer op 10 sitrus gashere om die matige status van die bron te bevestig sowel as die genotipe suiwerheid van die bron in verskillende gashere te bepaal. Geen strawwe simptome is op enige van die ontvanger plante gevind nie, en meeste plante het amplikone in die generiese CTV, T36 and NZRB2 PKR toetse getoon wat aangedui het op die teenwoordigheid van slegs ʼn NZRB2-agtige genotipe, soos ook aangetoon die die Illumina volgordebepalingstoets op die oorvleulende amplikone. Twee plante het egter ook lae vlakke van amplikone tot gevolg gehad in die B165 PKR toetse. Hierdie sal verder geanaliseer word.

Patogenisiteit van die *A. tumefaciens* ras wat tans gebruik word om *Nicotiana benthamiana* plante te transformeer, kortwiek vordering in die vestiging van ʼn transformasie pyplyn om hibried infektiewe CTV klone te kan maak en toets. Hierdie is om die algemene p33 geen genotipe-spesifisiteit by superinfeksie uitsluiting van Foliminova verder te toets op genotipies wat Foliminova nie getoets het nie, en wat van belang vir die Suid-Afrikaanse pomelo bedryf is.

4.2.9 PROGRESS REPORT: Differential cultivar selection or suppression of *Citrus tristeza virus* (CTV) genotypes

Project 1056 (2012 - 2014) by G. Cook, J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Summary

Citrus tristeza virus (CTV) is a complex of variants. This insight and the subsequent development of diagnostics for strain determination enable the analysis of mixed populations. We have expanded on a published CTV strain detection system and determined the strain composition of most of the CTV sources maintained at CRI using this methodology, including the sources used within this trial. In addition we tested various maintenance source plants of GFMS12 and GFMS35 pre-immunisation sources at 3 different institutions including all grapefruit mother trees maintained at the Citrus Foundation Block. This investigation indicated that segregation of strains within these primary sources has occurred and that various maintenance sources lack different strains that are detected as components of the original sources. These findings could potentially explain the cross-protection break down experienced in the field. Four different, single strain CTV sources were used to inoculate various citrus cultivars and transmission and symptom expression of each strain was investigated. ELISA tests to detect CTV within each trial plant were done at 7, 13 and 24 weeks after inoculation by sampling at 15cm, 30cm and at the shoot tip respectively. Plant growth measurements were also done after six months where after plants were cut back and allowed a second regrowth cycle to permit sufficient time for symptom expression. A final analysis including plant growth measurements and stem pitting ratings was done 12 months after initial inoculation. A Quantitative RT-PCR was optimised for testing the relative titres of the CTV strains in selected citrus varieties. This work is still being finalised. A final report detailing the final trial analysis will be presented in 2015.

Opsomming

Sitrus tristeza virus (CTV) bestaan uit ʼn kompleks van rasse. Hierdie insig gekoppel met die ontwikkeling van diagnostiese toetse verskaf die vermoë om gemengde CTV bevolkings te ontleed. Ons het uitgebrei op ʼn gepubliseerde opsporingstelsel vir CTV rasse en het die rasprofiele opgestel van meeste van die CTV bronne wat by CRI instand gehou word, insluitend die bronne wat gebruik is in die huidige proef. Daarbenewens het ons verskeie instandhoudingsbronne van GFMS12 en GFMS35 pre-immuniseringsbronne getoets by 3 verskillende instellings, asook alle pomelo moederbome by die Sitrus Grondvesblok wat met GFMS 35 gepre-immuniseer is. Hierdie ondersoek het aangedui dat segregasie van rasse plaasgevind het en dat verskeie instandhoudingsbronne nie meer die volle komponente van rasse besit wat in die ouer bronne teenwoordig is nie. Hierdie bevindinge is ʼn moontlike verduideliking vir die kruisbeskerming-afbraak wat in boorde ervaar word. Vier verskillende, enkelras CTV bronne is gebruik om verskeie sitrus kultivars te inokuleer en transmissie, translokasie en simptoom uitdrukking van elke ras te bestudeer. CTV ELISA toetse is op elke proefplant gedoen. Monsterneming is 7, 13 en 24 weke na inenting gedoen en is onderskeidelik op 15cm, 30cm en by die groeipunt geneem. Plantgroei metings is ook gedoen ná 6 maande. Alle plante is teruggesny en ʼn tweede hergroei toegelaat om voldoende tyd te laat vir simptoom uitdrukking. Finale proef analise het hergroei metings na ʼn verdere 6 maande ingesluit asook stamgleuf evaluasies op 12 maande na die aanvanklike inenting. ʼn Kwantitatiewe PKR toets was ontwikkel om die relatiewe titers van die verskillende rasse van CTV in sekers kultivars te bepaal. Hierdie werk word nog gefinaliseer. ʼn Finale gedetailleerde verslag sal in 2015 verskaf word.

4.2.10 **PROGRESS REPORT: Evaluation of citrus material for greening resistance**
Project 815 (2006 - 2016) by S.P. van Vuuren, J.H.J. Breytenbach & G.Cook (CRI)

Summary

Attempts are made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. Two embryo rescue clones, GTC-E2 and GTC-T2 were identified symptomless in 2006 after exposure to the 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. After 6 years no greening symptoms were observed on the trees and PCR tests were also negative. The fourth crop was harvested from the trees and 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 four years of all the ER clones was significantly better than that of the Midknight Valencia. External and internal qualities were within the export requirements.

Opsomming

Daar word 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 geïdentifiseer as simptoomloos na blootstelling aan besmette insekte. 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 uitgeplant vir verdere evaluasie. Na 6 jaar is nog geen vergroeningsimptome waargeneem nie en PKR toetse was ook negatief. Die vierde oes is van die bome verkry en vruggehalte vergelyk goed met die Midknight Valencia kontrole. Kloon GTC-E2 het die beste produksie gelewer en was betekenisvol beter as die van Midknight Valencia. Die kumulatiewe produksie oor vier jaar van al die embrio-herwinningsklones was betekenisvol beter as die van Midknight Valencia. Uiterlike en inwendige gehalte het aan die uitvoer vereistes.

4.2.11 **PROGRESS REPORT: Epidemiology of greening disease – alternate hosts and spread**
Project 886B (2013 - 2015) by G. Pietersen & R. Roberts (ARC-PPRI & UP)

Summary

Citrus greening has been reduced to 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 hosts other than citrus to '*Candidatus* Liberibacter africanus' (Laf). During a previous project (886) large numbers 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 the Rutaceae analysed, supporting the general hypothesis that Laf may have derived from indigenous African members of the Rutaceae. It is important to do controlled transmission tests to test the possibility that these bacteria may infect Citrus. We also need to determine the potential occurrence of Liberibacters in some of the remaining indigenous Rutaceous tree hosts genera (*Teclea* and *Oricia*). With the introduction to the USA of "*Candidatus* Liberibacter asiaticus" (Las), associated with huanglongbing disease, research effort, supported by millions of dollars of funding, is being used to develop fundamental understandings and control strategies for this disease. Novel control strategies are being evaluated, many based on molecular interventions. To this end it is important that we stay up to date with the latest international developments regarding Liberibacter detection, control and epidemiology and also characterize the local sources of Liberibacters by whole genome sequencing in order to exploit the sequence differences amongst the Liberibacters. Data obtained thus far suggest that the Liberibacter variants observed in South Africa appear to be restricted to specific Rutaceous hosts. If greater understanding is obtained regarding the genes that regulate host range we may be able to exploit this in the long term in control.

Opsomming

Die voorkoms van sitrus vergroening in Suid Afrika is veminder 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 daarop dui dat '*Candidatus* Liberibacter africanus' (Laf) moontlik voorkom op alternatiewe gashere. Gedurende ŉ vorige projek (886) is ŉ groot aantal *Calodendrum, Clausena, Vepris* en *Zanthoxylum* monsters getoets vir Liberibacters. Tipiese Laf was nie

geidentifiseer op enige van die monsters nie. Tog is ander Laf verwante Liberibacters geidentifiseer in elk van die geanaliseerde genera. Die bestaan van alternatiewe Liberibacters ondersteun die hipotese dat Laf se oorsprong vanaf ŉ inheemse Rutaceae bron kan wees. Dit is belangrik dat gekontroleerde oordragingstoetse na sitrus van die inheemse Liberibacters gedoen word om te bepaal of hierdie Liberibacters sitrus kan infekteer. Daar moet ook vasgestel word of Liberibacters in die oorblywende inheemse Rutaceae genera, *Teclea* en *Oricia,* voorkom. Met die voorkoms van '*Candidatus* Liberibacter asiaticus' (Las), die oorsaak van Huanglongbing, in Amerika, word baie geld en tyd bestee aan die navorsing van verskeie aspekte van hierdie bakterium. Alternatiewe beheer strategiëe word tans ondersoek met die klem op molekulêre sisteme. Dit is belangrik om by te bly met alle internasionale verwikkeling rakende Liberibakter diagnostiese toetese, beheer en epidemiologie. Addisioneel moet klem daarop geplaas word om die genome van inheemse Liberibakters te sekwens sodat verskille tussen die genome geidentifiseer kan word. Sulke verskille kan lei tot die ontwikkeling van beter beheer strategiëe.

4.2.12 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 & 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. Bud wood 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 bud wood sources were established by reverse transcription polymerase chain reaction (RT-PCR). The CTV severity in each source was also established by inoculating Mexican lime (ML) indicator plants and measuring growth as well as rating the stem pitting development. The old clone sources contained a greater variety of CTV genotypes and stem pitting development was more severe and growth retarded than that of the CFB material. Old clone Benny Valencia was infected with citrus exocortis viroid (CEV) and CVd-III (dwarfing viroid). Old clone Cambria navel was infected with CEV, CVd-II (Hop stunt viroid) and CVd-III. Old clone Glen Ora Late navel was infected with CVd-II and CVd-III. Trees from the CFB and old clone material are being prepared for field trials.

Opsomming

Sommige cultivar eienaars en agente maak aanspraak daarop dat ou klone materiaal meer winsgewend is as Sitrus Verbeteringskema materiaal vanaf die Grondvesblok (GVB). GVB material is skoongemaak van alle entoordraagbare siektes deur middel van groeipunt-enting en is daarna geïnokuleer met ŉ goedgekeurde *Citrus tristeza virus* (CTV) bron vir kruisbeskerming. Die doel van die studie is om boom en vrug eienskappe van GVB materiaal met die van ou kloon material te vergelyk. Drie kultivars is betrokke, nl. Benny Valencia, Cambria nawel en Glen Ora Late nawel. Okuleerhout is van oorspronklike bronne van die cultivars versamel en volgens normale kwekery praktyke op Swingle citrumelo, Carrizo citrange en C35 citrange onderstamme ge-okuleer. Dieselfde is gedoen met material wat vanaf die GVB ontvang is. Streng sterilisasie voorsorgmaatreels van snygereedskap is gevolg tydens okulering. Die CTV en Citrus viroid (CVd) status van al die enthout bronne is bepaal deur middel van polimerase kettingreaksie (PCR). Die strafheid van die CTV in elke bron is ook bepaal deur die inokulasie van sensitiewe Meksikaanse lemmetjie indikator plante en om die groei en ontwikkeling van stamgleuf te bepaal. Die ou kloon bronne het ŉ groter verskeidenheid van CTV genotipes gehuisves en stamgleuf ontwikkeling was meer straf en groei gestrem in vergelyking met die van GVB materiaal. Ou kloon Benny Valencia was besmet met eksokortis viroid (CEV) en CVd-III (verdwergingsviroid). Ou kloon Cambria nawel material was besmet met CEV, CVd-II (Hop stunt viroid) en CVd-III. Ou kloon Glen Ora Late navel was besmet met CVd-II en CVd-III. Bome van GVB en ou kloon materiaal word gemaak vir veld evaluasie.

4.3 PROGRAMME: FRUIT AND FOLIAR DISEASES

Programme coordinator: G.C. Schutte (CRI)

4.3.1 Programme summary

Results from field trials where new systemic and contact fungicides alone or in combination with registered fungicides were tested on 'Nova' mandarins for the control of Alternaria brown spot, showed that eight applications of copper oxychloride performed the best but also resulted in serious copper stippling. Combinations and altering of different fungicides with different modes of action showed promise and should be investigated on a commercial scale.

The deposition quality parameter of the spray deposition assessment protocol was corrected to a working format based on the interquartile coefficient of dispersion. This now allows for deposition quality to be determined. Previous spray trial with the Martignani and Nieuwoudt spray machines for the evaluation of potential of low-energy/low cost/low input sprayers was repeated on leaves. Results were similar to that of previous trials showing promising deposition quantity, uniformity and efficiency results at low volumes (2000 to 4000 L/ha), lower spray pressures (15 bar) and adequate, efficient spraying speeds (1.9 to 2.9 km/h). 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). Distinct differences in deposition quality were observed between machinery used. Negative effects on deposition parameters as influenced by density of canopy were also noted.

Evaluation of different fungicides in the Sundays River Valley could not prevent the excessive appearance of Botrytis-like ridges on lemon fruit. Application of excessive nitrogen can be attributed to this phenomenon. Lemon flowers sprayed with a fluorescent pigment in water showed poor coverage of all flower parts.

Programopsomming

Nuwe sistemiese en kontak swamdoders is op hul eie asook in kombinasies met geregistreerde swamdoders vir die beheer van Alternaria bruinvlek op 'nova' mandaryne getoets. Resultate toon dat sewe tot agt maandelikse koperoksichloried bespuitings die beste gevaar het, maar gepaardgaande met erge stippelvorming. Kombinasies en afwisseling van verskillende swamdoders met verskillende meganismes van werking toon groot belofte en moet verder op kommersiële skaal ondersoek word.

Die deposisiekwaliteit parameter van die spuit deposisie asseserings protokol was verbeter tot 'n werkende formaat deur dit te baseer op die interkwartiel koëffisiënt van verspreiding. Dit stel ons in staat om die deposisiekwaliteit parameter akkuraat te bepaal. Vorige spuitproewe met die Martignani en Nieuwoudt spuitmasjiene vir die evaluaering van lae energie/lae koste en insetting potensiaal is herhaal. Resultate was soortgelyk van die van vorige spuitproewe en het gewys op belowende deposisiekwantiteit, uniformiteit sowel as effektiwiteit by lae toedienings volumes (2000-4000 L/ha), laer spuit druk (15 bar) en aanvaarvare, effektiewe spuitspoed (1.9 tot 2.9 km/h). Klein verskille was waargeneem in deposisiekwaliteit soos beïnvloed deur spuitvolumes. Laer spuitvolumes het in die algemeen beter deposisiekwaliteit tot gevolg gehad, maar hoër spuitvolumes was meer konstant deur die boomlower (was-effek). Merkwaardige verskille was waargeneem in terme van deposisiekwaliteit tussen twee spuitmasjiene. Die negatiewe effekte van 'n digte boomlower op deposisie parameters was weereens uitgewys in hierdie proewe.

Evaluasie van verskillende swamdoders in die Sondagsriviervallei kon nie die buitensporige hoë voorkoms van Botrytis-agtige riwwe op suurlemoene voorkom nie. Toediening van te veel stikstof kon blykbaar bygedra het tot die verskynsel. Suurlemoenblomme wat gespuit is met fluoriserendepigment in water toon powere bedekking op alle blomdele.

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 & C. Kotze (CRI)

Summary

New systemic and contact fungicides alone or in combination with registered fungicides were tested on 'Nova' mandarins for the control of Alternaria brown spot. Results showed that copper oxychloride applied as seven to eight monthly applications performed the best, but accompanied with serious copper stippling. Combinations and altering fungicides with different modes of action showed promise and should be investigated on a commercial scale.

Opsomming

Nuwe sistemiese en kontak swamdoders is op hul eie asook in kombinasies met geregistreerde swamdoders vir die beheer van Alternaria bruinvlek op 'nova' mandaryne getoets. Resultate toon dat sewe tot agt maandelikse koperoksichloried bespuitings die beste gevaar het, maar gepaardgaande met erge stippelvorming. Kombinasies en afwisseling van verskillende swamdoders met verskillende meganismes van werking toon groot belofte en moet verder op kommersiële skaal ondersoek word.

Introduction

Alternaria brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. Susceptibility to ABS is a dominant trait that is transferred from 'Dancy' mandarin to its progency (Dalkilic *et al.*, 2005). Dancy mandarin hybrids and some cultivars of unknown origin, such as 'Murcott', 'Emperor' and 'Ponkan', are affected by the disease. The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the 'Nova', 'Minneola' and 'Mor'.

The causal agent of ABS was designated originally as *Alternaria citri* Ellis & N. Pierce (Pegg, 1966) and later renamed *A. alternata* (Fr.:Fr.) Keissl. pv. *citri*, based on the production of a toxin specific to mandarin fruit (Solel, 1991). Later, eight species were described among *Alternaria* isolates pathogenic to mandarins based on morphological and biochemical traits (Andersen *et al*., 2005; Simmons, 1999). However, all small-spored *Alternaria* spp. from citrus are closely related by molecular analysis and they have been placed into a single phylogenic species, *A. alternata* (Peever *et al*., 2002, 2004 & 2005).

ABS attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. ABS sporulates most abundantly on lesions on mature leaves remaining in the canopy (Reis *et al*., 2006) The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Leaves are susceptible until they are fully expanded and hardened. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer. Leaves are susceptible to infection from the time of formation until they are fully expanded and hardened, and fruit are susceptible from petal fall until harvest. In the USA, however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter.

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

Objectives

New spray programmes containing different fungicides with a broad spectrum of activity will be evaluated. We are also looking for alternative fungicides to be used in alternation with copper fungicides that can serve as an alternative for mancozeb.

Materials and methods

Ten single-tree plots per treatment were randomly selected in a 'Nova' orchard at Belmont 50 km west of Nelspruit. The trees were 17 years old and planted in 2x5 m tree spacing in rows that ran from North to South. Trees were selected for uniformity in canopy density and tree size. Guard trees were located between plots within rows. Only two rounds of fungicidal spays were applied with a trailer-mounted, high-volume, high-pressure (2500-3000 kPa) sprayer with two hand-held spray guns on the dates mentioned in Table 4.3.2.1, Table 4.3.2.3, Table 4.3.2.5 and Table 4.3.2.6. The weather was fine and dry on all occasions with minimal wind. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off.

Results and discussion

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

2010-2011 season

Results from the field trial conducted at Belmont (Table 4.3.2.2) were influenced by the heavy rain experienced during early part of January 2011. Serious flooding of the access roads prevented us from crossing the Crocodile river and the orchard was also flooded, preventing us from applying the January application. Due to this, we could only apply the next application after 9 weeks instead of the planned 4 weeks and this also resulted in one less spray application. Nonetheless, results showed that there was a significant difference ($P < 0.05$) between the standard registered copper oxychloride treatment and all the other treatments. Copper oxychloride resulted in 91.6% clean exportable fruit with the other standard, mancozeb, and Fighter resulting in 62.6% and 63.0% clean exportable fruit respectively.

This was quite surprising because in treatments 3 and 6 where every second Fighter treatment was altered with copper hydroxide (Copstar) and the rate of Fighter was lowered in treatment 6 to 400ml/hℓ water instead of 570ml/hℓ water (which is the recommended rate for root rot control) they only resulted in 46% and 47% clean exportable fruit. This poor performance shows that a single copper application (a SC formulation with less metallic copper than the WP formulations) do not have the legs to prevent infection, whereas copper oxychloride had 4 top-up applications up to 8 December 2011 and still had enough copper residues left on the fruit surface to prevent Alternaria brown spot from developing on the fruit. However, copper stippling still remained a problem with the frequent applications of copper oxychloride (Fig. 4.3.2.1).

Where the rate of Fighter was lowered from 570ml/hℓ water in treatment 4 to 400ml/hℓ water in a tank mix with Sporekill in treatment 5, treatment 5 resulted in 17% less clean exportable fruit; not significantly different from each other. In similar spray programmes (treatments 3 and 6), but with the exclusion of Sporekill from the tank mixture with Fighter, showed that they were equally effective in the control of Alternaria brown spot, even where the rate of Fighter was reduced to 400 ml/hℓ water (treatment 6).

For the criterion, fruit with 1 to 5 lesions per fruit, copper oxychloride, the untreated control and Fighter (400 mℓ/hℓ water) in tank mixtures with Sporekill alternated with copper hydroxide were not significantly different from each other. On the other hand, the untreated control resulted in 92.4% fruit with 6 and more Alternaria brown spot lesions, which was significantly different from all the treatments, demonstrating the high incidence of the disease during this extremely wet season (Table 4.3.2.2.).

2011-2012 season

Results from the field trial conducted at Belmont (Table 4.3.2.3) showed that there was a significant difference ($P < 0.05$) between the standard registered copper oxychloride treatment and all the other treatments. Copper oxychloride resulted in 92% clean exportable fruit with the other standard, mancozeb, resulting in 76% clean exportable fruit.

For a second year in a row was this quite surprising because in treatments 7, 8, 9 and 10 where every second Fighter treatment was altered with copper hydroxide (Copstar) and the rate of Fighter was lowered in treatment 6 to 400ml/hℓ water instead of 570ml/hℓ water (which is the recommended rate for root rot control) they resulted in 94.6%, 84.6%, 52.2% and 84.0% clean exportable fruit, respectively (Fig. 4.3.2.3).

Where Sporekill (100 ml/hℓ water)(treatment 11) and Fighter (570 ml/hℓ water)(treatment 12) were sprayed solo, they resulted in 2.2% and 64.4% clean exportable fruit which was significant different from each other. However, when they were mixed and alternated with copper hydroxide (treatments 3, 4, 5 and 6), they resulted in much more clean exportable fruit showing that there might be a synergistic effect between them. During the 2010-2011 the same treatments only resulted in 37.6% and 63.0% clean exportable fruit.

Products A and B sprayed solo and at two different rates, performed poorly. The 2x rate of Product A performed the better of the two and the higher rate also showed a dosage response. The disease pressure was extremely high as the untreated control only had 8.6% clean exportable fruit, 5.0% fruit with 1 to 5 ABS lesions and 86.4% fruit with 6 and more ABS lesions.

For the criterion, fruit with 1 to 5 ABS lesions per fruit, Fighter sprayed solo, Fighter + Sporekill (400 mℓ/hℓ + 100 mℓ/hℓ water) as a tank mix alternated with copper hydroxide and the 2x rate of Product A had the highest incidence of ABS were not significant different from each other. On the other hand, the untreated control, Sporekill and Products A and B at their lowest rate resulted in the most fruit with 6 and more Alternaria brown spot lesions (Table 4.3.2.4.).

2012-2013 season

Results from the field trial conducted at Belmont (Table 4.3.2.5) showed that there was not a significant difference ($P < 0.05$) between the standard registered copper oxychloride treatment and all the other treatments, resulting in 96.2% clean exportable fruit. Using a CBS spray programme consisting of Cabrio and the new fungicide BAS703 with mineral spray oil (treatments 1 and 2 with four applications only) resulted in 83.4% and 97.0% clean exportable fruit, respectively. Although not significantly different from the control, the spray programme consisting of Cabrio resulted in 11.6% fruit with five and more ABS lesions.

In comparison, a similar spray programme was followed but the first mancozeb spray application was replaced with copper oxychloride while the third and fourth application was also switched around (treatments 3 and 4). Both these spray programmes performed well resulting in 94.8% and 96.2% clean exportable fruit respectively.

During 2012-2013 season two new products from River Bioscience was also evaluated in spray programmes. The programmes started with a copper oxychloride and alternated with RB1 and RB2. In total, 4 copper oxychloride treatments were alternated with four RB1 and RB2 treatments. The latter were sprayed at 1x and 2x rates required for registration purposes. Also included was a tank mixture of Sporekill with copper hydroxide where copper oxychloride was sprayed at a reduced rate. Results showed that all these spray programmes (Treatment 5 to 10) performed very well resulting in more than 85% clean exportable fruit (Table 4.3.2.8).

The disease pressure was high as the untreated control only had 53.4% clean exportable fruit, 7.2% fruit with 1 to 5 ABS lesions and 39.4% fruit with 6 and more ABS lesions which was significantly different from all the spray programmes.

Conclusion to date

Three new fungicides were evaluated for the control of Alternaria brown spot during the previous seasons. None have been reported to be registered to date yet.

Technology transfer

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

Future objectives and work plan

Any new fungicides in spray programmes of different modes of action have to be evaluated depending on the MRL requirements for each and every market. Boscalid for instance, is being tested in the USA for control of the disease. Compatibility studies of strobilurins with RB1 and RB2 will also be executed to see if they can serve as alternatives for mancozeb and copper.

Table 4.3.2.1. Evaluation of different fungicidal spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' mandarins at Belmont, Schagen during 2010 and 2011.

Treatment	**15 September 2009**	**13 October 2009**	**10 November 2009**	**8 December 2009**	**9 February 2010**	**8 March 2010**	**5 April 2010**
1	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g
2	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g
3	Fighter 570ml	Copstar 350 ml	Fighter 570ml	Copstar 350ml	Fighter 570ml	Copstar 350ml	Mancozeb 200g
4	Fighter + Sporekill 570ml +100ml	Copstar 350 ml	Fighter + Sporekill 570ml+100ml	Copstar 350ml	Fighter +Sporekill 570ml+100ml	Copstar 350ml	Mancozeb 200g
5	Fighter + Sporekill 400ml +100ml	Copstar 350ml	Fighter + Sporekill 400ml+100ml	Copstar 350ml	Fighter +Sporekill 400 ml+100ml	Copstar 350ml	Mancozeb 200g
6	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Mancozeb 200g
7	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml
8	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml
9	Control						

Table 4.3.2.2. Results of various spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, Schagen.

Treatment	**Percentage of fruit in each class** [z]		
	Lesions/fruit		
	0	**1-5**	**≥6**
1	62.6 b	15.0 ab	22.4 de
2	91.6 a	3.2 c	5.2 e
3	46.0 bc	14.4 ab	39.6 bcd
4	52.8 bc	19.8 a	27.4 cde
5	35.8 c	9.0 bc	55.2 b
6	47.0 bc	16.4 ab	36.6 bcd
7	37.6 c	12.4 ab	50.0 bc
8	63.0 b	16.6 ab	20.4 de
9	4.8 d	2.8 c	92.4 a

[z] 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.

Table 4.3.2.3. Evaluation of different fungicidal spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' mandarins at Belmont, Schagen during 2011 and 2012.

Treatment	12 September 2011	10 October 2011	7 November 2011	5 December 2011	3 January 2012	30 January 2012	27 February 2012	26 March 2012
1	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g
2	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g
3	Product A	Product A	Product A	Product A	Product A	Product A	Product A	Product A
4	Product A	Product A	Product A	Product A	Product A	Product A	Product A	Product A
5	Product B	Product B	Product B	Product B	Product B	Product B	Product B	Product B
6	Product B	Product B	Product B	Product B	Product B	Product B	Product B	Product B
7	Fighter 570ml	Copstar 350 ml	Fighter 570ml	Copstar 350ml	Fighter 570ml	Copstar 350ml	Mancozeb 200g	Mancozeb 200g
8	Fighter + Sporekill 570ml +100ml	Copstar 350 ml	Fighter + Sporekill 570ml+100ml	Copstar 350ml	Fighter +Sporekill 570ml+100ml	Copstar 350ml	Mancozeb 200g	Mancozeb 200g
9	Fighter + Sporekill 400ml +100ml	Copstar 350ml	Fighter + Sporekill 400ml+100ml	Copstar 350ml	Fighter +Sporekill 400 ml+100ml	Copstar 350ml	Mancozeb 200g	Mancozeb 200g
10	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Mancozeb 200g	Mancozeb 200g
11	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml
12	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml
13	Control							

Table 4.3.2.4. Results of various spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, Schagen during 2011 - 2012.

Treatment	**Percentage of fruit in each class** [z]		
	Lesions/fruit		
	0	**1-5**	**≥6**
1	76.0 ab	10.4 bc	13.6 cd
2	92.0 a	4.0 cde	4.0 d
3	9.0 def	0.8 e	90.2 a
4	27.4 de	13.8 ab	58.8 b
5	29.2 d	8.4 bcd	62.4 b
6	4.6 f	5.6 cde	89.8 a
7	94.6 a	3.2 de	2.2 d
8	84.6 ab	6.0 cde	9.4 cd
9	52.2 c	18.4 a	29.4 c
10	84.0 ab	6.6 bcde	9.4 cd
11	2.2 f	2.4 de	95.4 a
12	64.4 bc	13.4 ab	22.2 cd
13	8.6 ef	5.0 cde	86.4 a

[z] 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.

Table 4.3.2.5. Evaluation of new BASF spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' mandarins at Belmont, Schagen during 2012 and 2013.

No.	19 September 2012	18 October 2012	15 November 2012	22 November 2012	21 December 2012	3 January 2013	7 February 2013	20 February 2013	21 March 2013
1		MZ 200g	Cabrio + oil 10 ml + 250 ml		Cabrio + oil 10 ml + 250 ml			MZ 200g	
2		MZ 200g	BAS 703 + oil 10 ml + 250 ml		BAS 703 + oil 10 ml + 250 ml			MZ 200g	
3		Demildex 200 g		Cabrio+ oil 10 ml + 250 ml		Demildex 200 g	Cabrio + oil 10 ml + 250 ml		Demildex 200 g
4		Demildex 200g		BAS703 + oil 10ml +250 ml		Demildex 200 g	BAS703 + oil 10ml +250 ml		Demildex 200 g

Table 4.3.2.6. Evaluation of different fungicidal spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' mandarins at Belmont, Schagen during 2012 and 2013.

No.	19 September 2012	18 October 2012	24 October 2012	21 November 2012	20 December 2012	17 January 2013	24 January 2013	14 February 2013	28 February 2013	21 March 2013
5	Demildex 200 g	Demildex 200 g		Demildex 200 g	Demildex 200 g		Demildex 200 g		Demildex 200 g	
6	Demildex 200 g		RB1 100ml	Demildex 200 g	RB1 100ml	Demildex 200 g		RB1 100ml	Demildex 200 g	RB1 100ml
7	Demildex 200 g		RB1 200ml	Demildex 200 g	RB1 200ml	Demildex 200 g		RB1 200ml	Demildex 200 g	RB1 200ml
8	Demildex 200 g		RB2 1L	Demildex 200 g	RB2 1L	Demildex 200 g		RB2 1L	Demildex 200 g	RB2 1L
9	Demildex 200 g		RB2 2L	Demildex 200 g	RB2 2L	Demildex 200 g		RB2 2L	Demildex 200 g	RB2 2L
10	Demildex + SK 100 g + 100 ml		RB1 100ml	Demildex + SK 100 g + 100 ml	RB1 100ml	Demildex + SK 100 g + 100 ml		RB1 100ml	Demidex + SK 100 g + 100 ml	RB1 100ml
11	Demildex + SK 100 g + 100 ml		RB2 1L	Demildex + SK 100 g + 100 ml	RB2 1L	Demildex + SK 100 g + 100 ml		RB2 1L	Demildex + SK 100 g + 100 ml	RB2 1L
12	Untreated control									

Table 4.3.2.7. Results of various spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, Schagen during 2012 - 2013.

Treatment	**Percentage of fruit in each class** [z]		
	Lesions/fruit		
	0	**1-5**	**≥6**
1	83.4b	5.4cd	11.6a
2	97.0d	1.4a	1.6a
3	94.8cd	2.2abc	3.0a
4	96.4cd	1.4a	2.2a
5	96.2cd	2.0abc	1.8a
6	93.2bcd	3.0abc	3.8a
7	97.0d	1.2a	1.8a
8	89.4bcd	1.8ab	8.8a
9	92.0bcd	3.6abc	4.4a
10	91.4bcd	3.4abc	5.2a
11	85.8bc	5.2bcd	9.0a
12	53.4a	7.2d	39.4b

[z]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.

Fig. 4.3.2.1. 'Nova' mandarin fruit samples taken at harvest after 7 field applications with the standard mancozeb (A) and 8 x copper oxychloride (B) treatments; 3 x Fighter (@ 400 mℓ (C) and 570 mℓ/hℓ water (D)) in tank mixtures with Sporekill alternated with copper hydroxide (3 x applications) and 3 x Fighter (@ 400 mℓ (E) and 570 mℓ/hℓ water (F)) without Sporekill alternated with copper hydroxide (3 x applications).

Fig. 4.3.2.2. 'Nova' mandarin fruit samples taken at harvest after 7 field applications with the untreated control (A) and 8x copper oxychloride (B) treatments; 4x copper oxychloride alternated with 4 x RB1(@ 100 mℓ (C) and 4x copper oxychloride alternated with 4x RB1 (@ 200 mℓ (D) treatments; 4x copper oxychloride alternated with 4 x RB2(@ 1000 mℓ (E) and 4x copper oxychloride alternated with 4x RB2 (@ 2000 mℓ (F) treatments

Fig.4.3.3.3. 'Nova' mandarin fruit showing copper stippling after 8 field applications with copper oxychloride.

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2015 and Jan-Mar 2016

Apr-Jun:

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

Jul-Sep:

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

Oct-Dec:

- Continue at pre-determined intervals as registered/recommended

Jan-Mar:

- Continue at pre-determined intervals as registered/recommended

Apr-Jun:

- Evaluate current field trial

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4.3.3 PROGRESS REPORT: Optimisation of fungicide spray applications in citrus orchards

Project PPL 891 (April 2007 - March 2010) by J.G. van Zyl & Paul Fourie (CRI at SU)

Summary

In South Africa, fungicide spray application at medium to high cover (±9 000 L/ha) is recommended to control fruit and foliar diseases. A large proportion of excessive spray volume is, however, lost to run-off and drift, which results in considerable environmental pollution of soils and air. These factors together with improper calibration, machinery and equipment use and wrongful and neglected application techniques, leads to reduced spray efficiency and poor disease control. This study uses an improved spray deposition assessment protocol and previously developed deposition benchmarks indicative of the biological effectiveness of depositions to improve spray application and possibly through cost-saving lower volume foliar application. From various spray trials using various machines at spray volumes from 1000 to 24000 L/ha, it was clear that excessively high spray volumes (>10 000 L/ha) did not result in better spray deposition. 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. In the past season, the deposition quality parameter was refined to accurately express deposition variation on leaf surfaces in a timely and efficient manner. The potential of low-energy sprayers was evaluated on leaves. Results showed promising deposition quantity, uniformity and efficiency results at low volumes (2000 to 4000 L/ha), lower spray pressures (15 bar) and adequate, efficient spraying speeds (1.9 to 2.9 km/h). 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). Distinct differences in deposition quality were observed between machinery used. The negative effects on deposition parameters as influenced by density of canopy were also shown in this trial. The denser a canopy, the harder it is to penetrate it with the spray mixture and the more uneconomical it is to spray. The effect of canopy density was also seen with field evaluation of adjuvants Break-Thru S240 and Break-Thru Union where spray application was generally improved in spray-friendly canopies, but also that super spreader adjuvants may negatively affect canopy penetration in dense canopies. From field spray trials it is also evident for the need of the development for a tree-row-volume sprayer calibration system, since canopy geometry and density clearly influences spray application dramatically. Certain objectives are being concluded and a final report will be submitted in 2015.

Opsomming

In Suid-Afrika word swamdoders teen medium dek-bespuitings (± 9 000 L/ha) aanbeveel om vrug- en blaarsiektes te beheer. ʼn Groot deel van oormatige spuitvolumes gaan egter verlore a.g.v afloop en drif wat tot aansienlike omgewingsbesoedeling van die grond en lug lei. Hierdie faktore saam met met onvoldoende kalibrasie, masjien- en toerustinggebruik, sowel as verkeerdelike en swak toedieningstegnieke, lei tot verlaagde spuit effektiwiteit en swak siektebestuur. Hierdie studie gebruik ʼn verbeterde, unieke spuit neerslag-assesseringsprotokol vir beter interpretasie van spuit deponeringsresultate asook drempelwaardes vir die aanduiding van biologiese doeltreffendheid van spesifieke deponeringshoeveelhede om verbeterde of kostebesparende spuit-toediening op blare te bestudeer. Na verskeie spuitproewe met verskillende masjiene by spuitvolumes vanaf 1 000 tot 24 000 L/ha, was dit duidelik dat die uitermate hoë spuitvolumes (> 10 000 L/ha), nie beter spuit neerslag op blare realiseer het nie. Soortgelyke en selfs beter spuitdeponering, eenvormigheid en

beter spuitdoeltreffendheidsvlakke kan d.m.v laer volume toedienings tesame met optimale gebruik van toerusting of deur die gebruik van meer doeltreffende spuit-tegnologie op blare verkry word. Tydens die afgelope seisoen is die protokol vir meting van bedekkingskwaliteit verbeter om variasie van bedekking op die blaaroppervlak akkuraat aan te dui. Die potensiaal van lae-energie spuitmasjiene is bestudeer op blare. Resultate dui op belowende deponeringskwantiteit, uniformiteit en spuit-effektiwiteit teen laer volumes (2000 tot 4000 L/ha), laer spuit-druk (15 bar) en spuit-spoed (1.9 tot 2.9 km/h). Klein verskille is waargeneem in bedekkingskwaliteit soos beiinvloed deur spuit volume. Laer spuit volumes het beter bedekkingskwaliteit tot gevol gehad terwyl hoër spuit volumes meer konstant was in herhaalbaarheid deur die boom lower. Duidelike verskille kon waargeneem word in bedekkingskwaliteit soos beïnvloed deur verskillende spuitmasjiene. Die negatiewe effekte van boomdigtheid is duidelik gewys, siende dat digter bome moeiliker penetreer word, en dus duurder is om effektief te spuit. Proewe met Break-Thru S240 en Break-Thru Union wys dat benatters ("super-spreaders") algemene spuitbedekking op blare in spuitvriendelike bome kan bevoordeel, maar penetrasie in digte bome kan benadeel. Hierdie proewe wys duidelik dat ŉ boom-ry-volume kalibrasie-sisteem ontwikkel moet word siende dat boom-vorm en -digtheid spuittoediening dramaties beïnvloed. Sekere doelwitte word nog afgehandel en ŉ finale verslag sal in 2015 ingedien word.

4.3.4 **PROGRESS REPORT**: **Control of *Botrytis cinerea* Pers. on lemons**

Project 1015 (April 2011 - March 2014) by G.C. Schutte, C. Kotze & P.H. Fourie (CRI)

Summary

An orchard evaluation of different fungides in the Sundays River Valley during September 2013 showed an excessive appearance of Botrytis-like ridges on lemon fruit which none of the treatments could prevent. This phenomenon can be attributed to application of excessive nitrogen. Conditions were also not favourable for the development of the disease. Lemon flowers sprayed with a fluorescent pigment in water showed poor coverage of all flower parts and should be investigated as soon as possible before new field trials are planned.

Opsomming

ŉ Veldproefbespuiting van drie swamdoders in die Sondagsriviervallei in September 2013 het ŉ uitermatige hoë voorkoms van Botrytis-agtige riwwe op suurlemoene gehad en geeneen van die swamdoderbespuitings kon dit verhoed nie. Uitermatige toedienings van stikstof word as rede hiervoor aangevoer. Toestande was ook nie gunstig vir die ontwikkling van die siekte nie. Suurlemoenblomme wat met ŉ fluoriserende pigment in water gespuit is, toon swak bedekking van alle blomdele en moet ondersoek word alvorens nuwe veldproewe bespuit word.

4.4 **PROGRAMME: SOILBORNE DISEASES**

Programme coordinator: M.C. Pretorius & J.M. van Niekerk (CRI)

4.4.1 **Programme summary**

Different projects within the soilborne diseases program address various soil related disease problems. Some projects are investigating more sustainable or softer chemicals to use in the management of *Phytophthora* and citrus nematode in the orchard; others are looking at *Phytophthora* resistant rootstocks while the different factors involved in citrus decline are also being research extensively. Apart from investigating soil-related industry problems, new diseases, with yet unknown etiology are also placed under the spotlight of research.

Two projects (762 and 1030) have been running for some time and in these projects alternative chemicals are evaluated with the aim of finding softer, less dangerous chemicals that are effective in managing citrus nematodes and *Phytophthora* in the orchard. In the evaluation of various pre-plant and post plant treatments (project 762) results that have been gathered since 2011 indicated that based on nematode juvenile counts in the soil, female counts in the roots and *Phytophthora* levels in the soil are indicating that a pre-plant treatment with Midas 50:50 are just as effective as MeBr fumigation in controlling nematodes and *Phytophthora*. In project 1030, various chemical, non-chemical and a new organic nematicide product from Bayer was evaluated also for nematode and *Phytophthora* control. For nematode control, the best treatments were the ones containing the new Bayer product. These trials are ongoing and final conclusions will be drawn later.

Having a *Phytophthora* resistant rootstock is seen as one of the most cost effective methods to manage root rot. In a project conducted at the University of Pretoria (UP_CRI1-09) a wide range of rootstocks were screened for

resistance along with any biochemical mechanisms that could be involved in resistance. The rootstocks were ranked with regards to *Phytophthora* resistance as tolerant, intermediately tolerant or susceptible to *Phytophthora*. Biochemical and statistical analyses identified three markers that may correctly classify tolerance / susceptibility in citrus rootstocks with > 90% accuracy.

Root disease related decline in South African citrus orchards are still poorly understood. Project 910 investigated the various edaphic factors, and their interactions, that might be involved in citrus tree decline. Two orchards displaying decline symptoms were identified and a total of 105 trees from these orchards were grouped in one of 4 different decline groups based on their visual decline symptoms. For each decline group various parameters were monitored for a period of two years. Intricate statistical analyses of the data indicated that several factors are involved in tree decline and that the interaction of these factors, rather than any single one, are at the root of the observed tree decline. Hopefully these indicators can be used in future to identify declining orchards at an early stage in order for proactive management strategies to be employed.

In a project aimed at the management of *Armillaria* root rot, two distinct diseases have been observed in orchards in the Kirkwood and Patensie areas of the Eastern Cape province. Isolations from diseased trees did not yield any *Armillaria* isolates, which led to the conclusion that the observed symptoms are possibly not caused by this pathogen. This project (1068) will now continue with extensive sampling of diseased trees displaying the different symptoms along with isolations from this material to determine which pathogens are involved in the soft wood rot seen in Kirkwood and the sudden decline seen in Patensie. Only once the etiology of the diseases has been established can management practices be developed.

Opsomming

Verskillende projekte binne die grondgedraagde siekte program spreek verskeie grondverwante siekte probleme aan. Sommige projekte ondersoek meer volhoubare of sagter chemiese middels om te gebruik in die bestuur van *Phytophthora* en sitrus nematode in die boord. Ander kyk na *Phytophthora* weerstandbiedende onderstamme terwyl die verskillende faktore wat betrokke is by sitrus agteruitgang ook intensief nagevors word. Apart van die ondersoek van grondverwante probleme in die industrie, word nuwe siektes met huidig onbekende oorsake, ook onder die soeklig van navorsing geplaas.

Twee projekte (762 en 1030) is al vir ʼn geruime tyd aan die gang. In hierdie projekte word alternatiewe chemiese middels geëvalueer met die doel om sagter, minder gevaarlike middels te vind wat effektief is in die bestuur van sitrus nematodes en *Phytophthora* in die boord. In die evaluering van verskeie voor-en na-plant behandelings (projek 762) het resultate wat sedert 2011 versamel is aangedui dat op grond van nematode tellings in die grond en wortels, en ook *Phytophthora* vlakke in die grond, die voor plant behandeling met Midas 50:50 net so effektief is as MeBr beroking in die beheer van nematodes en *Phytophthora*. In projek 1030 is verskeie chemiese, nie-chemiese en ʼn nuwe organiese nematisied van Bayer ook geëvalueer vir nematode en *Phytophthora* beheer. Nematode beheer was die beste in die behandelings wat die nuwe Bayer produk bevat het. Die proewe duur voort en finale gevolgtrekkings sal later gemaak word.

Phytophthora weerstandbiedende onderstamme word gesien as en van die mees koste effektiewe metodes om wortelvrot te bestuur. In ʼn projek wat by die Universiteit van Pretoria gedoen is (UP_CRI1-09), is ʼn wye reeks van onderstamme ondersoek vir weerstand tesame met enige biochemiese meganismes wat by weerstand betrokke kan wees. Die onderstamme is geklassifiseer ten opsigte van *Phytophthora* weerstand as tolerant, matig tolerant en vatbaar. Biochemiese en statistiese analise het drie merkers geïdentifiseer wat moontlik die toleransie/vatbaarheid van sitrus onderstamme met >90% akkuraatheid kan bepaal.

Wortelsiekte verwante agteruitgang in Suid-Afrikaanse sitrusboorde word nog swak verstaan. Projek 910 het die verskillende edafiese faktore, en hulle interaksies, wat moontlik by sitrus agteruitgang betrokke kan wees, ondersoek. Twee boorde wat agteruitgang simptome getoon het is geïdentifiseer en ʼn total van 105 bome uit hierdie boorde is geklassifiseer in een van 4 verskillende klasse op grond van hulle agteruitgang simptome. Vir elke klas is verskeie parameters vir ʼn periode van twee jaar gemonitor. In diepte statistiese ontleding van die data het getoon dat verskeie faktore betrokke is by die agteruitgaan van bome en dat die interaksie van hierdie faktore, eerder as enkele faktore, die oorsaak is van die waargenome boomagteruitgang. Hierdie indikators kan hopelik in die toekoms aangewend word om boorde wat agteruitgaan vroeg te identifiseer sodat proaktiewe bestuurstrategië toegepas kan word.

In ʼn projek gemik op die bestuur van *Armillaria* wortelvrot is twee duidelik verskillende siektes in boorde in die Kirkwood en Patensie areas van die Oos-Kaap provinsie waargeneem. Isolasies uit simptomatiese bome het geen *Armillaria* isolate opgelewer nie, wat tot die gevolgtrekking gelei het dat die waargenome simptome moontlik nie deur hierdie patogeen veroorsaak word nie. Hierdie projek (1068) sal nou voortgaan met intensiewe monsterneming van bome wat die verskillende simptome toon. Isolasies sal dan van hierdie materiaal gemaak word ten einde te bepaal watter patogene betrokke is by die sagte houtvrot waargeneem in Kirkwood en die skielike agteruitgang waargeneem in Patensie. Sodra die etiologie van die siektes vasgestel is, kan bestuurspraktyke ontwikkel word.

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 – 2014) by J.M. van Niekerk, M.C. Pretorius & 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 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 were 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, the best treatments since 2011 have been the MeBr and the two Midas 50:50 treatments. In terms of average percentage *Phytophthora* infested leaf discs resulting from soil baiting from treated soil, the best treatments were MeBr, the Midas 50:50 treatments and Telone. This indicates that these products are the best in reducing *Phytophthora* levels in the soil. From the tree measurement data no clear conclusion could be made as to the best treatment. Unfortunately no yield data is available yet, with the first harvest of the trial trees to occur in 2014. At this stage it seems as if the treatments with Midas 50:50 are just as good as the MeBr treatment. However, the trial will be monitored for at least another three years. Hopefully this will give a clear indication as to the best treatment that are cost effective and provide long term post plant freedom from *Phytophthora* and citrus nematodes.

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 sitrus aalwurm en *Phytophthora*. Die proef is al sedert 2010 aan die gang. Verskeie grondbehandelings is gedoen voor plant in Januarie 2010 terwyl sommige behandelings jaarliks in Januarie en November toegedien word. Parameters wat jaarliks gemonitor is sedert die begin van die proef sluit in stam deursnee, boom hoogte, nematode tellings in die grond en wortels, *Phytophthora* status in die grond en ʼn visuele boom gradering. Die MeBr en twee Midas 50:50 behandelings was sedert 2011 die drie beste behandelings op grond van nematode tellings in die grond en boomwortels. Behandelde grond is ontleed met behulp van die grondlokaas metode. Die gemiddelde persentasie *Phytophthora* besmette blaarskyfies het aangedui dat die beste behandelings die MeBr, die Midas 50:50 en Telone behandelings was. Hierdie behandelings was dus die beste om die vlakke van *Phytophthora* in die grond te verminder. Die boommetings wat gedoen is het nie duidelik aangedui watter behandelings die beste is nie. Ongelukkig is nog geen opbrengsdata beskikbaar nie met die bome wat die eerste keer in 2014 geoes sal word. Op die oomblik kom dit voor asof die behandelings met Midas 50:50 net so goed is soos die MeBr. Die proef sal egter vir minstens nog drie jaar gemonitor word. Dit sal dan hopelik duidelik wees watter behandeling is die beste en mees koste effektief om grond langduring na plant vry te hou van *Phytophthora* en sitrus nematodes.

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

Project 1030 (2008 – 2015/2016) by J.M. van Niekerk, M.C. Pretorius & 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 evaluated: Nontox–Silica (silica), Biolan (ZZ2, Product), CRI nematode egg stimulating product (DL), a combination of a nematode egg stimulating product with a nematicide (DL+), standard nematicide application (cadusaphos; Rugby), Messenger, Orosoil, Foodprint and Diatomied. A new organic nematicide from Bayer (BCS AR 83685 SC 500) was also included. Based on the results from the three samplings done, the best treatments in reducing juvenile and female nematode counts were the treatments containing the Bayer product. The harvest data from 2013 unfortunately did not support the nematode data. The harvest data indicated that treatments that performed poorly in terms of nematode control gave the best results. This is possibly due to some products having a physiological effect on the treated trees which led to good yield results despite poor nematode control. No clear conclusion as to the best nematode treatment could therefore be made. The glasshouse trial, testing *Phytophthora* control agents, from 2012/2013 need to be repeated before a field trial can be established.

Opsomming

Die sitrus nematode, *Tylenchulus semipenetrans*, infekteer sitrus wêreldwyd en is die volopste plantparasitiese nematode in sitrus boorde. Die gebruik van toksiese nemtisiedes kom toenemend plaaslik en internasionaal onder druk. Ontwikkeling van alternatiewe tot chemiese nematisiedes is dus noodsaaklik. Produkte wat gevalueer is sluit in Nontox–Silica (silika), Biolan (ZZ2, produk), CRI nematode eier stimulerende produk (DL), ŉ kombinasie van ŉ nematode stimulerende produk met ŉ nematisied (DL+), standaard nematisied toediening (cadusaphos; Rugby), Messenger, Orosoil, Foodprint en Diatomied. ŉ Nuwe organisiese nematisied van Bayer (BCS AR 83685 SC 500) is ook ingesluit. Gegrond op resultate verkry uit die drie monsternemings tydens die proef, was die beste behandelings ten opsigte van nematode tellings die behandelings waar die Bayer produk toegedien is. Ongelukkig het die oesdata van 2013 nie die nematode data ondersteun nie. Die oesdata het getoon dat die behandelings wat swak resultate gelewer het in terme van nematode beheer, die beste vertoon het in terme van die oesdata. Dit kan moontlik toegeskryf word aan die produk wat ŉ fisiologiese effek het op die boom wat lei tot goeie oesopbrengs ten spyte van swak nematode beheer. ŉ Duidelike gevolgtrekking aangaande die beste nematode behandeling kon dus nie gemaak word nie. Die glashuisproef van 2012/2013, waarin *Phytophthora* beheeragente getoets word, moet eers herhaal word voordat ŉ veldproef met die middels kan begin.

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 J.M. van Niekerk, M.C. Pretorius and C. Kotze (CRI)

Summary

Two symptomatically distinct diseases were observed in orchards in the Eastern Cape. These orchards were located in the Patensie and Kirkwood areas. The one disease is characterised by a soft wood rot of the trunks and/or roots with the tree falling over. The fallen trees sometimes continue growing and bearing fruit indicating that only a part of the trunk and possibly root system is affected. The other disease observed is characterised by a rapid decline and death of affected trees. This decline has been described as being so rapid that it almost happens overnight. The initial idea was that these diseases are caused by *Armillaria* spp. however; more than 400 isolations made from material from symptomatic trees yielded no *Armillaria* isolates. Study of the symptom pictures by an *Armillaria* expert also led to the conclusion that the observed symptoms are possibly not caused by *Armillaria* spp. The etiology of the two diseases is therefore at the moment unknown. Extensive sampling and isolation will now be undertaken from trees expressing the different symptoms in order to identify the pathogens involved. Once the causal organisms have been identified control strategies will be developed.

Opsomming

Twee siektes wat simptomaties duidelik verskillend is, is in boorde in die Oos-Kaap waargeneem. Hierdie boorde was in die Patensie en Kirkwood areas. Die een siekte word gekenmerk deur ŉ sagte houtverrotting in die stam en of wortels met die boom wat dan omval. Die omgevalle bome hou soms aan met groei en vrugte dra wat aandui dat net ŉ deel van die stam of wortels geaffekteer is. Die ander waargenome siekte word gekenmerk deur ŉ vinnige agteruitgang en vrek van die aangetasde boom. Hierdie agteruitgand is beskryf dat dit so vining gebeur dat dit amper oornag plaasvind. Aanvanklik was die idee dat hierdie siektes deur *Armillaria* spp. veroorsaak word. Meer as 400 isolasies is egter gedoen uit materiaal versamel van simptomatiese bome en geen *Armillaria* isolate kon verkry word nie. Die simptoomfotos is ook deur ŉ *Armillaria* kenner bestudeer wat ook tot die gevolgtrekking gekom het dat die simptome moontlik nie deur *Armillaria* spp. veroorsaak is nie. Die etiologie van die twee siektes is dus op die oomblik onbekend. Uigebreide versameling en isolasies sal nou gedoen word van bome wat die verskillende simptome toon ten einde die veroorsakende patogeen te identifiseer. Wanneer die oorsakende patogene geïdentifiseer is sal beheermaatreëls ontwikkel word.

4.4.5 PROGRESS REPORT: Investigation into edaphic factors and their interactions on citrus tree decline

Project 910 (2008 – 2013) by M.C. Pretorius, C. Kotze (CRI) & A. McLeod (SU)

Summary

Symptoms associated with root disease-related citrus decline include sparse foliage, twig die-back and reduced growth, yield and fruit size as well as root rot. The causal factors involved in root disease related decline have not yet been fully elucidated. Invariably tree decline is noticed too late for the implementation of **Summ**preventative management strategies. The aims of the study are to elucidate biotic and abiotic factors associated with citrus decline. Two declining orchards were selected for the study. Based on visual decline ratings of tree canopies, trees from three to four decline categories were selected. For each tree category several parameters were measured including yield, soil and leaf characteristics, soilborne pathogens and root disease associated symptoms. Multivariate analyses indicated that the two orchards were distinct. Subsequent analyses were therefore conducted separately for the two orchards. Distinct groups were identified for each tree category, indicating that most trees were visually correctly selected. Principal component analyses (PCA) indicate a very small difference within the sites among the different categories but the changes appear in chronological order according to the level of decline despite the differences between the 2 sites. The same parameters were concerned in the 2 sites such as clay content for the healthy trees and silt, %P and Mo for the declining trees. However none of these parameters were directly responsible for the decline due to the fact that it was shown along the lesser F2 (vertical) axis which is only described by about 11% of the total variability but collectively most of these parameters are related to the decline process. Several of these indicator characteristics identified in the study can now be utilised to determine the initial process of decline when the tree characteristic will change even before any decline symptoms are visual in the orchard.

Opsomming

Sitrusbome met agteruitgang simptome lei tot groot verliese tov vruggroottes, oes opbrengste, tak terugsterwing asook wortelvrot. Die doel van hierdie projek was om abiotiese en biotiese faktore en hul interaksies te identifiseer wat moontlik tot sitrusagteruitgang kan lei. ŉ “Multi-parameter” model is gebruik om resultale wat oor twee seisoen gemonitor is, te analiseer. Twee boorde met bome wat agteruitgang simptome getoon het, is volgens hul lower dighthede in 3-4 katagorieë onderverdeel. Verskeie parameters is per kategorie gemonitor; hierdie sluit in grond- en blaareienskappe, grondgedraagde patogene en wortelvrot geassosieerde simptome. Analise van die al die parameters het getoon dat die twee persele van mekaar verskil en is daarvolgens as aparte eenhede ontleed. Afsonderlike groeperings kon uit die ontledings gevorm word en dui dat die boom klassifikasie korrek gedoen was. Alhoewel die resultate getoon het dat daar ŉ baie klein verskil tussen die kategorieë is, kom die verskille wel kronologies van aard voor en volg dit die graad van agteruitgang. Parameters wat uitgestaan het by gesonde bome was ŉ hoër klei inhoud persentasie, terwyl slik inhoud, % P en Mo uitgestaan het by bome wat agteruitgang toon. Hierdie faktore is egter nie alleenlik verantwoordelik vir agteruitgang nie, maar dra egter by tot die proses. Hierdie verskille was langs die vertikale as van die faktoriale plan (F2) gelokaliseer en het 11% van die totale veranderlikheid bygedra. Deur gebruik te maak van hierdie bydraende faktore, kan agteruitgang in die toekoms moontlik meer akkuraat bepaal word nog voordat die visuele simptome waargeneem kan word.

4.4.6 FINAL REPORT: Rootstock resistance against *Phytophthora nicotianae* root rot

Project UP_CRR1-09 by N. Labuschagne, Z. Apostolides & M. Sakupwanya (UP)

Summary

Resistant rootstocks are one of the most cost effective ways of combating Phytophthora root rot of citrus. The main aims of the current project were screening of a wide range of rootstocks for tolerance, identify metabolite marker compounds for resistance and elucidating the biochemical mechanisms involved in resistance / tolerance of citrus rootstocks against Phytophthora root rot. More than eight separate greenhouse experiments have been conducted over three seasons to assess the relative tolerance of 13 citrus rootstocks to Phytophthora root rot. The aim of the screening experiments was to rank the rootstocks according to their response to *P. nicotianae*, that being tolerant, intermediate or susceptible. The greenhouse results were used to cross-check results from a metabolomics study to confirm whether the clustering of rootstocks during the multivariate analysis (Principle Component Analysis) corresponded with the tolerance rankings in the greenhouse trials. Root samples from selected experiments have been systematically analysed by means of thin layer chromatography (TLC), inhibition bioassays against an indicator fungus and the pathogen, as well as Ultra-High Performance Liquid Chromatography – Mass Spectroscopy (UPLC-MSe) to determine the phytochemical profiles in the various rootstocks. Inhibitory bioassays on TLC plates using *Cladosporium* as indicator fungus indicated that citrus roots contain constitutive compounds with toxicity against certain fungi. However, crude extracts from some of the samples did not inhibit *P. nicotianae* on potato dextrose agar during *in vitro* challenge plate bioassays. To identify biochemical markers for tolerance / susceptibility, root extracts from 13 uninfected rootstocks from a greenhouse experiment were analysed by means of UPLC-MSe, with results indicating 32 potential markers for tolerance among all cultivars (i.e. constitutive compounds). From the 13 uninfected samples, extracts of eight rootstocks that consistently produced a tolerant response either from this study or the literature (including Benton citrange, Flying Dragon, Swingle citrumelo and Terra Bella citrange) or a susceptible response (including Carizzo citrange, Rough lemon, Volkameriana and X639-hybrid) produced 366 markers. The best markers were selected through a three-step process during which markers were to meet three specific conditions. A total of 22 markers met all three conditions and were accepted as potential markers that may distinguish between susceptible and tolerant rootstocks. Multivariate principle component analysis was performed on the 22 marker compounds revealing clustering of metabolite markers according to tolerance and susceptibility. Step-forward discriminant analysis narrowed this down to three markers that may correctly classify tolerance / susceptibility in citrus rootstocks with > 90% accuracy. Following further validation, these markers may potentially be used in a high throughput protocol assisting future breeding programs to select for Phytophthora tolerance. The current study succeeded in identifying marker compounds associated with tolerant citrus rootstocks up to the level of the compounds' mass spectrum features. The metabolomics approach applied during this study constitutes a novel approach to the study of mechanisms of resistance in citrus rootstocks, and has opened the way for characterisation of these marker compounds in future. Although the original objective of elucidating the biochemical mechanism(s) of resistance has not been fully achieved, a solid foundation has been laid for an in-depth metabolomics study of resistance in citrus rootstocks.

Opsomming

Weerstandbiedende onderstamme is een van die mees koste effektiewe maniere om Phytophthora wortelvrot van sitrus te bestry. Die hoof doelstellings van hierdie projek was die assessering van ŉ wye reeks onderstamme vir weerstandbiedendheid, identifisering van metaboliet-merkerverbindings vir weerstandbiedendheid en bepaling van die biochemiese meganismes betrokke by onderstam weerstandbiedendheid teen Phytophthora wortelvrot. Meer as agt afsonderlike glashuisproewe is oor drie seisoene uitgevoer om die relatiewe weerstandbiedendheid van 13 sitrus onderstamme teen Phytophthora wortelvrot te evalueer. Die doel van die siftingsproewe was om die onderstamme in ŉ rangorde te plaas van vatbaar, intermediêr en weerstandbiedend. Hierdie glashuis resultate is gebruik om resultate van die "metabolomics" studie te bevestig, nl. om vas te stel of die groeperings van onderstamme tydens die multiveranderlike analise (prinsiep komponent analise) ooreenstem met die weerstandsrangorde in die kweekhuisproewe. Wortelmonsters van geselekteerde eksperimente is sistematies geanaliseer d.m.v. dunlaag-chromatografie (DLC), inhibisie-assessering teen ŉ indikator swam en *P. nicotianae* sowel as Ultra-Hoë-Uitset-Vloeistofchromatografie-Massaspektrometrie (UHVM) om die fitochemiese profiele in die onderskeie onderstamme te bepaal. Inhibisie-assessering met *Cladosporium* as indikator swam op dunlaag-chromatografie-plate het aangedui dat sitrus wortels vooraf bestaande verbindings bevat wat toksies is teen sekere swamme. Kru-ekstrakte van sekere van die monsters het egter nie *P. nicotianae* geïnhibeer tydens *in vitro* assessering op aartappel dekstrose agar nie. Om biochemiese merkers vir weerstandbiedendheid /vatbaarheid te identifiseer, is wortelekstrakte van 13 ongeïnfekteerde onderstamme

vanaf ʼn kweekhuisproef geanaliseer d.m.v. UHVM. Resultate het 32 potensiële merkers vir weerstandbiedendheid aangetoon binne die onderstamme (m.a.w. voorafbestaande verbindings). Vanuit die 13 ongeinfekteerde monsters het ekstrakte van agt onderstamme wat konsekwent ʼn weerstandbiedende reaksie getoon het in die huidige studie of volgens die literatuur (insluitend Benton citrange, Flying Dragon, Swingle citrumelo en Terra Bella citrange) of ʼn vatbare reaksie (insluitend Carizzo citrange, Growweskil suurlemoen, Volkameriana en X639-hibried) het 366 merkers opgelewer. Die beste merkers is geselekteer d.m.v. ʼn drie-stap proses waartydens merkers aan drie spesifieke kondisies moes voldoen. ʼn Totaal van 22 merkers het aan al drie kondisies voldoen en is aanvaar as potensiële merkers wat tussen vatbare en weerstandbiedende onderstamme kan onderskei. Multiveranderlike prinsiep komponent analise is uitgevoer op die 22 merkerverbindings. Dit het aangetoon dat merkers gegroepeer het volgens weerstandbiedendheid en vatbaarheid. Voorwaartse diskriminant-analise het hierdie verfyn tot drie merkers wat weerstandbiedendheid of vatbaarheid in sitrusonderstamme met > 90% akkuraatheid kan klassifiseer. Met verdere toetsing kan hierdie merkers potensieël in ʼn hoë-uitset protokol gebruik word wat toekomstige telingsprogramme vir Phytophthora weerstandbiedendheid sou kon ondersteun. Hierdie studie het daarin geslaag om merker-verbindings geassosieer met weerstandbiedende sitrus onderstamme te identifiseer tot op die vlak van die verbindings se massa-spektrum kenmerke. Die “metabolomics” benadering wat tydens hierdie studie toegepas is, verteenwoordig ʼn nuwe benadering tot die studie van weerstandsmeganismes in sitrusonderstamme en het die weg gebaan vir karakterisering van hierdie verbindings in die toekoms. Alhoewel die oorspronklike doelwit om die biochemiese meganismes van weerstandbiedendheid te ontrafel nie ten volle bereik is nie, is ʼn soliede grondslag gelê vir ʼn in-diepte “metabolomics” gebaseerde ondersoek van weerstandbiedendheid in sitrus onderstamme.

Introduction

The most serious soilborne diseases of citrus trees are caused by *Phytophthora* species (Timmer and Menge, 1988). *Phytophthora nicotianae* and *P. citrophthora* are responsible for fibrous root rot and gummosis, which are both devastating diseases causing major losses in production world-wide (Afek and Sztejnberg, 1990). In South Africa, *Phytophthora nicotianae* is the most important pathogen for root rot disease (Thompson *et al.*, 1995; Meitz-Hopkins *et al.*, 2014). *Phytophthora* diseases can be controlled by various methods such as cultural practices and chemical control, but these measures are expensive and difficult to sustain. Rootstock resistance remains the most effective and economical way of combating *Phytophthora* root rot in citrus. Planting of resistant rootstocks is one of the best ways to prevent root rot of citrus (Afek and Sztejnberg, 1990). Unfortunately, no rootstocks that are 100% immune to *Phytophthora* exist (Castle, 1987). Tolerance may be related to the accumulation of phytoalexins that suppress infection or disease escape through re-growth of new roots (Graham, 1995). The ultimate objective in control of *Phytophthora* root rot is to replace the currently existing rootstocks with those of greater tolerance/ resistance to the disease. The overall aim of the current study is to elucidate the biochemical mechanisms of resistance in tolerant citrus rootstocks and identify metabolite markers that are positively or negatively correlated with tolerance to assist in selection of tolerant varieties.

The biochemical basis for disease resistance in plants can be based on constitutive (pre-formed) and infection induced secondary metabolites with anti-microbial activity (Hammerschmidt, 1999). Determining the role and function of these groups of defense related metabolites is increasingly studied through the application of metabolomics based approaches (Fernie and Schauer, 2008; Hadacek, 2002; Hall, 2011). Secondary metabolites related to resistance are infection induced metabolites and such markers are best assessed during the first few days to two weeks of infection (Afek and Sztejnberg, 1988). Possible biochemical metabolites for resistance / tolerance are typically phytoalexins (Hammerschmidt, 1999) which accumulate or are over expressed in plants in response to infection and such metabolites have been assigned the term pathogenicity related metabolites (Kumaraswamy *et al.*, 2011). Identifying biochemical markers for resistance / tolerance is best achieved through assessing constitutive secondary metabolites associated with tolerant cultivars. This approach will yield ‘resistance related metabolites’, which are secondary metabolites detected in higher concentrations in resistant plants as opposed to susceptible plants (Kumaraswamy *et al.*, 2011). The best chromatography available today for secondary metabolite analysis is based on metabolomics tools such as Ultra-High Performance Liquid Chromatography (UPLC) coupled to Mass Spectroscopy instrumentation and associated software including biomathematical data analysis and online database libraries (Patti *et al.*, 2013). Coupling of UPLC with mass spectrometry instrumentation in a non-targeted metabolomics approach provide data that can be analysed by multivariate analytical or biomathematical procedures. This will lead to discovery of metabolite markers that correlate with tolerance / susceptibility. Once the markers have been identified and putative compound names assigned, subsequent steps include comparisons with historical data on cultivar

response to a pathogen infection along with validation bioassays and use of standards to confirm marker identity (Steinfath *et al.*, 2010; Patti *et al.*, 2013).

Previous studies in our laboratories have shown that scoparone, a phytoalexin implicated in the defense response of citrus cultivars to *P. citrophthora* infection (Afek and Sztejnberg, 1988), does not have a role in the defense response of citrus against *P. nicotianae* (Aucamp *et al.*, 2000). Fourie (2004) determined a correlation between resistance and total phenolics. Here we report on the metabolomics analysis that targeted 'constitutive resistance metabolites' which are secondary metabolites detected in higher concentrations in resistant plants as opposed to susceptible plants.

Objectives

During the course of this study it became apparent that the original objectives 2 and 3 are almost inseparable. Therefore we combined these into one objective with sub-objectives:

1. To screen a wide range of rootstocks for resistance / tolerance against *Phytophthora nicotianae*.
2. a) To determine whether a consistent correlation exists between any phytochemical compound and rootstock tolerance / resistance.
   b) To determine whether any of the identified phytochemicals can be used as marker compounds for resistance in rootstocks and to develop a rapid screening method for rootstock resistance.
   c) To elucidate the biochemical mechanisms involved in resistance.

3. To assess the importance of root growth potential / root regeneration in rootstock tolerance against root rot.

Materials and methods

1. Screening of rootstocks for resistance / tolerance against Phytophthora nicotianae.

The citrus cultivars evaluated in the greenhouse against *P. nicotianae* are listed in Table 1.

Table 4.4.6.1. Citrus rootstocks evaluated for tolerance/susceptibility against *P. nicotianae* root rot during this study.

ROOTSTOCK	ABBREVIATION
X639-hybrid	X639
Benton citrange	BC
Cairn Rough lemon	CRL
Carrizo citrange	CC
C-35 citrange	C-35
Cleo x Swingle citrumelo	CxS
Esselen Rough lemon	ERC
Minneola x Trifoliate	MxT
Volkamer lemon	VOLK
Flying Dragon	FD
Swingle citrumelo	SwC
Sunki X Benecke	SxB
Terra Bella citrange	TB
Yuma citrange	YC

Plants

Seed from Citrus Research Internationals' Foundation Block (Uitenhage, South Africa) were germinated in containers (44 x 190 x 20 cm) filled with sterile vermiculite under growth cabinet conditions at 28°C and a 12 hour light dark cycle. The growth medium was kept moist by watering with sterile water. Seedlings were fertilized fortnightly with a water soluble fertilizer (6-1-4, N-K-P, 1g/L, Hygrotech). Four months post sowing, all plants were moved from the growth cabinets to a greenhouse. Seedlings were transplanted into 15 cm plastic pots

containing heat pasteurized sand/peat potting mixture (3:1 v/v) and maintained under greenhouse conditions until inoculation with the pathogen. Plants were watered twice weekly with the inclusion of the water soluble fertilizer once a fortnight.

Pathogen inoculum preparation

P. nicotianae was isolated from citrus orchard soils (Nelspruit) by means of the citrus leaf-disk method. Virulence was confirmed by infecting citrus seedlings in a hydroponics system. The *P. nicotianae* isolate was grown in pure culture on clear V8 juice agar plates. Millet seed inoculum was prepared as previously described by Fourie (2004). Briefly, autoclave bags containing 200g millet seed plus 100 ml distilled water were triple autoclaved, inoculated with *P. nicotianae* and incubated for 4 weeks at room temperature in the dark.

Inoculation procedure

The citrus seedlings growing in sand/peat mixture were transplanted into soil/sand (2:1 v/v) medium augmented with either *P. nicotianae* millet seed inoculum (5% v/v) or sterile millet seed (5% v/v) i.e. controls.

Treatments

For each cultivar an uninoculated control was included as well as a pathogen inoculated treatment with at least four replicates (one pot containing one plant representing one replicate). The experimental design was a completely randomised (block less) design. Soils were kept moist throughout the experiment to provide favourable conditions for infection.

Evaluation

Plants were harvested 8 weeks after inoculation by gently removing them from their pots, rinsing the soil from the roots in running tap water, then excising the roots from the stems followed by weighing. Disease severity was determined by means of a root rot assessment according to a rating scale of 0 to 4 where 0 = no sign of root rot, resistant response; 1 = 25% root rot, tolerant response; 2 = 50% root rot, intermediate response; 3 = 75% root rot and 4 = 100% root rot susceptible response. After the assessments all plant roots were transferred to a -20°C freezer for storage prior to conducting biochemical analysis. Rank transformation of raw data was performed prior to one-way analysis of variance (ANOVA).

2. a) To determine whether a consistent correlation exists between any phytochemical compound and rootstock tolerance / resistance.

b) To determine whether any of the identified phytochemicals can be used as marker compounds for resistance in rootstocks and to develop a rapid screening method for rootstock resistance.

c) To elucidate the biochemical mechanisms involved in resistance.

Biochemical analysis of roots:

Extraction procedure

Frozen root samples from the pathogen inoculated and uninoculated (controls) rootstock cultivars from the soil/sand 2012 experiment were removed from freezer storage, flash frozen in liquid nitrogen and crushed to a powder. Subsequently 0.5 g powder was transferred to test tubes and 3 ml ethyl acetate-ethanol (1:1) added to the tubes (Chong *et al.*, 2006). Tubes were plugged, vortexed for 30 s and extraction conducted overnight in the dark at room temperature. After 24 hrs the extract was recovered from the tubes and transferred to clean, labelled tubes before being evaporated. Resultant residues were resuspended in 0.5 ml (500 µl) ethanol to produce crude extracts representative of 0.5 g (500 mg) plant material. Aliquots of the crude extracts were then prepared as in Figure 1 for biochemical analysis.

UPLC-MS and data analysis

The extracts were kept frozen at -20°C until UPLC/MS analysis on a Waters Synapt 2 instrument with MassLynx software. Two cocktails of commercially (Sigma) available flavonoid standards were prepared. The cocktails were injected before and after every eight samples, as technical repeats to confirm the stability of the LC/MS system. The samples were analysed over a period of five consecutive days to minimize process variance.

Ion-features (retention time and molecular mass data) from uninoculated and inoculated plant root extracts were analysed with the MarkerLynx software. Excel was used to calculate descriptive and inferential statistics of the markers. Multivariate analysis was undertaken with MassLynx to identify the markers that clearly distinguish between tolerant and susceptible cultivars from uninoculated samples. Statistical analysis of the features was done by principal component analysis (PCA). The features that correlated with tolerance were identified by Step-forward Discriminant Analysis. These features are currently being identified by data mining of the online databases, METLIN (Scripps Center for Metabolomics, USA) and MetFrag (Metabolite Fragments, Germany) using the KEGG, ChemSpider or PubChem databases.

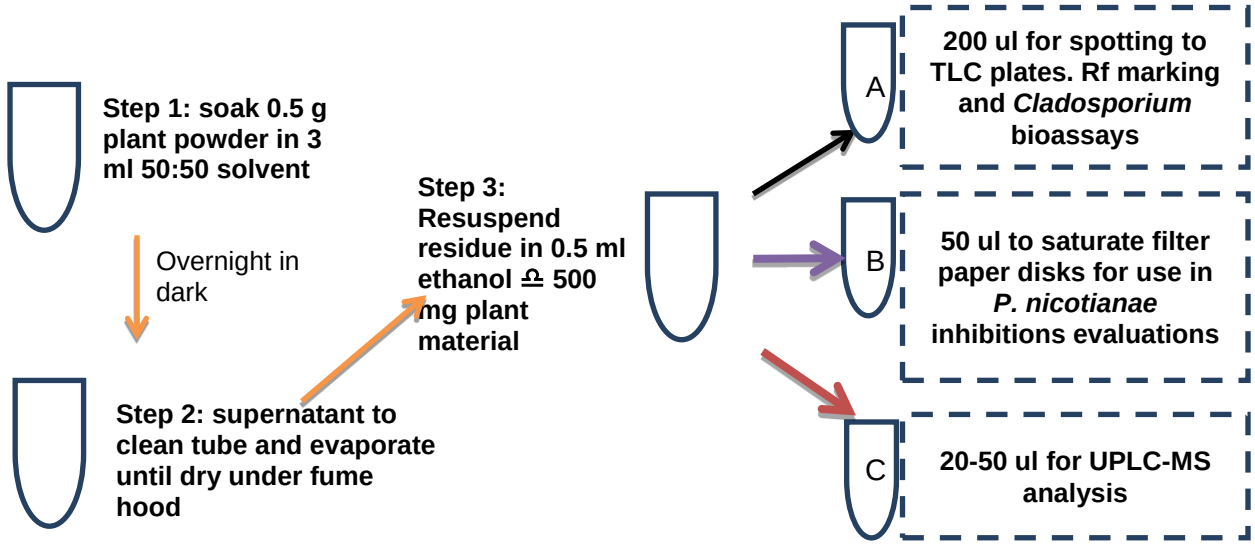

Figure 4.4.6.1. Flow chart of extraction steps. Remaining extracts were re-dried and stored at -20°C.

3. To assess the importance of root growth potential / root regeneration as a marker for in rootstock tolerance against root rot.

The first experiments to assess root regeneration of rootstocks were designed to test root regeneration under conditions of disease pressure, i.e. root regeneration was assessed on pathogen inoculated plants. These experiments were, however, inconclusive due to poor quality seedlings and the fact that root tips of vigorous rootstocks rapidly became infected. Root regeneration experiments were repeated in the absence of the pathogen by assessing root regeneration on pathogen-free plants of which 50% of the root systems were pruned off. Experiments were conducted in sand/peat growth medium using 10 replicate plants per treatment in the green house. Percentage root growth potential was calculated according to the formula A-B/A where A=dry weight of cut root system, B= dry weight of uncut root system.

Results and discussion

1. Screening of rootstocks for resistance / tolerance against Phytophthora nicotianae.

Results indicate that although there were significant interactions between rootstocks, Duncan's multiple range test indicated that there are narrow differences in the response of the rootstocks to the pathogen. In our experiments the different potting mixtures appear to be a factor in cultivar response to infection particularly with Rough lemon and Volkamer lemon, which produce a susceptible response in soil/sand potting mixtures. In the 2012 soil/sand experiment CC, VOL, SxB, C+S, FD and CRL produced a susceptible response which was significantly different to BC, ERL, SwC and TB which showed a tolerant response to infection. MxT and C-35 were not significantly different from BC and ERL. Although FD produced a susceptible response in this experiment, the rootstock is typically reported as tolerant (Grosser *et al.*, 1992). Ranking the rootstocks using Duncan's multiple range test indicate that rootstocks previously determined as tolerant in previous screening experiments (with exception of the 2012 exp. indicated in Table 4.4.6.3) were similarly tolerant in this instance.

TB, FD, AT, BC and SwC were significantly more tolerant than CRL ranked here as susceptible. In this case SxB is noted as having an intermediate response to *P. nicotianae* infection.

Root samples from the 2012 soil/sand experiment described above were used for biochemical analysis to determine constitutive and induced resistance related phytochemicals as described below.

Table 4.6.6.2. Summary of the response of different citrus rootstock cultivars to *Phytophthora nicotianae* in the green house obtained in the current study.*

Potting Mixture & Year of Experiment	**Sand/Peat 2011**	**Sand/Peat 2012**	**Soil/Sand 2011**	**Soil/Sand 2012**	**Soil/Sand 2013**
Rootstock	**Response**	**Response**	**Response**	**Response**	**Response**
Australia trifoliate	I-T	NA	T	NA	T
Benton citrange	I	T	T	I-T	T
Carrizo citrange	S	S	S	S	NA
C35-citrange	I-T	I	T	I	NA
C-Rough lemon	I-T	I-T	S	S	S
Flying Dragon	I-T	I-T	NA	S	T
MxT	I-T	I-T	T	I	NA
SxB	I-T	I-T	S	S	S
Swingle citrumelo	I	I-T	T	T	T
Terra Bella citrange	I	T	T	T	T
Troyer citrange	S	S	S	NA	NA
Volkamer lemon	I-T	I-T	S	S	NA
X639-hybrid	I	S	S	I	NA
Yuma citrange	I	NA	T	NA	NA

S= susceptible response; I= intermediate response; T= tolerant response. NA= not available for the season. For soil/sand 2012, two cultivars CxS and ERL omitted having been available and evaluated for one season.
*Although some of this data has been shown in previous reports, the current table is intended as a summary of the various experiments in order to enable cross-checking with metabolomics data.

Table 4.4.6.3. Summary of screening trails evaluating citrus rootstock tolerance against *Phytophthora nicotianae* under greenhouse conditions with different pathogen inoculation methods and potting mixtures.

	Soil/sand +millet seed inoc.			**Sand/peat+ millet seed inoc.**			**Soil/sand +millet seed inoc.**			**Sand/peat zoospore-drench inoc.**		
Plant material	3 m.o.seedl.			6 m.o.seedl.			5 m.o.seedl.			9 m.o seedl.		
	C*	R*	Rwr	C*	R*	Rwr	C*	R*	Rwr	C*	R*	Rwr
Australian trifoliate	***/***			Tol	**0.8**	12%	Tol	**1.3**	39%	Tol	**0.7**	-21%
Benton citrange	Int	**2.3**	58%	Int	**1.9**	31%	Tol	**1.5**	35%	Tol	**1.2**	-41%
C-35 citrange	Int	**2.0**	0%	Tol	**1.3**	41%	Tol	**1.1**	23%	Tol	**0.5**	12%
Carrizo citrange	Sus	**3.0**	68%	**Sus poor con.**			Sus	**2.8**	58%	Sus	**2.6**	-12%
Cairn rough lemon	***/***			Tol	**1.4**	20%	Sus	**2.6**	41%	Sus	**3.5**	41%
Flying dragon	Tol	**1.6**	47%	*Tol	**0.6**	5%	***/***			***/***		
Minneola X Trifoliate	Int	**2.5**	45%	Tol	**1.3**	23%	Tol	**1.3**	40%	Int	**1.9**	-7%
Sunki X Benece	Sus	**2.6**	54%	Tol	**1.4**	5%	Sus	**3.0**	57%	Int	**1.7**	-6%
Swingle citrumelo	Tol	**0.8**	17%	Int	**1.6**	38%	Tol	**1.0**	35%	Int	**2.3**	30%
Tera Bella citrange	Tol	**0.5**	41%	**Poor seedlings**			Tol	**0.8**	42%	Int	**1.7**	49%
Troyer citrange	Sus	**3.1**	53%	**Sus poor con.**			Sus	**2.8**	41%	Int	**2.2**	20%
Volckameriana	Sus	**2.5**	35%	Tol	**1.0**	0%	Sus	**2.5**	17%	Sus	**3.1**	37%
X639	Sus	**2.6**	34%	Int	**2.7**	12%	Sus	**2.3**	56%	Sus	**3.0**	-29%
Yuma citrange	***/***			Int	**1.8**	19%	Tol	**0.8**	48%	Int	**1.8**	-6%

C* represents susceptibility rating category where Tol=tolerant; Sus=susceptible; Int=intermediate;
R* indicate root rot rating.
Rwr indicate % root weight reduction.
/ indicate not included in experiment.

Table 4.4.6.4. Effect of *Phytophthora nicotianae* on fresh root weight of inoculated and uninoculated citrus rootstocks 8 weeks after inoculation with millet seed inoculum of the pathogen in soil / sand growth medium in the greenhouse. The table indicate % reduction in root weight as well as statistical significance of this comparing inoculated with uninoculated plants within each rootstock.

	Root weight (g)			
Rootstock	Inoculated	Control	*P*	% Root Weight reduction
Australian trifoliate	7.6	12.4	0.0057 **	39%
Benton citrange	9.0	13.9	0.0180 *	35%
C-35 citrange	10.9	14.1	not significant	23%
Carrizo citrange	7.4	17.9	0.0004 ***	58%
Cairn rough lemon	18.8	32.2	0.007 **	41%
Minneola X Trifoliate	15.4	25.5	0.0128*	40%
Sunki X Benece	4.3	10.1	0.0042**	57%
Swingle citrumelo	14.4	22.1	0.01*	36%
Troyer citrange	5.5	9.3	not significant	41%
Volckameriana	17.8	21.5	0.075*	17%
X639	5.5	12.6	0.0009***	56%
Yuma citrange	9.7	18.6	0.035*	48%

T-test *** *P, <0.0001*; ** *P, <0.001*; * *P, <0.05*; *P> 0.5* not significant. (analysis of Soil/sand +millet seed inoculum).

No clear correlation was observed between root rot rating and reduction in root fresh weight (Table 4.4.6.3). In this context the ability of rootstocks to regenerate roots should be taken into account. Also, a high % reduction of root fresh weight was not necessarily significant within a rootstock cultivar (Table 4.4.6.4).

Based on the current data, the ranking of rootstocks according to susceptibility or tolerance corresponds to a large extent with the findings of Burger (2001, MSc dissertation, University of Pretoria) on most but not all of the cultivars. It should be noted that various researchers from different countries have reported different, sometimes conflicting findings regarding rootstock tolerance or susceptibility, as summarised by Burger (2001). Variations in ranking in the current study may also stem from the different inoculation methods applied (e.g. millet seed v zoospores), with repeat experiment in the following season expected to yield more raw data for analysis and comparison.

2. a) To determine whether a consistent correlation exists between any phytochemical compound and rootstock tolerance / resistance.

b) To determine whether any of the identified phytochemicals can be used as marker compounds for resistance in rootstocks and to develop a rapid screening method for rootstock resistance.

c) To elucidate the biochemical mechanisms involved in resistance.

Biochemical analysis of roots

The markers identified by MassLynx across the 124 samples were aligned by retention time and base peak m/z. The peak areas of an early, middle and late eluting peak of the quality control cocktail (QCC) were plotted against chromatogram sequence number to test the reproducibility of the peak area. No correction was made for peak area of the citrus samples, as this change, in the QCC standards, was less than 30% for the late eluting peak and less than 10% for the middle and early eluting peaks, and the CV were low, as shown in Figure 2. Three-hundred-and-sixty-six markers were identified in the 124 citrus samples by means of MassLynx analysis.

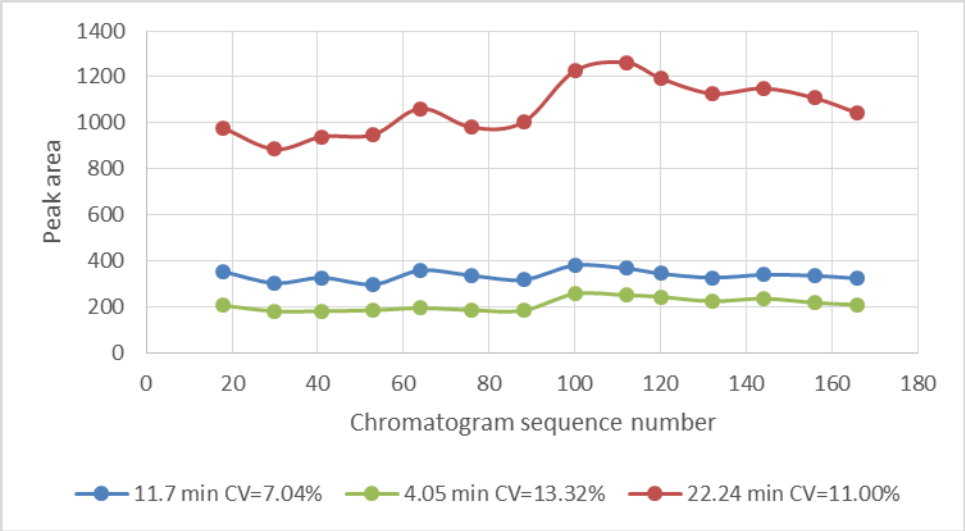

Figure 4.4.6.2. Peak area vs chromatogram sequence number, for an early middle and late eluting peak from the technical repeats of Standard Mix B.

Tolerant vs. Susceptible Rootstocks (uninfected controls)

The first analysis was to select all the uninfected (control) samples and compare the tolerant to the susceptible cultivars (as found in the screening experiments) to identify markers that may discriminate between these two groups, before inoculation with the pathogen. These may be called constitutive markers and may be the most important for the plant breeder because such markers will allow screening of new cultivars without the complications of preparing viable and axenic pathogen inoculum.

The selection of these markers was based on a univariate Student's t-test with $p < 0.05$ and average signal strength of Tolerant/Susceptible of > 2 fold and the marker was present in all 20 injections of the tolerant cultivars. This allowed selection of 14 such markers. There were 18 markers with $p < 0.05$ and average signal

strength of Susceptible/Tolerant > 2 fold and present in all 19 injections of the susceptible cultivars. These 32 markers are shown in Table 4.4.6.5. The markers in Table 5 that were not identified by the multi-variate analysis are underlined, while the remaining markers are shown in bold in Table 4.4.6.6.

The best markers were selected using a three step method in Excel as follows. Since there were five repeats and four susceptible cultivars, ideally there should be 20 values, but if only one value was missing from each cultivar, i.e. 16 or more values, then the marker met condition 1. The same applies to the four tolerant cultivars. The average of the susceptible and the tolerant values were calculated. If the fold of average S/T or T/S was > 2, then the marker met condition 2. The Student's t-test was performed to compare the susceptible to the tolerant values. If the $p < 0.05$, then condition 3 was met. The markers that met all three conditions were accepted as top markers that may distinguish between susceptible and tolerant cultivars.

The markers identified by means of MassLynx with multivariate analysis are shown in an S-plot in Figure 4.4.6.3. The markers at the bottom left and top right of the curve, with p corr[1] < -0.5 and > 0.5, that occur predominantly in the tolerant and susceptible cultivars are shown in Table 4.4.6.6. There is good agreement between the markers identified by multivariate analysis and the top markers. Figure 4.4.6.4 illustrates how principle component analysis discriminates between features unique to tolerant rootstocks (left hemisphere) and susceptible rootstocks (right hemisphere). Figure 4.4.6.5 highlights three top metabolite markers identified in Tables 4.4.6.5 and 4.4.6.6. Using stepwise variable analysis the markers with the smallest P values are selected in step-forward selection to identify the minimum number of ion-features or markers required in trait determination. When the curve evens out with the number of misclassification levelling off the best markers are identified. The biochemical compounds that are represented by these markers need to be identified from their low and high energy mass spectra. The mass spectra of these markers must now be used to make putative identifications of the biochemical compounds that they represent. The compounds must be purchased, if commercially available, and re-chromatographed with the samples to confirm the retention times and MS spectra under identical LC/MS conditions.

Table 4.4.6.5. Potential marker compounds that discriminate between four tolerant and four susceptible citrus cultivars which were uninfected (i.e. constitutive compounds).

t-test Control T vs S	Average Control T	N Control T	Average Control S	N Control S	Average T/S	Average S/T	Retention Time	Mass	T or S Marker	Number	Putative Biochemical Compound
0.0147	2.91	20	1.08	10	2.70	0.37	22.35	540.2206	t	1	
0.0167	3.33	20	1.16	11	2.86	0.35	22.35	494.2325	t	2	
0.0020	31.09	20	9.51	13	3.27	0.31	22.21	625.2799	t	3	
0.0166	8.63	20	2.79	13	3.09	0.32	22.35	539.2184	t	4	
0.0434	4.61	20	1.93	15	2.38	0.42	22.44	584.1923	t	5	
0.0006	122.40	20	55.28	17	2.21	0.45	22.21	314.1478	t	6	
0.0005	12.28	20	5.26	18	2.34	0.43	14.45	715.3837	t	7	
0.0016	5.41	20	1.97	18	2.75	0.36	23.51	403.1883	t	8	
0.0039	548.54	20	130.64	18	4.20	0.24	22.15	324.1241	t	9	
0.0002	659.94	20	275.78	19	2.39	0.42	22.21	313.1445	t	10	4'-prenyloxy resveratrol
0.0013	32.32	20	15.18	19	2.13	0.47	14.45	714.382	t	11	
0.0038	102.16	20	22.70	19	4.50	0.22	22.14	325.1273	t	12	
0.0044	144.39	20	36.04	19	4.01	0.25	22.02	260.1008	t	13	
0.0048	964.32	20	248.87	19	3.87	0.26	22.03	259.0975	t	14	
0.0000	3.66	20	11.41	19	0.32	3.12	22.21	514.1504	s	1	
0.0000	21.97	20	55.27	19	0.40	2.52	22.12	243.1024	s	2	

0.0000	53.20	20	131.45	19	0.40	2.47	21.49	231.0991	s	3	
0.0001	2.64	20	7.51	19	0.35	2.85	21.33	1031.454	s	4	
0.0001	14.58	20	42.32	19	0.34	2.90	21.33	1029.449	s	5	
0.0265	0.09	20	0.21	19	0.43	2.32	23.49	637.5906	s	6	
0.0298	10.22	20	40.44	19	0.25	3.96	22.31	340.1552	s	7	
0.0568	23.39	20	48.90	19	0.48	2.09	22.14	258.1498	s	8	
0.0000	5.02	19	17.15	19	0.29	3.42	21.44	232.1056	s	9	
0.0005	86.18	19	727.81	19	0.12	8.44	22.19	245.1182	s	10	
0.1286	3.68	19	11.91	19	0.31	3.24	22.46	265.1805	s	11	
0.0002	9.18	18	24.05	19	0.38	2.62	21.33	1030.451	s	12	
0.0091	1.22	18	2.59	19	0.47	2.13	22.35	408.276	s	13	
0.1521	0.88	18	1.95	19	0.45	2.23	22.46	663.4229	s	14	
0.0977	0.57	17	2.03	19	0.28	3.54	22.45	266.1837	s	15	
0.0007	2.24	16	11.42	19	0.20	5.10	22.38	370.1653	s	16	
0.0005	0.72	14	2.67	19	0.27	3.69	22.38	371.1686	s	17	
0.0260	6.44	14	40.97	19	0.16	6.36	22.06	356.15	s	18	

Table 4.4.6.6. The marker compounds obtained with multivariate analysis, with p(corr)[1]P < -0.5 or > 0.5 representing tolerant and susceptible cultivars respectively, with the markers in bold obtained with Students t-test, fold increase> 2 and presence in all 20 tolerant or all 19 susceptible samples. The three highlighted features were found by step forward discriminant analysis to be able to classify > 90% of samples correctly.

Row	Primary ID	Retention Time	Mass	CoeffCS[2](Group)	p(corr)[1]P
31	21.89_213.1281	21.89	213.1281	0.000536587	0.510378
53	18.85_229.0866	18.85	229.0866	0.00447096	0.60479
64	21.49_231.0991	**21.49**	**231.0991**	0.0053897	0.711474
67	21.44_232.1056	**21.44**	**232.1056**	0.00229388	0.740884
48	21.75_242.1182	21.75	242.1182	0.00178223	0.565774
51	16.18_243.1019	16.18	243.1019	0.00213581	0.588721
59	21.97_243.1024	21.97	243.1024	0.00317549	0.671676
70	22.12_243.1024	**22.12**	**243.1024**	0.00206227	0.790236
60	22.11_244.1055	22.11	244.1055	0.000800409	0.672361
58	21.96_244.1057	21.96	244.1057	0.00110855	0.66403
57	19.79_244.1336	19.79	244.1336	0.00279272	0.644859
40	18.79_245.0815	18.79	245.0815	0.0019554	0.544969
56	15.72_245.0815	15.72	245.0815	-0.000965107	0.635038
49	22.19_245.1182	**22.19**	**245.1182**	0.0061677	0.580371
39	22.21_245.2126	22.21	245.2126	0.000437577	0.544208
42	15.72_246.0847	15.72	246.0847	-9.23E-05	0.549294
46	22.19_246.1214	22.19	246.1214	0.00221084	0.562936
50	22.21_247.1238	22.21	247.1238	0.000807441	0.581688
47	21.44_253.0837	21.44	253.0837	0.000973916	0.564554
30	22.14_258.1498	**22.14**	**258.1498**	0.00383864	0.504621
23	21.85_259.0974	**21.85**	**259.0974**	-0.00481941	-0.528361
21	21.85_260.1007	**21.85**	**260.1007**	-0.00198731	-0.544815
28	21.85_261.1031	21.85	261.1031	-0.000618426	-0.507002

54	16.20_261.1126	16.2	261.1126	0.00355367	0.612554
25	22.04_264.1392	22.04	264.1392	-0.000669548	-0.517603
37	21.96_265.0843	21.96	265.0843	0.000936801	0.537354
24	22.02_265.1424	22.02	265.1424	-0.000371546	-0.520721
34	18.24_277.1076	18.24	277.1076	0.00125081	0.528022
1	21.84_281.0791	21.84	281.0791	-0.00104878	-0.709988
3	22.21_313.1445	**22.21**	**313.1445**	-0.00986958	-0.656458
13	22.21_313.2512	22.21	313.2512	-0.00105194	-0.604267
4	22.21_314.1478	**22.21**	**314.1478**	-0.004273	-0.6517
7	22.22_315.1514	22.22	315.1514	-0.00162388	-0.629964
8	22.15_324.1241	**22.15**	**324.1241**	-0.00413958	-0.620396
9	22.14_325.1273	**22.14**	**325.1273**	-0.0018099	-0.616989
14	22.13_326.1299	22.13	326.1299	-0.000719641	-0.596425
32	22.31_341.1583	22.31	341.1583	0.00176415	0.513502
41	22.06_356.1500	**22.06**	**356.15**	0.0044316	0.545541
45	22.06_357.1533	22.06	357.1533	0.00198825	0.555562
27	21.87_370.1352	21.87	370.1352	-0.000147098	-0.507612
52	22.38_370.1653	**22.38**	**370.1653**	0.00126699	0.600972
55	22.38_371.1686	**22.38**	**371.1686**	0.000620073	0.633513
26	14.46_376.6689	14.46	376.6689	-0.00133588	-0.512229
18	1.04_381.0785	1.04	381.0785	-0.000330998	-0.554342
17	23.50_381.2067	23.5	381.2067	-0.000943357	-0.562059
16	23.51_403.1883	**23.51**	**403.1883**	-0.000819414	-0.576294
33	22.35_408.2760	**22.35**	**408.276**	0.000863025	0.520311
36	21.96_485.1970	21.96	485.197	0.00149814	0.536449
38	21.96_486.2000	21.96	486.2	0.000756516	0.53866
44	21.96_487.2019	21.96	487.2019	0.00031092	0.550995
35	22.02_488.1710	22.02	488.171	0.00189644	0.531984
15	22.35_494.2325	**22.35**	**494.2325**	-0.000388128	-0.584485
65	21.96_503.1711	21.96	503.1711	0.00154535	0.726268
68	22.21_514.1504	**22.21**	**514.1504**	0.00123677	0.787402
66	22.20_515.1539	22.2	515.1539	0.000525805	0.739919
71	21.33_515.2285	21.33	515.2285	0.00237624	0.798203
69	21.33_516.2315	21.33	516.2315	0.00125399	0.790186
43	21.95_520.1972	21.95	520.1972	0.000894352	0.550137
20	21.84_539.1686	21.84	539.1686	-0.000734648	-0.551999
19	22.35_539.2184	**22.35**	**539.2184**	-0.000516428	-0.552375
10	21.84_540.1717	21.84	540.1717	-0.00041397	-0.61087
12	22.35_540.2206	**22.35**	**540.2206**	-0.000386837	-0.606747
29	22.44_584.1923	**22.44**	**584.1923**	-0.000219715	-0.505082
11	21.87_605.2375	21.87	605.2375	-0.000106592	-0.608673
5	22.21_625.2799	**22.21**	**625.2799**	-0.00239273	-0.643712
6	22.21_626.2836	22.21	626.2836	-0.0014173	-0.640683
2	21.86_627.2189	21.86	627.2189	-0.000164849	-0.672296
22	14.45_715.3837	**14.45**	**715.3837**	-0.00189658	-0.529813

61	21.33_1029.4489	**21.33**	**1029.449**	0.00230462	0.69509
62	21.33_1030.4512	**21.33**	**1030.451**	0.00174346	0.700045
63	21.33_1031.4541	**21.33**	**1031.454**	0.000934172	0.709147

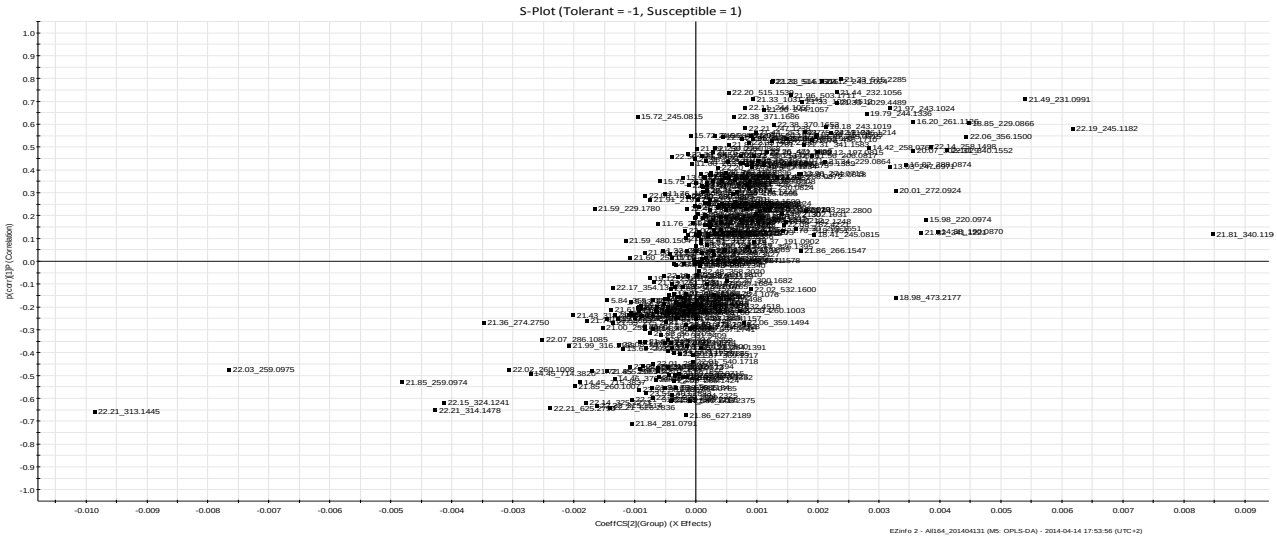

Figure 4.4.6.3. S-plot for the markers that differentiate tolerant (lower left hemisphere) and susceptible (top right hemisphere) cultivars.

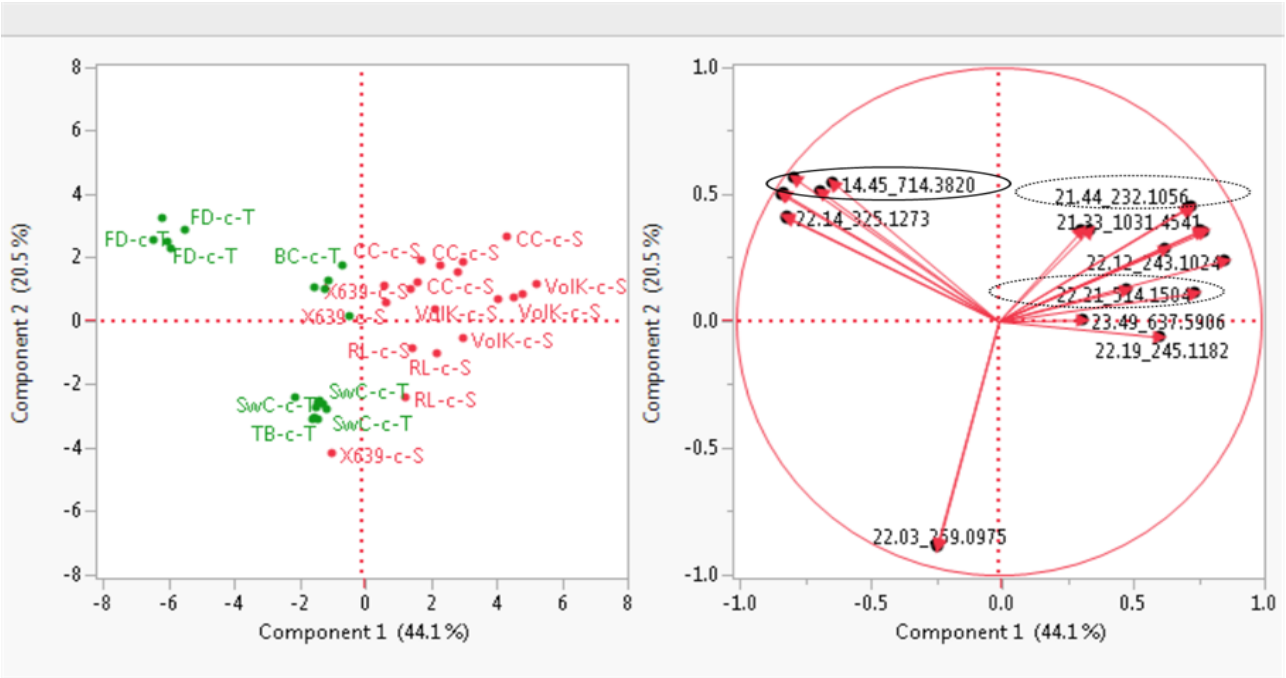

Figure 4.4.6.4. Left: Principle Component Analysis plot for discrimination and clustering of tolerant (left hemisphere: green labelled) and susceptible (right hemisphere: red labelled) cultivars. Right: example of principle component for tolerance related markers circled in black. Example of principle component for susceptibility related markers circled in dash-line.

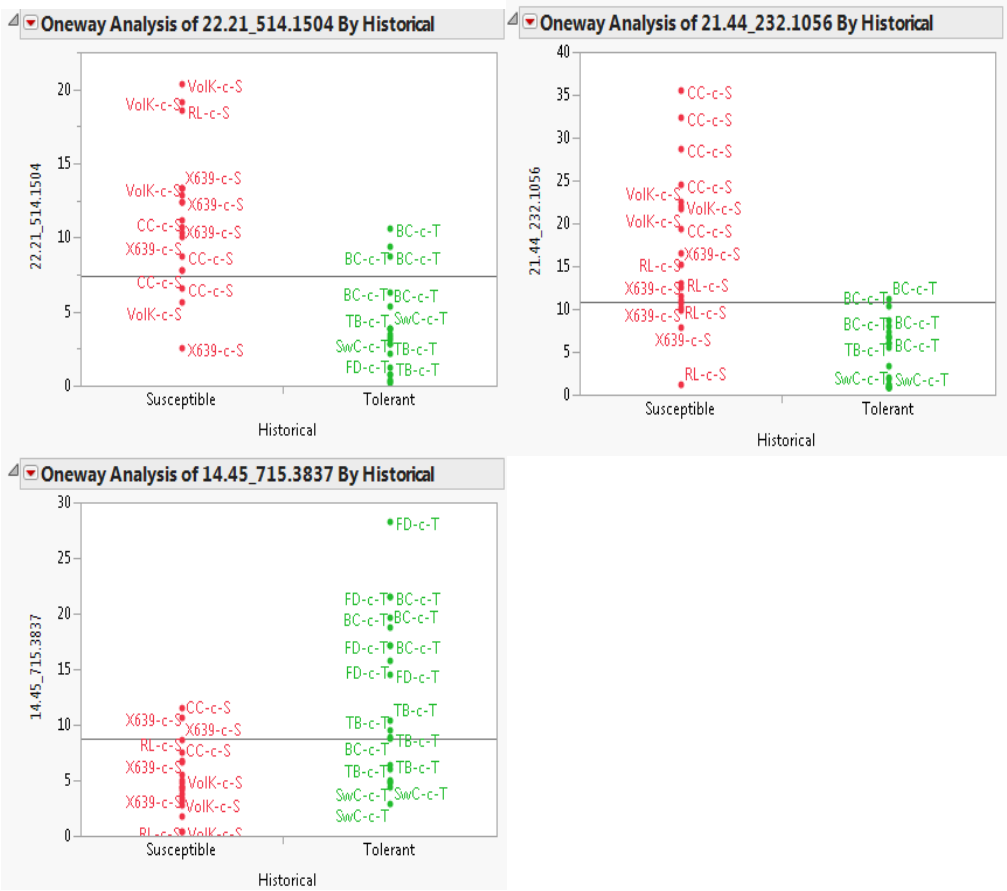

Figure 4.4.6.5. Fit Y by X plots - Ion features 21.44_232.1056 & 22.21_514.1504 (top left & right) detected in higher concentrations in susceptible rootstocks. Top marker Ion feature 14.45_715.3837 (bottom left) detected in higher concentration in all 4 tolerant rootstocks.

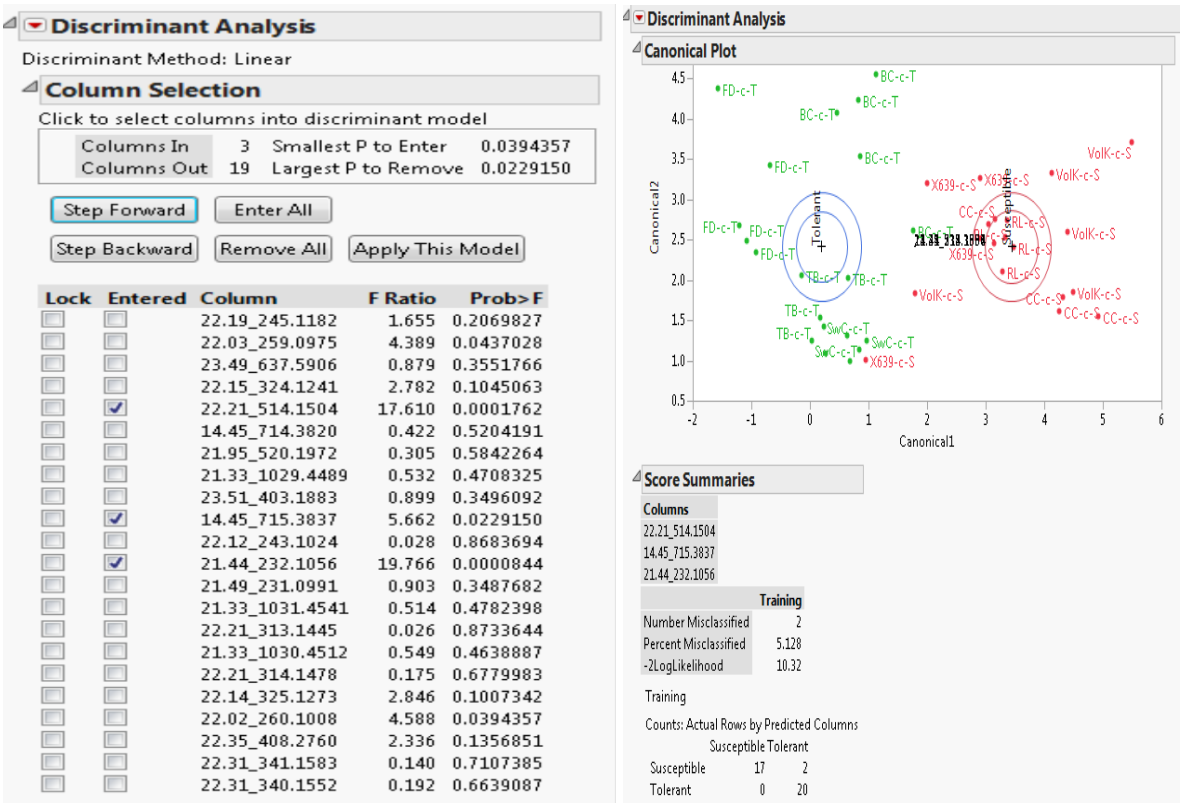

Figure 4.4.6.6. Example of Stepwise variable analyses using step-forward selection of top three markers to select for tolerance / susceptibility among citrus rootstocks.

Three ion features have thus far been identified as the most suitable metabolite markers correlating with tolerance or susceptibility. Using step-forward discriminate analysis (Figure. 6) it was possible to allocate the ion feature detected at 14.45_715.3838 minutes as a tentative metabolite marker for tolerance, and the features detected at 21.44_232.1050 and 22.21_514.1504 minutes as tentative metabolite markers for susceptibility as visualised in Figure 4.4.6.5. To fully achieve the aim of determining whether the identified markers can be used in breeding programs for the rapid screening of tolerance / susceptibility, the validation of accumulated data is required. Steinfath *et al.*, (2010) and Patti *et al.*, (2012) amongst others discuss the in-depth processes required to confirm the function of detected markers for example their presence in the same cultivars from a different test group. Following the confirmation with authentic standards of the ion-features several more steps, including determining their role and function within metabolic pathways and against pathogens *in vitro* using commercial standards, is necessary to validate these discoveries.

3. To assess the importance of root growth potential / root regeneration as a marker for rootstock tolerance against root rot.

Table 4.4.6.7. Results of assessment of root growth potential of citrus rootstocks in the greenhouse: mean dry root weights for cut & uncut root systems.

Rootstock	Cut root system Ave. dry weight(g)	Uncut root system Ave.dry weight(g)	%Growth Potential
Australian trifoliate	3.22	4.03	20
Benton citrange	4.3	6.6	33
C-35 citrange	7.14	8.35	14
Carrizo citrange	6.25	6.7	6
Cairn rough lemon	8.19	9.516	14
Minneola X Trifoliate	7.25	8.66	16
Swingle citrumelo	7.37	7.36	-

Sunki X Benece	5.2	5.31	1
Tera Bella citrange	1.67	1.35	-
Troyer citrange	2.92	5.157	44
Volckameriana	8.65	9.0375	3
X639	4.05	5.05	19
Yuma citrange	3.65	3.21	-

Rootstocks were ranked in order from highest to lowest root growth potential as follows: Troyer citrange, Benton citrange, Australian trifoliate, X639, MXT, Cairn rough lemon and C-35, Carrizo citrange, Volckameriana, Sunki X Benecke, Terrabella, Yuma citrange (Table 4.4.6.7). There did not appear to be a clear relationship between root growth potential and tolerance against Phytophthora root rot in our study. Some of the rootstocks which showed a high root growth potential were susceptible to *P. nicotianae* (eg. Troyer citrange) whist others were tolerant (e.g Benton citrange). **Conclusions**

Root samples from *P. nicotianae* susceptible and tolerant rootstocks which were either uninfected or infected were systematically analysed for their metabolite profiles with the objective of identifying specific marker compounds associated with rootstock tolerance against *P.nicotianae*. Figure 4.4.6.7 gives an indication of the overall process and our current position therein.

The current analyses enabled us to identify 22 constitutive marker compounds associated with tolerant rootstocks. Current software allowed us to narrow down this figure to select the top three biochemical markers to accurately select for tolerance / susceptibility among citrus rootstocks. Further statistical analysis will be done to investigate how these markers were modulated by inoculation with the pathogen. The most important markers will be selected for further characterization.

The current data obtained from the study appear to indicate that root growth potential and tolerance related metabolites are two separate and probably unrelated aspects with regards to Phytophthora resistance in citrus rootstocks.

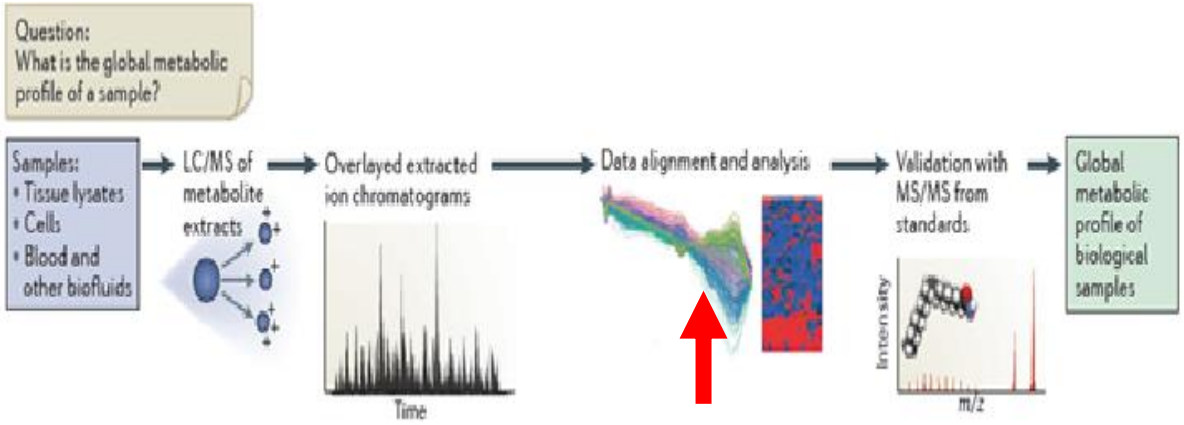

Figure 4.4.6.7. Flow schematic for untargeted metabolomics (Source: Patti *et al*., 2012). The red arrow indicates where we currently are in the process.

Future research

The current study succeeded in identifying metabolite marker compounds associated with tolerant citrus rootstocks up to the level of the compound' mass spectrum features. The metabolomics approach applied during this study constitutes a novel approach to the study of mechanisms of resistance in citrus rootstocks, and has opened the way for characterisation of these marker compounds in future. Furthermore, although the original objective of elucidating the biochemical mechanism(s) of resistance has not been fully achieved, a solid foundation has been laid.

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4.4.7 **PROGRESS REPORT: Evaluation of a new nematicide for the control of the citrus nematode**
Project 950 by M.C. Pretorius & C. Kotze (CRI)

Summary

A contract trial for Makhteshim, Israel, was laid out in Komatipoort and in the Western Cape. A progress report was sent to the company. Two seasons' data were captured in the Western Cape and the trial was terminated. The company requested that the Komatipoort trial continue for one more season.

Opsomming

ʼn Evaluasieproef is vir Makhteshim, Israel, met ʼn nuwe nematisied uitgevoer. Twee proef persele is uitgelê; een in Komatipoort omgewing en een in die Wes-Kaap. ʼn Vorderingsverslag is aan die maatskappy gestuur. Twee seisoene se data is in die Wes-Kaap versamel, en die proef is daar afgehandel. Daar is versoek dat die Komatipoort perseel vir nog ʼn seisoen herhaal word.

4.5 **PROGRAMME: POST-HARVEST PATHOLOGY**
Programme coordinator: Arno Erasmus (CRI)

4.5.1 **Programme summary**

Good progress has been made in the field of citrus postharvest pathology during 2013/14. A resistance monitoring protocol for packhouses has been developed and can now be offered as a service to the industry by the CRI DC (Project 123). The development of a molecular method to assess and quantify imazalil (IMZ) has been initiated (Project 936) and will be continued in a future project (Project 1102). Project 936, a very fruitful project in terms of publications, student training and the development of recommendations to the industry has now been finalised. With 1 honours, 2 masters and 1 PhD degree successfully completed as part of this project it has also resulted in 6 internationally peer reviewed publications so far and a whole list of presentations nationally and internationally this project was industry money well spend. The greatest value from this project lies in the recommendations being practiced by industry today. Project 1034 has also been finalised showing that imazalil (IMZ) resistant isolates of *Penicillium digitatum* cannot be managed by higher residues of IMZ. The combination with alternative actives is vitally necessary to preserve IMZ as long as possible. The JBT flooder has been shown to be a real effective alternative for the fungicide dip tank (Project 1050); results were presented at the CRI Packhouse Workshops and will be published. Preharvest risk indicators have been identified and measured in 6 orchards (Project 1073). However, the relevance of these indicators still needs to be validated. Work has been done to develop an integrated treatment protocol that will use warm water as a curative treatment and a biocontrol yeast as protective (Project UKZN1). This protocol should now be tested on a larger scale.

Programopsomming

Goeie vordering is gemaak in die gebied van sitrus naoes patologie gedurende 2013/14. ʼn Moniteringsprotokol vir swamdoder bestandheid is vir sitrus pakhuise ontwikkel en kan nou as ʼn CRI DC diens aan die bedryf aangebied word (Projek 123). Die ontwikkeling van ʼn molekulêre metode om imazalil (IMZ) bestandheid te bepaal en te kwantifiseer is geïnisieer (Projek 936) en sal in ʼn toekomstige projek (Projek 1102) voortgesit word. Projek 936, ʼn vrugbare projek in terme van publikasies, studente-opleiding en die ontwikkeling van aanbevelings aan die bedryf is nou gefinaliseer. Met een honneurs-, twee meesters- en een PhD-graad suksesvol voltooi as deel van die projek, ses internasionaal eweknie geëvalueerde publikasies tot dusver, en ʼn hele lys van nasionale en internasionale aanbiedings is hierdie projek ʼn voorbeeld van bedryfsgeld goed bestee. Die grootste waarde van hierdie projek is in die aanbevelings wat vandag deur die industrie toegepas word. Projek 1034 is ook gefinaliseer en toon dat IMZ bestande isolate van *Penicillium digitatum* nie deur hoër residue lading van IMZ bestuur kan word nie. Die kombinasie met alternatiewe aktiewes is uiters noodsaaklik om IMZ vir so lank moontlik te bewaar. Die JBT vloedtoediener is bevestig as ʼn werklike en effektiewe alternatief vir die swamdoder dompelbad (Projek 1050); die resultate is tydens die CRI Pakhuis Werkswinkels aangebied en sal gepubliseer word. Vooroes risiko-aanwysers is geïdentifiseer en gemeet in ses boorde (Projek 1073). Die relevansie van hierdie aanwysers moet nog bewys word. Werk is gedoen om ʼn geïntegreerde behandelingsprotokol te ontwikkel met warm water as ʼn kuratiewe behandeling en ʼn biobeheer gis as beskermende behandeling (Projek UKZN1). Hierdie protokol moet nou op ʼn groter skaal getoets word.

4.5.2 **PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided**

Project 123 (Ongoing) by Arno Erasmus, Catherine Savage & Paul H. Fourie (CRI)

Opsomming

Produkte wat waterstofperoksied met asynsuur kombineer (HPPA) is ondersoek as alternatiewe sanitasie agente en vir die verenigbaarheid met die belangrikste deurdrenking (drench) swamdoders. HPPA het geen uitwerking op die konsentrasie van tiabendasool en 2,4-D gehad nie, maar was nadelig vir pyrimethanil. Dit sal verder in 2014 ondersoek word. ʼn Protokol is suksesvol ontwikkel vir die assessering van bestandheid teen swamdoders in pakhuis omgewings en is nou gereed om deur die CRI DC geïmplementeer te word en sal gelewer word as ʼn diens aan die industrie. Twee lug reinigingstelsels is ondersoek. Glocaut PC (met kwaternêre ammoniakverbindings, DDAC en BAC) is in ʼn fyn mis in die lug vrygestel. Daar was ʼn aanduiding van ʼn verlaging in inokulumlading, maar die resultate is nog voorlopig en verdere assessering sal gedoen word in 2014. ʼn Osoon generator is gebruik om ʼn konstante konsentrasie van 0,5 dpm in ʼn besmette koelkamer te handhaaf. Hierdie behandeling was in staat om die inokulumlading in die lug te verminder met 22 - 49%. Hierdie opsie moet verder in 2014 ondersoek word. In 2014 sal die fokus meer op die alternatiewe vir guazatine vir die beheer van suurvrot en ander produkte vir die beheer van Phytophthora bruinvrot wees. Verdere betrokkenheid met analitiese laboratoriums sal gepoog word om variasie te verminder. Laastens is ʼn opname gedoen deur middel van ʼn vraelys wat deur 85 verteenwoordigers van pakhuise landwyd by die 2013 CRI pakhuis werkswinkels voltooi is; hierdie resultate sal nuttig wees vir die beplanning van toekomstige navorsing en die aktiwiteite van pakhuise te bepaal.

Summary

Products that combine hydrogen peroxide with parasitic acid (HPPA) as alternative sanitation agents was assessed for compatibility with the main drench fungicides for the use. HPPA had no effect on the concentration of thiabendazole and 2.4-D, but was detrimental to pyrimethanil. This will be investigated further in 2014. A protocol has been successfully developed for assessing fungicide resistance in packhouse environments and is now ready to be implemented by the CRI DC and will be rendered as a service to industry. Two aerial sanitising systems have been assessed. Glocaut PC (containing quaternary ammonium compounds, DDAC and BAC) are released in a fine miss into the air. There was some indication of reduction of inoculum load, but results are still preliminary and further assessment will be conducted in 2014. An ozone generator was used to apply a constant concentration of 0.5 ppm in a contaminated cold room. This treatment was able to reduce the inoculum load in the air by 22 – 49%. This option needs to be explored further in 2014. In 2014 the focus will be more on investigating alternatives for guazatine for controlling sour rot and other products for controlling Phytophthora brown rot. Further engagement will be attempted with the analytical laboratories to reduce variability. Lastly a survey was conducted by means of a questionnaire completed by 85 representatives from packhouses nationwide at the 2013 CRI packhouse workshops; these results will be useful for planning of research and to gage the activities of packhouses.

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 2013/14 report year.

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides, the bulk of the work will be done by QMS and CRI postharvest plant pathology will have limited involvement. CRI will be involved in setting up protocols and interpreting the data and findings	1. QMS had a change of ownership and management, this means the relationship between QMS and CRI needs to be reviewed. CRI will selectively conduct pilot and registration trials in the interest of the industry.

2. Seek and test alternative actives for the control of sour rot and Phytophthora brown rot	1.No trials were done on Phytophthora brown rot or sour rot during 2013
3. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	1. No progress were achieved during 2013, part of this objective is now included in a new project (1103). 2. Communication and a relationship have been established with a packhouse that is willing to conduct the first industry trials on the integrations of GRAS chemicals as part of an industry application.
4. Analytical lab focus – ring test with the aim to reduce variability	1. Analytical labs have received results from the 2012 ring test with no response. More effort needs to be implemented.
5. Develop and implement a DC protocol for assessing fungicide resistance in citrus packhouses	1. The protocol has been completed and is ready for implementation.

Test compatibility of two products containing hydrogen peroxide and parasitic acid with fungicides applied in packhouse drench systems

Due to the withdrawal of quaternary ammonium compounds (BAC and DDAC) from drench solutions the needs arose for alternative sanitation agents. This is necessary to reduce the build-upp of spores in the solution. Products containing hydrogen peroxide combined with parasitic acid (HPPA) are proclaimed as such alternatives by certain researchers and chemical companies. Two products are currently available, Citrocide (Citrosol, Spain) and Tsunami (Ecolab, USA). These products were combined with thiabendazole (TBZ), pyrimethanil (PYR) and 2.4-D in the recommended concentrations. Trials were conducted at the CRI Postharvest laboratory in Nelspruit and repeated at the USPP Postharvest laboratory at Stellenbosch University. The findings were that both products reduced the PYR concentration by 70 – 94%, while having no effect TBZ and 2.4-D. This needs to be investigated further, but at this stage these products cannot be recommended as sanitising agents for fungicide solutions. Detailed reports are shown in Addendum 1 (Citrocide) and 2 (Tsunami).

Latent pathogens

As in 2013 an attempt was made to test different actives against the latent pathogens, but the trial was not successful due to the controls having no latent pathogen infection. This will be repeated in the 2014 season.

Sour rot

No work was done on sour rot in 2013.

Phytophthora brown rot

No work has been done on this disease due to time constraints.

Fungicide resistance management

A protocol for rendering a resistance testing service to the industry via the CRI DC has been completed. A draft Cutting Edge explaining resistance testing and protocols for trappings spores is shown in Addendum 3. A trial run was conducted on assessing swabs taken from a commercial packhouse situated in the Eastern Cape. Resistant isolates of *Pencillium digitatum* and *P. italicum* (green en blue mould, respectively) were detected in various areas in the packhouse. The management were advised to conduct a thorough cleaning and sanitation of the packhouse and to send swabs throughout the season. Results can be seen in Table 4.5.2.1.

Table 4.5.2.1. Colony count extracted from swabs taken in an Eastern Cape packhouse during the 2013 packing season. The extract was diluted and plated out on IMZ unamended (IMZ-) and amended (IMZ+) growth medium.

		Colony count					
		IMZ-			IMZ+		
	Sample taken	Total	*P. digitatum*	*P. italicum*	Total	*P. digitatum*	*P. italicum*
EC 1	Carton room 1	33	0	22	11	0	**1**
	Carton room 2	6	0	4	2	0	0
	Main sorting area 1	7	1	1	4	0	**1**
	Main sorting area 2	10	4	3	4	0	**2**
	Packline Class 1+2	36	0	4	31	**3**	**1**
	PPECB Inspection Room	29	3	5	3	0	**2**
	Pre sorting Area	3	0	0	1	**1**	0
	Shelf life 1	14	9	0	5	**1**	0
	Shelf life 2	n/a	n/a	n/a	1	0	0
EC 2	Carton Store floor	3	0	3	n/a	n/a	n/a
	Packhouse floor	2	2	0	3	**3**	**3**
	PPECB Area 1	1	1	0	5	0	**1**
	PPECB Area 2	n/a	n/a	n/a	4	0	**4**
	Pre sorting Area	3	1	2	1	**1**	0

Packhouse sanitation

Aerial sanitising systems were assessed.

Glocuat PC (Citrosol, Spain), containing benzalkonium chloride (BAC) and didecyl dimethyl ammonium chloride (DDAC), delivered in a fine miss by means of nebuliser type nozzles and a compressor to the air. This application was assessed in an empty degreening room and a cold room at a commercial packhouse in Nelspruit. As this needs to be repeated, the results are still preliminary and no conclusions can be made yet. Results to date are shown in Table 4.5.2.2.

Table 4.5.2.2. Colony count obtained from swabs taken at different target sites in a degreening and cold room in a commercial packhouse before and after aerial treatment with Glocuat PC. The spores obtained from swabs were diluted and plated out on imazalil unamended (IMZ-) and amended (IMZ+) growth media.

Location	Target site	Colony count			
		IMZ-		IMZ+	
		Before	After	Before	After
Degreening room	Front top	14	0	0	0
	Front bottom	0	8	1	0
	Middle top	1	0	0	0
	Middle bottom	6	3	0	1
	Back top	2	1	0	0
	Back bottom	3	27	1	26
Cold room	Front top	0	0	0	0
	Front bottom	1	0	0	0

Middle top	7	0	1	4
Middle bottom	1	0	0	0
Back top	2	0	0	0
Back bottom	0	1	0	0

Ozone was tested in a contaminated cold room at CRI Nelspruit. Ozone was generated inside the cold room to reach a concentration of 0.5 ppm. Three potato dextrose agar (PDA) plates were exposed for 5 min after 0, 0.25, 0.5, 1, 2, 3, 4 and 24 h after initiation of the ozone application. Although there was a 22 – 49% reduction (Figure 4.5.2.1) in colony count after ozone release, statistically the difference was not significant. As this needs to be repeated, the results are still preliminary and no conclusions can be made yet.

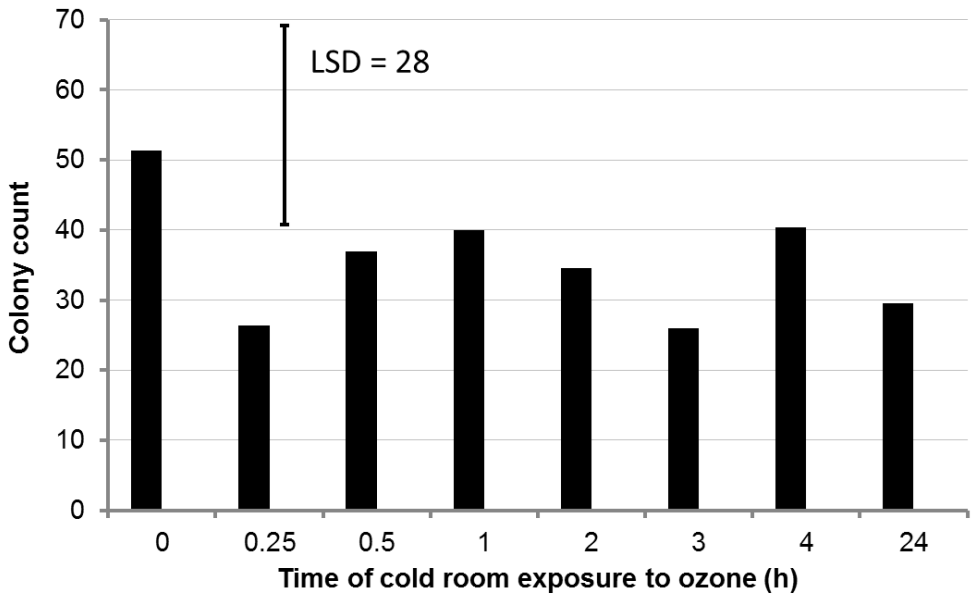


Figure 4.5.2.1. Colony count from PDA plates exposed for 5 min during various time increments during treatment in a cold room where the ozone level was maintained at 0.5 ppm.

Packhouse survey

A packhouse survey was conducted during the 2013 CRI packhouse workshops nationwide. The survey was conducted by means of a questionnaire to be filled in by a representative of the different packhouses it was voluntary and 85 packhouses took part. Data have been compiled (Addendum 4) and still needs to be analysed more intensively. Some highlights will be shared. More than 50% of the packhouses utilise drench systems. The majority of these are situated in the Western Cape, Eastern Cape and Limpopo provinces (32, 30 and 23%, respectively). The time from harvest to drenching is ≥ 24 h for the majority (74%) of packhouses. The majority (46%) drench 3 stacked crates. 65% of the packhouses utilise a wet tip dumping system, 94% use chlorine as sanitising agent and 73% utilise a dip tank for chlorine application. The majority (90%) of packhouses applies fungicides in a dip tank and 95% of these apply imazalil (IMZ) in these tanks. Around 20% of the packhouses apply no fungicides incorporated with the wax, 15% only IMZ and 17% IMZ and TBZ. This survey will be useful to plan future research and to gage the activities of the packhouses.

Further objectives (milestones) and work plan

1. New potential products will be tested as sanitation agents and/or fungicides.
2. Seek and test alternative actives for the control of sour rot and Phytophthora brown rot.
3. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry.
4. Analytical lab focus – ring test with the aim to reduce variability.

Addendum 1

The effect of Citrocide on the concentration of 2.4-D, pyrimethanil and thiabendazole in a drench solution

Arno Erasmus, Ncumisa Njombolwana and Paul Fourie

Introduction

Citrocide (hydrogen peroxide / peracetic acid; HPPA) is a sanitising agent, which is safe to use on citrus fruit. Currently, no sanitising agent is available or applied in South African citrus drench solutions. Citrocide has the potential to be applied for this purpose. However, there is a concern that the active ingredients will have an adverse effect on the fungicide in the drench solution. The effect of Citrocide on the concentration of 2.4-D, pyrimethanil (PYR) and thiabendazole (TBZ) were investigated.

Materials and methods

Four samples were prepared with the three products as per recommended concentration. 2.4-D at 250 $\mu g.mL^{-1}$, PYR at 1000 $\mu g.mL^{-1}$ and TBZ at 1000 $\mu g.mL^{-1}$. For Sample A, a sub-sample was taken at 0 h before 0.8% Citrocide was added, another sub-sample was taken after 3 h before another 0.8% Citrocide was added, the last sub-sample was taken at 24 h. For samples B, C and D a sub-sample was taken at 0 h before 0.8% Citrocide was added and the final sub-sample was taken after 24 h. All samples were sent to Hearshaw and Kinnes (Cape Town) for analyses through LCMS/MS. Samples A and B were prepared in the CRI Nelspruit laboratory and Samples C and D in the University Stellenbosch Plant Pathology laboratory.

Results and discussion

Results are presented in Table 1. The concentration of 2.4-D in Samples A and B are low due to a miscalculation in the CRI Nelspruit laboratory. It is clear that Citrocide has little to no effect on the concentrations of 2.4-D and TBZ. The concentration of PYR were more than halved after the addition of 0.8% Citrocide and almost depleted after a second addition of 0.8% Citrocide. The reduction in PYR concentration was observed in both labs.

Table 1. Concentration of 2.4-D, pyrimethanil and thiabendazole before and after the addition of Citrocide to the solution.

		Concentration determined by LCMS/MS ($\mu g.mL^{-1}$)		
Sample	**Treatment**	**2.4-D**	**PYR**	**TBZ**
A	**0h + 0% Citrocide**	69	827	992
A	**3h + 0% Citrocide**	81	798	934
A	**24h + 0% Citrocide**	77	850	996
B	**0h + 0% Citrocide**	75	873	904
B	**3h + 0.8% Citrocide**	73	381	912
B	**24h + 0.8% Citrocide**	69	52	952
C	**0h + 0% Citrocide**	223	709	833
C	**24h + 0.8% Citrocide**	218	244	803
D	**0h + 0% Citrocide**	264	847	995
D	**24h + 0.8% Citrocide**	240	250	1028

Conclusion

Citrocide cannot be recommended at this stage as a sanitising agent in a drench mix due to the detrimental effect it has on PYR. Our findings are contrary to Kanetis et al. (2008) who found positive effects with mixing HPPA with pyrimethanil. The different and negative effect that our work show could be due to Citrocide having a different formulation than ZeroTol (27% hydrogen peroxide) which was investigated by Kanetis et al. (2008).

References cited

Kanetis, L., Förster, H. & Adaskaveg, J.E., 2008. Optimizing Efficacy of New Postharvest Fungicides and Evaluation of Sanitizing Agents for Managing Citrus Green Mold. *Plant Disease*, 92(2), pp.261–269. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-92-2-0261.

Addendum 2

The effect of Tsunami on the concentration of 2.4-D, pyrimethanil and thiabendazole in a drench solution
Arno Erasmus, Ncumisa Njombolwana and Paul Fourie

Introduction
Tsunami (hydrogen peroxide / peracetic acid) is a sanitising agent, which is safe to use on citrus fruit. Currently, no sanitising agent is available or applied in South African citrus drench solutions. Tsunami has the potential to be applied for this purpose. However, there was a concern that the active ingredients will have an adverse effect on the fungicides in the drench solution. The effect of Tsunami on the concentration of 2.4-D, pyrimethanil (PYR) and thiabendazole (TBZ) were investigated.

Materials and methods
Four samples were prepared with the three products as per recommended concentration. 2.4-D at 250 $\mu g.mL^{-1}$, PYR at 1000 $\mu g.mL^{-1}$ and TBZ at 1000 $\mu g.mL^{-1}$. For Samples A and B a sub-sample was taken at 0 h before 0.2% Tsunami was added and the final sub-sample was taken after 24 h. All samples were sent to Hearshaw and Kinnes (Cape Town) for analyses through LCMS/MS. The samples were prepared in the University Stellenbosch Plant Pathology (USPP) laboratory.

Results and discussion
Results are presented in Table 1. It is clear that Tsunami has little to no effect on the concentrations of 2.4-D and TBZ. The concentration of PYR was almost depleted in Sample A, but in Sample B it was reduced by 24% after the addition of 0.2% Tsunami. It is unclear why the PYR concentration of the 0 h samples were around 50% of the prepared 1000 $\mu g.mL^{-1}$.

Table 1. Concentration of 2.4-D, pyrimethanil and thiabendazole before and after the addition of Tsunami to the solution.

		Concentration determined by LCMS/MS ($\mu g.mL^{-1}$)		
Sample	**Treatment**	**2.4-D**	**PYR**	**TBZ**
A	**0h + 0% Tsunami**	207	473	729
A	**0h + 0.2% Tsunami**	201	75	744
B	**0h + 0% Tsunami**	254	674	967
B	**0h + 0.2% Tsunami**	227	510	1038

Conclusion
Tsunami cannot be recommended at this stage as a sanitising agent in a drench mix due to the detrimental effect it has on PYR. Kanetis et al. (2008) found that HPPA had no negative effect specifically on the concentration of PYR. Further investigation is necessary with this product due to the contrary effects found by Kanetis et al. (2008) with a similar formulation and concentration.

References cited

Kanetis, L., Förster, H. & Adaskaveg, J.E., 2008. Optimizing Efficacy of New Postharvest Fungicides and Evaluation of Sanitizing Agents for Managing Citrus Green Mold. *Plant Disease*, 92(2), pp.261–269. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-92-2-0261.

Addendum 3

Assessing the incidence of imazalil resistance in a citrus packhouse environment

Arno Erasmus[a], Elaine Basson[a], Mareli Kellerman[ab], Catherine Savage[a], Keith Lesar[a], Cheryl Lennox[b] and Paul Fourie[ab]
[a] Citrus Research International, Nelspruit
[b] Department Plant Pathology, University Stellenbosch

Background. Imazalil (IMZ) is currently our most important and frequently used fungicide applied against *Penicillium* green and blue mould (Figure 1 and 2, respectively). An attribute that makes it more favourable than other *Penicillium* fungicides, is its ability to inhibit sporulation (Figure 3). The prolonged use of IMZ makes development of IMZ resistance in *Penicillium* populations inevitable. However, the rate of resistance development can be reduced by applying a variety of different fungicides active against *Penicillium* and most importantly the inoculum load in the packhouse environment must be reduced through effective sanitation practices. This prevents the proliferation of resistant *Penicillium* isolates.

In the short term the efficacy of a specific sanitation regime can be assessed by capturing and counting spores (conidia) in the water, air and on surfaces before and after a specific sanitation action. If the second spore count is less than the first, the sanitation action has been effective.

In the medium to long term the fungicide resistance frequency should be assessed during the packing season and this should be repeated every season in order to get a good indication of the packhouse's specific resistance profile.

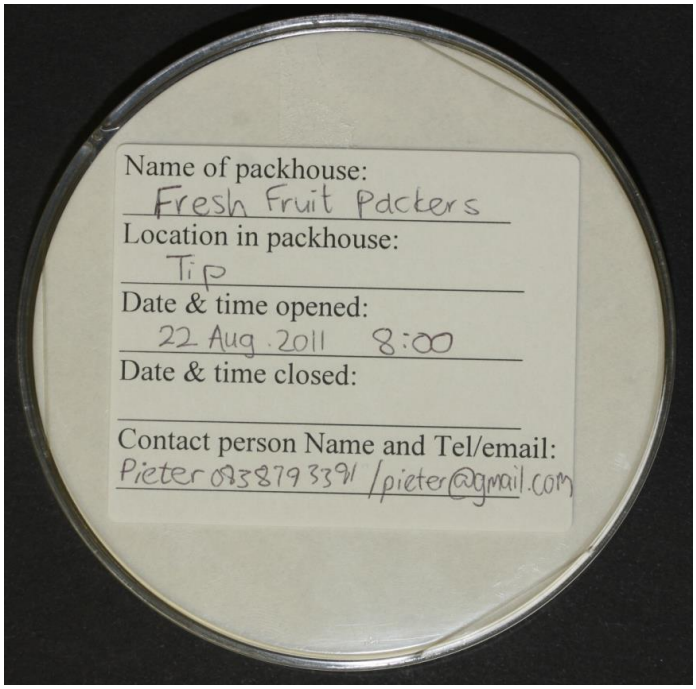
Current diagnostic protocols only allow for the detection of resistance, and not for quantification of that resistance. In other words, we can only detect whether resistant individuals are present at a certain time or not, but we don't know how many resistant individuals are present. To quantify the level of resistant individuals will require a very intensive assessment, which will entail a high number of assessments on a more frequent basis, which will be too costly to conduct as routine test. CRI is continuing funding a research project at Stellenbosch University that aims to develop new technologies that should enable the rapid and accurate determination of the IMZ resistance frequency in packhouses.

In the meantime, we encourage packhouses to determine their IMZ resistance status (presence or absence) at least 3 times a season.

Method. The CRI Diagnostic Centre (DC) has an IMZ-resistance detection assessment available. This can be done from either spore traps (Figure 4) or swabs (Figure 5). A spore trap consists of a petri dish containing a filter paper disc. The filter paper disc is treated with an adhesive to ensure that the captured spores stay on the filter paper. This method is ideal for trapping spores in the air. Swabs can be used to pick up spores from surfaces or rotten (sporulating) fruit. Spore traps can be ordered from the DC. Swabs can be bought at most pharmacies or from your chemical representative.

Instructions for using spore traps

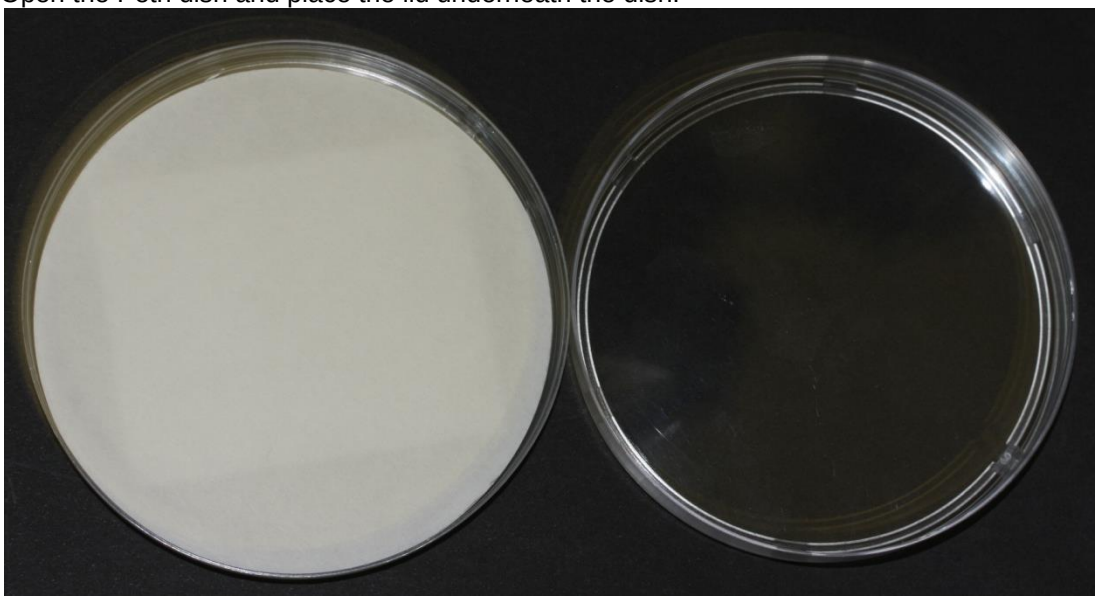
- Complete the label on all 4 of the Petri dishes. Please fill in your contact details so that we may email your packhouse's results to you.

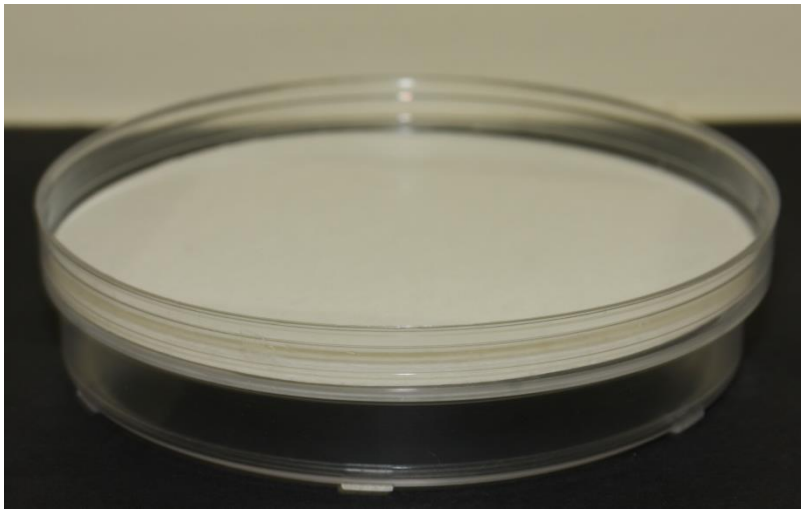


- Place one spore trap at each of the following locations in the packhouse:

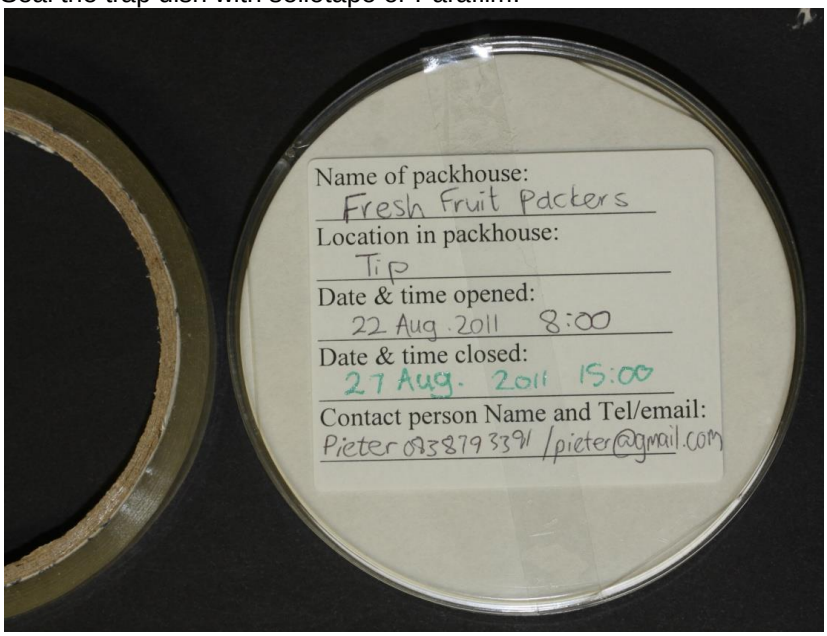
1. Degreening room
2. Tipping area
3. Wax applicator
4. Packing area

- The spore trap should be placed 1 m above the floor, away from possible liquid spills etc. **It is very important that the Petri dish does not get wet.**
  - A good place is to place the trap on a lattice above the designated area.
- Open the Petri dish and place the lid underneath the dish.





- Leave the trap open for 7 days.
- Close the trap and write the date and time it was closed.
- Seal the trap dish with sellotape or Parafilm.



- Send the traps inside provided containers to the CRI DC for assessment
- Do not send open spore traps (without lids) for assessment, this will not give an accurate result and will not be accepted by the DC

  Recommendation for using a swab
  - Sporulating fruit
- Rotten shelf life fruit (hold back fruit)
- Rejected fruit due to decay
- CAUTION! Do not stick the swab inside the rotten fruit. The aim is to only pick up the green mould spores and not from other moulds
- Lightly touch the sporulating surface to pick up only the green or blue spores (Figures 7 – 9)
- Swiftly place the swab pack into its holding capsule (Figures 10 & 11)
  - Do not send open swabs for assessment, this will not give an accurate result and will not be accepted by the DC. Swabs should preferably be sent within a “bubble” envelope to for protection

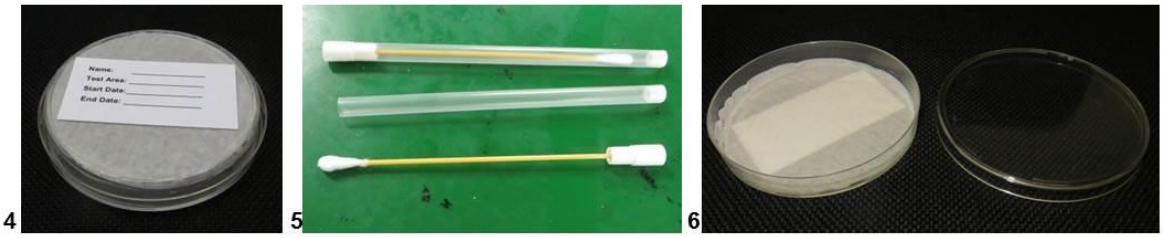
  At the DC, spores will be transferred from the traps or swabs to water. A dilution series will be plated out on PDA (growth medium) half of the PDA will be amended with IMZ. If *Penicillium* spores grow on the amended

media it will be regarded as resistant. Due to the process of plating out and growing of cultures it will take two to four weeks from intake of samples at the DC to the sending of the report.

Spore traps can be ordered from the diagnostician, Elaine Basson, either telephonically (013 759 8000) or by e-mail (elaineb@cri.co.za). Each spore trap will also have a label, which should be completed by the packhouse. The cost of one spore trap is R 7.00 (exl. VAT and postage). A "request for diagnostic analysis form" will be included with each consignment of spore traps. The form needs to be completed by the packhouse and sent back with the spore traps. If swabs are sent, please download the form from CRI's website (http://www.citrusres.com/research/disease/diagnostic) or contact the diagnostician. The cost of analyses for one sample is R185. If more than 4 samples are submitted from a packhouse, the cost will be 4 x R185 (= R740) plus R70 for every additional sample. All prices exclude VAT.

1 2 3

Figure 1 – 3. Groenskimmel (1), blouskimmel (2) en onderdrukking van sporulasie (3)
Figures 1 – 3. Green mould (1), blue mould (2) and sporulation inhibition (3)



4 5 6

Figure 4 – 6. 'n Spoorvanger met etiket (4), dempers wat in 'n kapsule behoort te wees om steriliteit te verseker (5) en 'n oop spoorvanger (6)
Figures 4 – 6. A spore trap with label (4), swabs that should come with a capsule to ensure sterility and an open spore trap

7 8 9

Figuur 7 – 9. Wanneer 'n monster met 'n demper geneem word behoort die demper slegs ligweg oor die sporuleerende vrug gerol te word
Figure 7 – 9. When sampling with a swab the swab should just be lightly rolled over the sporulating fruit

10 11

Figuur 10 & 11. Plaas die demper terug in die kapsule onmiddelik na monsterneming om kruis besmetting te verhoud
Figure 10 & 11. Place the swab back into the capsule immediately after sampling to prevent cross contamination

Addendum 4

1. Participation and length of packing season

Table 1. Provincial distribution of 85 representatives that completed a questionnaire about their specific packhouse.

Province	No of Packhouses	Percentage (%)
Eastern Cape	18	21.2
Kwazulu Natal	2	2.4
Limpopo	35	41.2
Mpumalanga	7	8.2
North West	1	1.2
Northen Cape	3	3.5
Swaziland	3	3.5
Western Cape	16	18.8
Total	85	

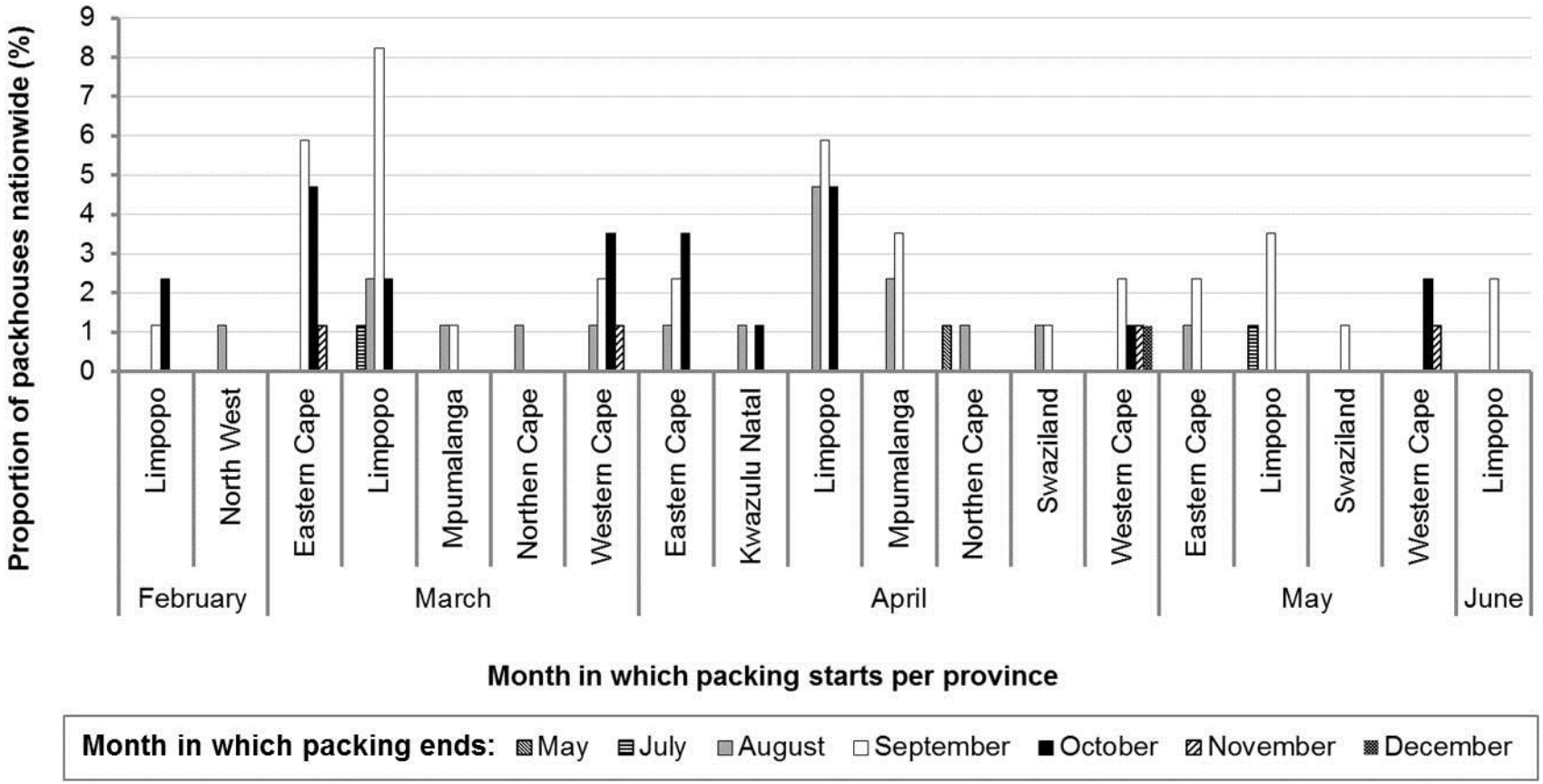


Figure 1. The start month and end month of 84 packouses as per province of packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Table 2. Length (months) of typical packing season of 84 packhouses surveyed during the 2013 CRI Packhouse workshops nationwide

Packing season (months)	Packhouses (%)
2	1.2
3	1.2
4	3.6
5	20.2
6	23.8
7	31.0
8	13.1
9	6.0

(*n* = 84)

2. Drenching

Table 3. Provincial distribution of drenching systems at various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Province	Drenching packhouses (%)
Eastern Cape	29.8
Kwazulu Natal	4.3
Limpopo	23.4
Mpumalanga	2.1
North West	2.1
Northen Cape	6.4
Swaziland	0.0
Western Cape	31.9

(*n* = 47)

Table 4. Hours from harvest to drench at various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Hours between harvest and drenching	Packhouses (%)
1	8.6
2	2.9
4	5.7
6	2.9
12	5.7
24	60.0
48	14.3

(*n* = 35)

Table 5. Application of drench solution on a single, double or triple bin stack at various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Bin stack	Packhouses (%)
Single	21.7
Double	32.6
Triple	45.7

(*n* = 47)

Table 6. Descriptive statistics of exposure time (s) and tank capacity (L) at various packhouses utilising drench systems that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	(*n*)	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Exposure time (s)	(41)	15	60	70	120	600
Capacity (L)	(46)	700	1000	1500	3000	18000

Table 7. Renewing frequency of drench solution at packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Renewing frequency	Packhouses (%)
20 bins	2.4
120 bins	7.1
140 bins	2.4
150 bins	16.7
160 bins	2.4
200 bins	4.8
250 bins	4.8
300 bins	11.9
400 bins	4.8
500 bins	4.8
600 bins	2.4
800 bins	2.4
6 h	2.4
24 h	19.0
72 h	4.8
300 tons	2.4
weekly	2.4
when empty	2.4

(*n* = 42)

Table 8. Time (h) from drench to degreening and duration (h) of degreening at various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	(*n*)	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Time from drench to degreening (h)	(38)	1	12	24	24	72
Duration (h)	(41)	24	48	72	72	96

3. Fruit washing and sanitation systems

Table 9. Type of dumping system, detergent and application utilised in the washing systems in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	Packhouses (%)	
Dump system	Dry	Wet
(*n* = 80)	35.0	65.0
Detergent	Chlorine	Other
(*n* = 65)	93.8	6.2
Application	Dip	Spray
(*n* = 33)	72.7	27.3

Table 10. Descriptive statistics of dump tank capacity, exposure time and the pH level of fresh water in the washing systems in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	(*n*)	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Capacity (L)	(49)	1000	2000	4000	6000	60000
Exposure time (min)	(45)	0.25	1.00	2.00	3.00	20.00
pH level of fresh water	(41)	6.0	6.5	7.0	7.6	11.0

4. Aqueous fungicide application

Table 11. Proportion fungicide dip tanks and those that apply imazalil (IMZ) in these dip tanks in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	Packhouses (%)	
Dip tank	Yes	No
(*n* = 84)	90.5	9.5
Imazalil in tank	Yes	No
(*n* = 77)	94.8	5.2

Table 12. Fungicide dip tanks applying different combination of imazalil (IMZ), guazatine (GZT), thiabendazole (TBZ), pyrimethanil (PYR) and 2.4-D in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Product combination	Packhouses (%)
IMZ alone	53.9
IMZ + GZT	22.4
IMZ + GZT + TBZ	1.3
IMZ + TBZ	15.8
IMZ + 2.4-D	5.3
IMZ + PYR	1.3

(*n* = 76)

Table 13. Descriptive statistics of different parameters of fungicide dip tanks in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	(*n*)	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Capacity (L)	(70)	600	2000	2750	3525	10000
Solution temperature (°C)	(57)	14	25	35	38	48
Exposure time (s)	(56)	5	46	60	90	300
Brushes after dip tank	(56)	0	0	4	12	36
Top-up frequency (h)	(46)	0	0	4	24	72
Renewing frequency (days)	(53)	0.3	3.0	5.0	5.0	20.0

Table 14. Energy source used to heat drying tunnels following fungicide dip tanks in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Energy source	Packhouses (%)
Electricity	29.2
Diesel	34.7
Paraffin	15.3
Gas	13.9
None	6.9

(*n* = 72)

Table 15. Descriptive statistics of temperature and exposure time in drying tunnels following the fungicide dip tank in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	(*n*)	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Temperature (°C)	(61)	25	35	40	45	65
Drying time (min)	(47)	0.3	1.0	1.5	3.0	8.0

5. Wax application

Table 16. Wax systems applying different combination of imazalil (IMZ), guazatine (GZT), thiabendazole (TBZ), pyrimethanil (PYR) and 2.4-D in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Product combination	Packhouses (%)
None (wax only)	19.8
IMZ only	14.8
IMZ+GZT	3.7
IMZ+GZT+TBZ	2.5
IMZ+GZT+TBZ+2.4-D	12.3
IMZ+TBZ+2.4-D	11.1
IMZ+TBZ	17.3
IMZ+2.4-D	8.6
GZT	2.5
GZT+TBZ	0.0
GZT+TBZ+2.4-D	3.7
TBZ+2.4-D	0.0
TBZ	3.7
2.4-D	0.0

(*n* = 81)

Table 17. Distribution of natural or poly ethylene waxes applied by various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Was type	Packhouses (%)
Natural	36.5
Poly	50.8
Natural and Poly	12.7

(*n* = 63)

Table 18. Descriptive statistics of the volume of wax applied by various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	*n*	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Volume (L/ton fruit)	43	0.6	1.1	1.2	1.3	3.5

Table 19. Energy source used to heat drying tunnels following the wax application systems in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Energy source	Packhouses (%)
Electricity	33.3
Diesel	30.6
Paraffin	15.3
Gas	13.9
None	5.6

(*n* = 72)

Table 20. Descriptive statistics of drying tunnels following the wax application systems in various packhouses that took part in a survey during the 2013 CRI packhouse workshops nationwide

Parameter	*n*	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Temperature (°C)	57	25	35	40	45	60
Drying time (min)	46	0.5	1.5	2.0	3.0	13.0

4.5.3 **FINAL REPORT: Optimisation of fungicide application in citrus packhouses**

Project 936 (April 2008 – March 2014) by Paul Fourie (CRI – SU)

Summary

Poor control of green mould, caused by *Penicillium digitatum*, is often the result of insufficient fungicide residue loading and/or fungicide resistance. The aim of this study is to optimise fungicide application by investigating application methods, concentration, exposure time, solution temperature and pH, as well as curative and protective control of fungicide sensitive and resistant *P. digitatum*. Fungicide residue loading was studied and benchmark residue values for green mould control following dip treatments with imazalil (IMZ), pyrimethanil (PYR) and thiabendazole (TBZ) were determined. Application of these fungicides in wax coatings or in drenches were also studied and optimised. Green mould caused by sensitive strains could be controlled by these fungicides, but their efficacy was influenced by application type. In general dip-treatments were most effective in terms of curative control, while wax application of the fungicide led to improved protective control and sporulation inhibition. Drench treatment proved to be an inferior application, mostly ascribed to insufficient coverage of fruit. In optimised drenchers, adequate curative control can be expected if infected fruit is treated within 24 hours after harvest. Synergism and improved control was observed between fungicides in drench mixtures, and use of mixtures in this application is recommended to prevent resistance development. It was clear from this study that fungicide resistance leads to control failure. Fungicides should therefore be optimally used, alternated and resistance frequencies monitored in packhouses. In developing a resistance assay, exposed-plate assays using semi-selective media were non-specific and unreliable, and a quantitative real-time PCR method is being developed. The findings of this 6-year research project are extensively reported in this final report.

Opsommming

Swak beheer van groenskimmel, veroorsaak deur *Penicillium digitatum*, is meestal die gevolg van onvoldoende fungisied residulading en/of fungisied weerstand. Die doel van hierdie studie is om fungisied toediening te optimaliseer deur aanwendingsmetodes, konsentrasie, bloodstellingstyd, oplossingstemperatuur en -pH, sowel as genesende en beskermende beheer van swamdoder sensitiewe en bestande *P. digitatum*, te ondersoek. Fungisied residulading is ondersoek en drempelwaarde residuwaardes vir groenskimmelbeheer, volgende op doopbehandelings met imazalil (IMZ), pyrimethanil (PYR) en thiabendasool (TBZ), is bepaal. Toediening van hierdie swamdoders in waks asook in stort-aanwenders ('drenchers') is ook bestudeer. Groenskimmel veroorsaak deur die sensitiewe isolate kon effektief deur hierdie swamdoders beheer word, maar hul effektiwiteit is deur toedieningsmetodes beinvloed. Doopbehandelings was in algemeen die mees effektiewe metode van toediening, spesifiek vir kuratiewe beheer. Waksaanwending het tot beter beskermende beheer en sporulasie inhibisie gelei. Stort behandelings was die swakste metode, grootliks gegewe onvoldoende bedekking van vrugte. In ge-optimiseerde 'drenchers' is aanvaarbare kuratiewe beheer gekry mits binne 24 uur na oes behandel is. Sinergisme en verbeterde beheer is waargeneem met sekere swamdoder mengsels; gebruik van mengsels in die stort-aanwending word aanbeveel om weerstandsontwikkeling te voorkom. Dit blyk, uit die werk tot op hede, dat fungisiedweerstand tot mislukte beheer lei. Fungisiedes moet dus optimaal gebruik word, afgewissel word, en weerstandsfrekwensies in pakhuise moet gemonitor word. In die ontwikkeling van ŉ weerstandstoets, was blootgestelde plaattoetse met ŉ semi-selektiewe media nie-spesifiek en onbetroubaar, en ŉ kwantitatiewe "real-time" PKR metode word tans ontwikkel.

Introduction

Excessive financial losses occur every year due to decay caused by postharvest pathogens such as *Penicillium digitatum* (causal agent of citrus green mould). Excellent fungicides such as imazalil are available and effective to control diseases such as Citrus green mould. An investigation was conducted to determine why this fungicide is not as effective as it should be; the main focus was on the application method and the subsequent residue loading in practice. Laboratory studies were conducted to study ways and methods in an effort to optimise fungicide application. If fungicide applications could be effective less resistance and waste would occur.

Management of post-harvest diseases of citrus involves several fungicide applications in packhouses, such as in drenches, dips, sprays and wax applications. Biological efficacy of the fungicides is, however, directly related to the adequacy of deposition of the active ingredient on the hydrophobic citrus fruit surfaces. By using green mould caused by *Penicillium digitatum* and the fungicide imazalil sulphate (IMZ) as model system optimisation of fungicide application in citrus packhouses was studied. Maximum residue level (MRL) for IMZ on citrus fruit is set at 5 µg/ml, whereas 2-3 µg/ml is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that citrus fruit from the majority of packhouses have residue levels below 1 µg/ml. Poor control of green mould as a result of insufficient residue loading might further be compounded by the presence of IMZ-resistant strains of *P. digitatum* in packhouses. The aims of this study are to determine the adequate residue levels needed for control and sporulation inhibition of IMZ-sensitive and -resistant strains, to investigate IMZ application and resultant residue levels in commercial citrus packhouses, and to study optimisation of IMZ application in citrus packhouses. Factors that will be studied include application type (spray *vs.* dip), exposure time, bath temperature, wound size, solution temperature and solution pH on the control of *Penicillium digitatum* with IMZ.

Stated objectives

1. Improve the deposition assessment protocol for use on mature citrus fruit.
2. Determine benchmark values for biologically effective fungicide residue loading [Imazalil sulphate and *Penicillium digitatum* (green mould) will be used as the model system].
3. Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses.
4. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.
5. *New objective:* Development of a user-friendly fungicide resistance assay for packhouses.

Materials and methods

1. **Determine benchmark values for biologically effective fungicide residue loading**
2. **Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses.**
3. **Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.**

Research has focused on residue loading and green mould control using imazalil, pyrimethanil and thiabendazole in drench, dip, spray and wax applications in citrus packhouses as affected by parameters such as solution pH, temperature and exposure time.

The research has mostly been concluded and materials and methods for these objectives are described in detail in the articles / addenda.

4. *New objective*: Development of a user-friendly fungicide resistance assay for packhouses.

Research was written up as 3rd chapter in MSc.Agric thesis of Mareli Kellerman; see Addendum K.

Results

Objective / Milestone	**Achievement**
1. Improve the deposition assessment protocol for use on mature citrus fruit	The deposition assessment protocol was optimised and is being used to study drench application, and was also configured to quantify shine on waxed fruit. Residue loading is studied through determination of fungicide residues using analytical methods. An in-house IMZ residue analysis protocol was developed, but to increase throughput and the scope of research, samples are analysed in a commercial laboratory.
2. Determine benchmark values for biologically effective fungicide residue loading 3. Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses 4. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality	Imazalil: IMZ application in dip and wax provides good curative and protective control and sporulation inhibition. Various factors influencing residue loading in dip and wax applications were studied to optimise and manage IMZ application. However, the IMZ resistant strain could not be controlled. Outcomes from this study were extensively communicated to industry.
	Pyrimethanil: PYR provided sufficient curative control and moderate protective control against green mould following dip applications, and was able to control an isolate of *P. digitatum* resistant to IMZ and TBZ. Sporulation was poorly and inconsistently inhibited. PYR provided relatively poor green mould control when applied with wax coating. PYR drench application provided good green mould control at lower inoculum concentrations as well as in mixture with TBZ.
	Thiabendazole: TBZ in dip application provided excellent curative control and poor protective control of the sensitive isolate. TBZ in wax provided moderate protective control, and sporulation inhibition. The TBZ resistant isolate could not be controlled. TBZ applied in dip or wax provided chilling injury control. TBZ applied in the drench lead to poor control on soft citrus and moderate to good control on oranges. Drench exposure time did not have an influence on TBZ residue, but it did have an effect on the efficacy of TBZ to control green mould.
2. New objective: Development of a user-friendly fungicide resistance assay for packhouses	An exposed-plate assay using published semi-selective media was studied but found to be insufficiently species-specific. A real-time PCR assay to quantify IMZ resistance in *P. digitatum* populations was developed; however, optimization of sporetrapping and DNA extraction methods have to be researched before practical implementation.

1. **Determine benchmark values for biologically effective fungicide residue loading**
2. **Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses.**
3. **Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.**

Imazalil

Addendum A

Imazalil residue loading and green mould control in citrus pack-houses

Arno Erasmus, Cheryl L. Lennox, Hennie Jordaan, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2011. *Postharvest Biology and Technology* 62: 193–203.

Imazalil (IMZ) is commonly applied in South African citrus packhouses for the control of green mould, caused by *Penicillium digitatum*, yet the disease still causes significant postharvest losses. Maximum residue limit (MRL) for IMZ on citrus fruit is 5 $\mu g.g^{-1}$, whereas 2-3 $\mu g.g^{-1}$ is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that fruit from the majority of packhouses have residues of ≈ 1 $\mu g.g^{-1}$. Poor disease control from insufficient residue loading might further be compounded by the presence of IMZ-resistant isolates of *P. digitatum* in packhouses. This study was conducted to assess the current status of IMZ application in South African packhouses, to determine the adequate residue levels needed to control green mould and inhibit its sporulation using both IMZ sensitive and resistant isolates, to investigate IMZ application methods and resultant residue levels in commercial citrus packhouses, and to study optimisation of modes of IMZ application in citrus packhouses. Factors studied were IMZ concentration, application type (spray *vs.* dip and drench), exposure time, solution temperature and pH, as well as curative and protective control of *P. digitatum*. The packhouse survey showed that the majority of packhouses applied IMZ in a sulphate salt formulation through a fungicide dip tank, and loaded an IMZ residue of ≈1 $\mu g.g^{-1}$. In dip applications, IMZ had excellent curative and protective activity against *Penicillium* isolates sensitive to IMZ. However, curative control of IMZ resistant isolates was substantially reduced and protective control was lost, even at twice the recommended concentration, nor was sporulation inhibited. The use of sodium bicarbonate (2%) buffered imazalil sulphate solutions at pH ±8, compared with pH ±3 of the unbuffered solutions, markedly increased IMZ residue loading on Navel and Valencia oranges and improved curative and protective control of IMZ resistant isolates. Exposure time did not affect IMZ residue loading in IMZ sulphate solutions at pH 3, although the MRL was exceeded after 45 s exposure in pH 8 solutions. Imazalil applied through spray or drench application improved residue loading, but green mould control was less effective than after dip application.

Addendum B

Imazalil residue loading on citrus fruit as affected by formulation, solution ph and exposure time in aqueous dip treatments

Arno Erasmus, Cheryl L. Lennox, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2013. *Postharvest Biology and Technology* 77: 43–49.

Green mould, caused by *Penicillium digitatum*, is responsible for major postharvest fruit losses on the South African fresh citrus export market. Some of these losses as well as fungicide resistance development can be attributed to sub-optimal imazalil (IMZ) residue loading on citrus fruit (<2 $\mu g.g^{-1}$), which is commonly the case in South African packhouses. This will result in loss of control and sporulation inhibition on decayed fruit. IMZ formulation [IMZ sulphate and emulsifiable concentrate (EC)], solution pH (IMZ sulphate at 500 $\mu g.mL^{-1}$ buffered with $NaHCO_3$ or NaOH to pH 6 and 8) and exposure time (15 to 540 s) were investigated in order to improve IMZ residue loading and the green mould control on Clementine mandarin, 'Eureka' lemon, and navel and Valencia orange fruit. Exposure time had no significant effect on residue loading in the unbuffered IMZ sulphate solution (pH 3). No differences were observed between the pH buffers used, but residue loading improved with increase in pH. The maximum residue limit (MRL) of 5.0 $\mu g.g^{-1}$ was exceeded following dip treatment in the IMZ EC (after 75 s exposure time), and IMZ sulphate at pH 8 using $NaHCO_3$ (77 s) or NaOH (89 s) as buffer. The MRL was exceeded after 161 s in IMZ sulphate solutions buffered at pH 6 with either $NaHCO_3$ or NaOH. An IMZ residue loading curve was prepared from which residue levels can be predicted for the control of IMZ-sensitive and IMZ-resistant isolates of *P. digitatum*. From this model the benchmark residue level for 95% control of an IMZ-sensitive isolate and of an IMZ-resistant isolate were predicted to be 0.81 and 2.64 ug g^{-1}, respectively. Residue loading can be improved by adjusting the pH upward of an IMZ sulphate solution or by using the IMZ EC formulation, but exposure time should be restricted so as not to exceed the MRL. The resistant isolate could not be controlled adequately with residue levels below the MRL, therewith indicating the practical relevance of IMZ resistance.

Addendum C

Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating

Ncumisa S. Njombolwana, Arno Erasmus, Paul H. Fourie. 2013. *Postharvest Biology and Technology* 77: 102-110.

Imazalil (IMZ) is widely used in citrus packhouses to manage green mould, caused by *Penicillium digitatum*. The aim of this study was to investigate green mould control efficacy of IMZ applied in a wax coating, and the combination of aqueous dip and coating IMZ applications. Single application of IMZ at 3000 µg mL^{-1} in carnauba wax coating at rates of 0.6, 1.2 and 1.8 L ton^{-1} of fruit gave better protective (mean 13% infection) than curative (mean 70% infection) control of the sensitive isolate. Imazalil residue levels increased (0.85 to 1.75 µg g^{-1}) with increasing coating load. However, the resistant isolate could not be controlled (>74% infection). Dip only treatment (IMZ sulphate at 500 µg mL^{-1} for 45 s and 90 s) gave good curative control (≈ 77%) of the sensitive isolate at residue loading of 0.12 to 0.73 µg g^{-1}. Wax coating only treatment (IMZ at 3000 µg mL^{-1} at 1.8 L wax ton^{-1}) gave good protective control and improved sporulation inhibition (≈ 80%) at residue loading of 1.32 to 7.09 µg g^{-1}. The MRL of 5 µg g^{-1} was exceeded at higher wax loads on navels and clementines. Double application with dip (45 s in IMZ sulphate at 500 µg mL^{-1}) followed by 2000 µg mL^{-1} IMZ in wax coating at 0.6, 1.2 and 1.8 L wax ton^{-1} resulted in residue loading of 1.42 to 2.83 µg g^{-1}, increased protective control (≈ 69%) as well as curative control (≈ 83%). In all treatments, poor curative and protective control of the resistant isolate was observed (<46% and <55%, respectively). Double application demonstrated superior green mould control by giving good curative and protective control and sporulation inhibition.

Addendum D

Effects of citrus wax coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges

Ncumisa S. Njombolwana, Arno Erasmus, J. Gideon van Zyl, Wilma du Plooy, Paul J.R. Cronje and Paul H. Fourie. 2013. *Postharvest Biology and Technology* 86: 362-371.

Wax application plays an important role in prolonging fruit quality, and the addition of imazalil (IMZ) furthermore protects fruit against green mould caused by *Penicillium digitatum.* The objectives of this study were to evaluate green mould control and quality preservation effects of carnauba or polyethylene citrus coatings supplemented with IMZ, as well as the effect of synthetic or horsehair brush types used on sweet orange fruit. Single application of IMZ at 3000 µg.mL^{-1} at rates of 0.6, 1.2 and 1.8 L.ton^{-1} resulted in residues that increased with increasing coating loads on navel oranges (1.31 to 3.32 µg.g^{-1}) and Valencia oranges (3.22 to 6.00 µg.g^{-1}). Coating with IMZ generally provided poorer curative control (≈ 14%) than protective control (≈ 58%), with less sporulation in treatments done using horsehair brushes (≈ 59%) than synthetic brushes (≈ 64%). More fruit weight loss and firmness loss were obtained in fruit treated with the polyethylene coating (≈ 1.18 and ≈ 0.93 ratios of treated *vs.* untreated, respectively) and lower in carnauba coating treated fruit (≈ 0.76 and ≈ 0.74 ratios, respectively). However, polyethylene coating resulted in shinier fruit before storage (≈ 10.85 shine ratio) and after storage (11.60), whereas carnauba coating resulted in lower shine ratios (≈ 7.45 and 10.15, respectively). Gaseous (CO_2) exchange ratios remained similar for both waxes (≈ 0.67). Higher polyethylene coating loads (1.8 L.ton^{-1}) resulted in off-tastes similar to uncoated control fruit (≈ 2.21 rating on a 5-point scale) and higher than the rating for carnauba coated fruit (≈ 1.82) at this rate. Scanning electron micrographs showed an amorphous crystallised natural wax layer with uncovered stomatal pores on the surface of uncoated fruit. The thickness of the applied wax layer increased with increasing coating load. A single application of IMZ in wax provided good protective green mould control and sporulation inhibition, with differing effects on some fruit quality parameters due to coating and brush types.

Addendum E

Curative control of citrus green mould by imazalil as influenced by infection age, wound size, fruit exposure time, solution ph and fruit brushing after treatment

Chapter 4 in PhD(Agric) dissertation of Arno Erasmus (Stellenbosch University)

Green mould (*Penicillium digitatum)* is a major cause of postharvest losses in citrus. Wounds and infection can be induced during the harvest process and should be controlled during postharvest stages to prevent decay. Imazalil (IMZ) in the sulphate formulation is currently applied by the majority of South African packhouses through an aqueous dip treatment. In this study the effects of incubation time (infection age), exposure time, solution pH, wounds size and fruit brushing after dip treatments on residue loading and curative green mould control were investigated. Exposure time did not have a significant effect on residue loading on fruit dipped in pH 3 solutions of IMZ (< 2.00 µg.g^{-1}). Increasing the pH to 6 resulted in significantly increased residue loading,

which increased with longer exposure time, but mostly to levels below the maximum residue level (MRL) of 5 µg.g^{-1} after 180 s. Post-dip treatment brushing reduced residue levels obtained in IMZ pH 3 solutions by up to 90% to levels < 0.5 µg.g^{-1}; however, curative control of the IMZ sensitive (S) isolate was mostly unaffected, but with poor sporulation inhibition. At pH 6, post-dip brushing reduced residues to ≈ 60%; again curative control of the sensitive isolate was unaffected, but with better sporulation inhibition than the pH 3 treatments. Wounded rind sections loaded higher residue levels compared with intact rind sections, and large wounds loaded higher levels than small wounds (≈ 10.19, ≈ 9.06 and ≈ 7.91 µg.g^{-1} for large, small and no wound, respectively). Curative control of infections originating from large wounds was significantly better than those from small wounds. The ability of IMZ to control sensitive green mould infections declined from 6 and 12 h after inoculation on Clementine mandarin fruit of infections induced by small and large wounds, respectively; on navel orange fruit, curative control declined 18 and 36 h after inoculation for the respective wound size treatments. This work shows the importance of timeous fungicide treatment after harvest especially on more susceptible citrus types. Results also indicate that excess residues can be stripped from the fruit, retaining residues necessary for curative control in the wound sites. However, reduced residue loading compromised the sporulation inhibition activity of IMZ.

Addendum F

The effect of temperature and pH on imazalil residue loading and green mould control on citrus through dip application

Report of 4th year student project by Jeanine Joubert, Plant Pathology, Stellenbosch University

Green mould disease causes substantial loss in the South-African and international citrus industry. Imazalil (IMZ) is the most effective fungicide applied for postharvest control. As various cases of resistance to IMZ has been reported, it is imperative to apply it in the most effective way in order to obtain good residue loading and green mould control. Therefore the aim of this study is to evaluate the influence of temperature and pH on residue loading and green mould control on citrus fruit using dip application. Sodium bicarbonate was used to buffer IMZ sulphate solutions at pH 6. Lemons, Clementines and navels loaded residues of 1.34, 1.27 and 1.29 µg.g^{-1} at pH 3 *vs*. 5.20, 6.96 and 4.82 µg.g^{-1} at pH 6, respectively. Likewise, increased temperature in the dip solutions led to increased residues loaded (1.85-4.80, 1.90-6.64 and 1.69-4.74 µg.g^{-1} on lemon, Clementine and navel fruit, respectively, when temperature was increased from 23°C to 45°C). This compared well with the increased residues loaded with increase in exposure times (2.32-4.25, 2.68-5.47 and 2.20-3.84 µg.g^{-1} on lemon, Clementine and navel fruit, respectively), for exposure times ranging from 15 to 90 s. Green mould control and sporulation inhibition improved significantly when fruit were treated with heated aqueous solution. The infection control increased significantly for Clementines with an increase in pH (39.9 to 46.5%), temperature (43.7 to 50.3%) and exposure time (34.2 to 53.1%). An increase in control was seen in increased pH (94.7 to 97.9 and 88.7 to 90.1%) as well as with an increased exposure time (94.3 to 97.9 and 84.7 to 92.5%), for lemons and navels respectively. A slight decrease in control were noted for both these cultivars, respectively (97.2 to 95.7 and 90.3 to 88.5%), when temperatures increased from 23°C to 45°C. There were some missing values with the sporulation incidence of lemons, but it was found to be 83.5% at pH 3, 57.2% (15 s) *vs*. 46.0% (45 s) and 50.7% (23°C) *vs*. 50.2 (45°C). A decrease in sporulation incidence was seen for Clementines and navels, respectively with pH 3 to 6 (63.8 to 27.2 and 77.2 to 4.6%), longer exposure time (49.2 to 41.4 and 47.4 to 36.2%) and increasing temperature (49.6 to 46.2 and 44.4 to 40.5%).

Thiabendazole

Addendum G

Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit

Mareli Kellerman, Arno Erasmus, Paul J.R. Cronje and Paul H. Fourie. 2014. *Postharvest Biology and Technology* (accepted for publication)

Green mould (caused by *Penicillium digitatum*) is a major cause of postharvest losses in citrus. Residue loading of thiabendazole (TBZ) with application methods typically used in South African packhouses and green mould control was studied. TBZ was applied curatively and protectively in dip, drench and wax coating treatments and fruit were inoculated with a TBZ-sensitive or a TBZ-resistant isolate of *P. digitatum*. The dip treatments consisted of TBZ concentrations of 0 – 2000 µg.mL^{-1}; fruit were dipped for 60 s at 22°C at a pH of 7. Residues differed between fruit batches and ranged from 0.5 – 1.7 µg.g^{-1} at 1000 µg.mL^{-1} TBZ. Curative dip treatments almost completely controlled green mould (>96% at 1000 µg.mL^{-1} TBZ). The residue level needed for 75% curative control ranged from 0.06 to 0.22 µg.g^{-1}, depending on citrus type. Protective treatments were unreliable and control varied from 17 to 97.9% at 1000 µg.mL^{-1} TBZ between fruit batches. Drench treatments consisted of

exposure times of 30, 60 and 90 s with 1000 or 2000 µg.mL^{-1} TBZ. Average TBZ residues were 2.14 µg.g^{-1} for Clementine mandarin fruit and 3.50 µg.g^{-1} for navel orange fruit. Green mould control on navel orange fruit resulted in 66 – 92%, 34 – 90% and 9 – 38% control for curative treatment after 6 and 24 h and protective treatments, respectively, depending on fruit batch. Wax with 4000 µg.mL^{-1} TBZ was applied at 0.6, 1.2 and 1.8 L wax.ton^{-1} fruit. Chilling injury was evaluated after fruit storage at -0.5°C for 40 days. Average TBZ residues loaded was 1.3, 1.3 and 2.7 µg.g^{-1} at the recommended 1.2 L.ton^{-1} for Satsuma mandarin, Clementine mandarin and Valencia orange fruit, respectively. Protective treatments showed lower infection levels (14 – 20%) than curative treatments (27 – 40%) for Valencia orange fruit. The same trend was observed with Satsuma (92 – 95% curative; 87 – 90% protective) and Clementine mandarin fruit (82 – 90% curative; 59 – 88% protective), but control was relatively poor. TBZ application in wax exceeded 5 µg.g^{-1} at higher wax loads (1.2 and 1.8 L/ton). Wax treatments showed a significant reduction in chilling injury; TBZ had an additive effect. TBZ resistant isolates could not be controlled.

<u>Pyrimethanil</u>

Addendum H

Optimal use of pyrimethanil, a new postharvest fungicide, for the control of green mould on citrus in South Africa

Hons.BSc. project report of Elbie Liebenberg (Stellenbosch University)

South Africa is currently the second largest exporter of fresh citrus fruit in the world. *Penicillium digitatum*, the cause of green mould on citrus, is the number one cause of postharvest losses. Increasing development of resistance in *P. digitatum* populations to commonly used fungicides emphasises the need to investigate alternative fungicides. Pyrimethanil (PYR), a recently registered fungicide for postharvest use against *P. digitatum* on citrus fruit, with its unique mode of action, is an ideal alternative to currently used fungicides; specifically for its usefulness in resistance management programmes. In this study, baseline sensitivity for PYR against 44 *P. digitatum* isolates and *in vivo* benchmark PYR residue values for effective green mould control following curative and protective dip applications were determined. Effective concentrations where *in vitro* mycelial growth was inhibited by 50% (EC_{50}) and 95% (EC_{95}) were 0.354 and 0.515 µg.mL^{-1}, respectively. *In vivo* trials involved infection studies on fruit following curative (24-h-old wound infections) and protective dip-treatment (60 s at 18°C) in range of PYR solutions (0 to 1000 µg.mL^{-1}) and were repeated on satsuma, clementine and twice on navel fruit. PYR residue loading and levels of control varied between fruit types (1.404 to 2.954 µg.g^{-1} for 1000 µg.mL^{-1}treatment) and also within the two navel batches, but trends were generally similar, but with predicted maximum levels of 1.636 to 7.177 µg.g^{-1}. Curative control was significantly better than protective control. Mean benchmark PYR residue values for 50% and 75% curative control were 0.268 and 0.905 µg.g^{-1}, respectively, while 50% protective control was predicted at 1.181 µg.g^{-1}. Sporulation was poorly and inconsistently inhibited. PYR provided sufficient curative control and moderate protective control against green mould, and was able to control an isolate of *P. digitatum* resistant to commonly used imazalil and thiabendazole.

Addendum I

Evaluation of green mould control following pyrimethanil application in wax coatings and drench applications

Combined reports from 4th year and researcher projects, Plant Pathology, Stellenbosch University

Wax application. Single application of PYR with wax coating and in the drench was evaluated. The application of PYR at 2000 µg.ml^{-1} in wax coating in navel oranges resulted in lower residue loading (2.31 to 3.44 µg.g^{-1}) than when applied at 4000 µg.ml^{-1} (3.18 to 5.46 µg.g^{-1}). Similar trends were observed in Valencia oranges (2.21 to 3.56 µg.g^{-1}) and (4.22 to 8.39 µg.g^{-1}). Generally, in both curative and protective treatments, there was poor control of green mould and sporulation inhibition in all the treatments on navel oranges. On Valencia oranges, poor curative control was observed at 2000 µg.ml^{-1} (10 to 21%) and slightly better at 4000 µg.ml^{-1} (22 to 27%). However, protective treatments resulted in 33 to 38% control at 2000 µg.ml^{-1} and significantly improved control with increasing coating load (41 to 54%) at 4000 µg.ml^{-1}. Sporulation was reduced in the protective treatments, resulting in decreasing level of sporulation with increasing coating load (79 to 61%) and increasing concentration (68 to 51%).

Drench application. Laboratory trials clearly showed that drench application with PYR was not as good as dip or wax application. Compared with previous experience with dip and wax applications, protective control following drench application with PYR was relatively poor despite comparable residue levels loaded on treated fruit. Curative control was significantly better than protective control, but only when infected fruit were treated 6 hours after infection; treatment after 24 hours gave relatively poor control. Drench trials were repeated on fruit inoculated at varying inoculum concentrations [10^6 (as used in previous trials), 10^5, 10^4 or 10^3 spores.mL^{-1}], 24

hours before drench treatment with 1000 µg.mL^{-1} PYR. On all fruit types, inoculum concentration had a significant effect on wound infection. Significantly better control was achieved on all fruit types when inoculated with 10^3 spores.mL^{-1} (90.6 – 98.6% control), compared to 10^6 spores.mL^{-1} (32.6 – 71.8%); intermediate control levels were obtained at 10^4 and 10^5 spores.mL^{-1}. Fruit drenched with PYR had significantly lower sporulation levels than control fruit (82.7 *vs.* 98.2% incidence).

Drench application with fungicide mixtures. Fruit were drench-treated with a mixture of the fungicides. Fruit were treated curatively or protectively. Exposure time was 60 s. Different drench mixes were tested: TBZ (1000 µg.mL^{-1}); IMZ (500 µg.mL^{-1}); Guazatine (GZT; 1000 µg.mL^{-1}); PYR (1000 µg.mL^{-1}); Philabuster (PLB; 500 µg.mL^{-1} PYR + 500 µg.mL^{-1} IMZ); Guazatine + TBZ (1000 µg.mL^{-1} each); PYR + Guazatine (1000 µg.mL^{-1} each); TBZ, PYR + Guazatine (1000 µg.mL^{-1} each); TBZ, PYR, Guazatine + Sporekill (1000 µg.mL^{-1} each, 1 mL/L^{-1} Sporekill); TBZ + PYR (1000 µg.mL^{-1} each); Philabuster, Guazatine + TBZ (500 µg.mL^{-1} IMZ, 500 µg.mL^{-1} PYR, 1000 µg.mL^{-1} GZT, 1000 µg.mL^{-1} TBZ). PYR residues ranged from 0.6 to 4.7 µg.g^{-1}, TBZ residues ranged from 0.2 to 3.5 µg.g^{-1}, and IMZ residues ranged from 1.1 to 2.2 µg.g^{-1}, depending on fungicide mixture. Curative control was generally better than protective control; however, in the second trial, protective control was similar to curative control. Treatments containing imazalil (IMZ, PLB, PLB + GZT + TBZ) generally provided the best curative and protective green mould control (47.9 – 96.5%) depending on fruit batch. Mixtures including TBZ and PYR (TBZ+PYR, TBZ+PYR+GZT, TBZ+PYR+GZT+SK) also resulted in good curative control (54.9 – 94.3%), and some protective control (27.8 – 98.5%), depending on fruit batch. In the first trial, sporulation incidence was lowest where IMZ was part of the mix curatively (38.9%, 72.2 and 20.0% for IMZ, PLB and PLB + GZT + TBZ, respectively) and more so protectively (0.0, 37.5 and 13.8% respectively). In the second trial, sporulation incidence was not lowered by any treatments.

Addendum J

Commercial drench evaluation

Investigation of a commercial drench using fluorescent pigment and residue results showed that factors such as bin position, fruit orientation and inclusion of Sporekill had significant effects. Without Sporekill (and its adjuvant effect), deposition and residues on lower halves of fruit was poorer than upward facing halves. Similar residue levels were loaded in all bins, whether stacked in the bottom, middle or top. However, green mould was more effective in top bins and improved with the addition of Sporekill. Two packhouses were evaluated in terms of residue loading in the drench in the Eastern Cape. Residues at packhouses A decreased sharply from the first bin drenched to the last bin drenched (6.4 µg.g^{-1} PYR at bin 1 and 0.5 µg.g^{-1} at bin 300; 5.8 µg.g^{-1} TBZ at bin 1 and 0.1 µg.g^{-1} TBZ at bin 300 for navel oranges). No difference in residue loading or green mould control was observed between the normal and top-up drench (94 – 98.5% for navel oranges). Packhouse B performed better in loading consistent residues over the course of 300 bins per drench mix (2.6 µg.g^{-1} PYR at bin 1 and 1.6 µg.g^{-1} at bin 300; 1.3 µg.g^{-1} TBZ at bin 1 and 0.9 µg.g^{-1} TBZ at bin 300 for navel oranges). This indicates the effect that the drench specifications and management practices during drenching can have on effective fungicide application. Further work needs to be done to assess the value of of adjuvants in drench mixtures, a top-up system, as well as to ascertain the rates of residue stripping from drench mixes in order to accurately and safely conduct top-up.

4. *New objective*: Development of a user-friendly fungicide resistance assay for packhouses

Addendum K

Classification and quantification of imazalil resistance in *Penicillium digitatum*

Chapter 3 in MSc.Agric thesis of Mareli Kellerman (Stellenbosch University)

Soon after the introduction of imazalil (IMZ) as a postharvest fungicide on citrus, IMZ resistant isolates of *Penicilium digitatum*, the causal agent of green mould, were identified with varying frequencies in citrus packhouses around the world. Imazalil resistance is a quantitative trait with multiple genes involved. Most information is available on the *Cyp51* gene family, where resistance is caused by the insertion of DNA elements in the promoter region of the *Cyp51A* or *Cyp51B* genes, which results in overexpression of the genes. Based on the insertions in the promoter region, these isolates can be classified into three groups, R1, R2 and R3. The relative prevalence of these resistance groups in IMZ resistant *P. digitatum* populations is largely unknown in most citrus producing countries. In this study, the resistance group of 230 IMZ resistant *P. digitatum* isolates from green moulded citrus fruit from several countries (South Africa, the USA, Uruguay, Spain, Israel, Cyprus, Chile, Australia and Argentina) was determined using a published multiplex PCR assay. The resistance group of 189 isolates was successfully determined, but 41 of the isolates could not be classified into any of the three groups, indicating that other uncharacterised resistance groups might be involved in IMZ resistance in *P. digitatum*. It was also shown for the first time that some of the latter isolates contain the *Cyp51B* gene only.

Isolates from the USA showed the highest resistance group diversity, with 13.7% of 73 isolates identified as R1, 12.3% R2 and 56.2% as R3, and 17.8% unclassified. From Chile, 1.5% of 67 isolates were R1, 76.1% were R3 and 22.4% were unclassified. All the isolates from the other countries were classified into the R3 group (83.3%) or were unclassified (16.7%). Due to the prevalence of the R3 group in all countries, a real-time PCR assay was developed for quantifying IMZ resistance in *P. digitatum* populations. The developed assay, which targets the promoter region involved in mediating resistance via the *Cyp51B* gene, was successfully optimized using mycelial DNA. However, quantification from spore suspensions could not be conducted reliably, as was observed for quantification of spores from filter paper spore traps and spore suspensions. Since previous reports showed that *Penicillium*-selective PDA-plate assays can be used for quantifying fungicide resistance in packhouses, this approach was also evaluated. The exposed plate assay was not reliable, since false positives were problematic for both of the evaluated selective media.

Conclusions to date

The conclusion to date from this study is briefly highlighted below as recommendations currently applied by industry:

Imazalil (IMZ)

- IMZ is more curative in aqueous dip application than protective with poor sporulation inhibition
- IMZ is more protective in wax application than curative with good sporulation inhibition
- Double application of IMZ is important to get both curative and protective action plus sporulation inhibition
- Imazalil sulphate is pH sensitive in terms of residue loading in combination with increased exposure time and/or solution temperature. At pH 3 exposure time and solution temperature has no effect on residue loading. Increasing the pH to 6 resulted in steep increases in residue loading as exposure time and/or temperature increased.
    - At pH 3 exposure time should be at least 60 s and solution temperature appeared not to be important in terms of green mould control
    - At pH 6 exposure time should be ≤ 45 s at solution temperatures between 23 and 45°C
    - Solution pH levels of 8 is not recommendable and should be avoided
- Post dip brushing can reduce IMZ residue loading by 80% when the solution pH level was at 3; increasing this level to 6 reduced the loss from brushing to 50%. The residue stripping did not affect curative control, but reduced sporulation inhibition.
- Large wounds loaded higher residue levels and infections in these wounds were better controlled than in small wounds.
- Increasing age of infection (time from harvest to treatment) had a detrimental effect on the curative ability of IMZ, which was also influenced by fruit susceptibility. Oranges should be treated within 24 h and soft citrus within 12 h.
- Imazalil residue levels of ≥ 2 $\mu g.g^{-1}$ gives good curative and protective control and sporulation inhibition of an imazalil sensitive isolate of *Penicillium digitatum*.
- IMZ resistant isolates could never be adequately controlled with IMZ, with the only exception being young infections (4 – 6 h) where higher residue levels (≥ 2 $\mu g.g^{-1}$) gave good control.

Thiabendazole (TBZ)

- TBZ applied in drench can load residues levels of > 2.00 $\mu g.g^{-1}$, but is more curative on younger (6 h) than older green mould infections (24 h). TBZ applied in drench gave poor protective control.
- TBZ applied in dip without brushing resulted in residue levels of < 2.00$\mu g.g^{-1}$, but is highly effective curatively, but gave poor protective green mould control.
- TBZ applied in wax results to residue levels of ≈ 2.00 $\mu g.g^{-1}$. Control was inconsistent and better protective than curative.
- TBZ reduced chilling injury better when applied in a dip treatment than in wax.

Pyrimethanil (PYR)

- PYR applied in drench resulted in residue levels of 2 to 8 $\mu g.g^{-1}$. Curative control is better on young infections (6 h) than on older (24 h) infections and protective control is poor regardless of infection age.

- PYR drench applications gave poor to average green mould control when inoculations were done using high inoculum loads; however, at lower inoculum loads control was very good, especially curative control.
- Evidence of synergistic action was observed between TBZ and PYR, as drench mixtures with these active gave improved green mould comparable with IMZ containing drench mixtures.
- PYR applied in dip without brushing resulted in residue levels of ≈ 2 $\mu g.g^{-1}$. These levels gave very good curative control and relatively poor protective control.
- PYR applied in wax resulted in residue levels of > 2.00 $\mu g.g^{-1}$, but gave poor curative and protective control and sporulation inhibition. From these results, PYR cannot be recommended for application in wax.

Commercial drench application

- Marked differences were observed between the commercial drenches evaluated. Factors such as flow rate, bin stacking, agitation and inclusion of adjuvants or anti-foaming agents played very important parts in effective fungicide application and should be carefully considered. These factors will be researched in continuing research.
- In triple stack drenches, exposure of fruit in middle and bottom bin was poorer than top bins.

Fungicide resistance

- IMZ and TBZ resistant strains could not be controlled by these fungicides, but could be controlled by PYR.
- Monitoring of fungicide resistance in packhouses is very important to effectively manage the situation and sustainably control green mould. A conventional resistance monitoring technique was evaluated in this study and a cutting-edge PCR technique was developed.

Future research

This research has mostly been concluded and will be scientific published. Research gaps have been identified and three project proposals have been accepted for CRI funding from April 2014:

- Project 1102 - Quantification of imazalil resistance in *Penicillium digitatum* populations in citrus packhouses.
- Project 1103 - Optimisation of postharvest drench application of fungicides on citrus fruit.
- Project 1104 - Further optimisation of in-line aqueous fungicide application in citrus packhouses.

Technology transfer

Research papers published:

1. Arno Erasmus, Cheryl L. Lennox, Hennie Jordaan, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2011. Imazalil residue loading and green mould control in citrus pack-houses. Postharvest Biology and Technology 62: 193–203.
2. Arno Erasmus, Cheryl L. Lennox, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2013. Imazalil residue loading on citrus fruit as affected by formulation, solution ph and exposure time in aqueous dip treatments. *Postharvest Biology and Technology* 77: 43–49.
3. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2013. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. *Postharvest Biology and Technology* 77: 102–110.
4. Ncumisa S. Njombolwana, Arno Erasmus, J. Gideon van Zyl, Wilma du Plooy, Paul J.R. Cronje and Paul H. Fourie. 2013. Effects of citrus wax coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges. *Postharvest Biology and Technology* 86: 362- 371.
5. Mareli Kellerman, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2014. Thiabendazole residue loading and control of green mould and chilling injury on citrus. *Postharvest Biology and Technology* (Accepted).

Presentations at international congresses:

1. Paul Fourie, Ncumisa Njombolwana, Arno Erasmus and Keith Lesar. 2011. Postharvest citrus green mould control in South Africa: integration of aqueous and wax fungicides application systems. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.

2. Ncumisa Njombolwana, Arno Erasmus and Paul Fourie. 2011. Evaluation of protective and curative control of *Penicillium digitatum* following imazalil application in wax. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.
3. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2011. Aqueous imazalil sulphate application in citrus packhouses, residue loading and green mould control. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.
4. Paul H. Fourie, GC Schutte, S Serfontein, SH Swart. 2011. Modelling of *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards. Oral presentation at the American Phytopathological Society Congress, 6-10 August 2011, Waikiki, Hawaii, USA (Phytopathology Vol. 101, No. 6 (Supplement), 2011 S54).
5. Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.
6. Njombolwana N.S., Erasmus A., and Fourie P.H. 2012. Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.
7. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.
8. Njombolwana, N.S., Erasmus, A., and Fourie, P.H. 2012. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.
9. Arno Erasmus, Vongani Rikhotso, Cheryl L. Lennox, Keith Lesar and Paul H. Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.
10. Mareli Kellerman, Ilse Beukes, Lindy J. Rose, Altus Viljoen, Geert de Wever, Arno Erasmus and Paul H. Fourie. 2013. Genotyping imazalil resistance in an international collection of *Penicillium digitatum* isolates. Poster presentation at the 2013 APS-MSA joint meeting, 10-14 August 2013, Austin, Texas, USA.

Presentations at local congresses:

1. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2010. Citrus green mould control by improved imazalil residue loading in fruit dip applications. Oral presentation at CRI Citrus Research symposium (Drakensberg, Aug 2010).
2. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2010. Imazalil *versus* green mould: effect of incubation time on curative control. Poster presentation at CRI Citrus Research symposium (Drakensberg, Aug 2010).
3. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2011. The efficacy of imazalil sulphate dip applications for the control of citrus green mould. Oral presentation at 47th SASPP conference (Kruger Park, Jan 2011).
4. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2011. Evaluation of protective and curative control of *Penicillium digitatum* following imazalil application in wax. Oral presentation at 47th SASPP conference (Kruger Park, Jan 2011).
5. Arno Erasmus, Vongani Rikhotso, Cheryl L. Lennox, Keith Lesar and Paul H Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
6. N.S. Njombolwana, A. Erasmus, P.H. Fourie. 2012. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
7. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
8. E. Liebenberg, A. Erasmus, P.H. Fourie. 2012. Optimal use of pyrimethanil, a new postharvest fungicide, for the control of green mould on citrus in South Africa. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
9. W. du Plooy, A. Erasmus, C. Jewell, P. Fourie. 2012. A Heated Imazalil Flooder – New technology for South African packing houses. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.

10. Ncumisa S. Njombolwana, Arno Erasmus, Paul Cronje, Wilma du Plooy and Paul H. Fourie. 2013. The effect of wax coating and brush type on imazalil residue loading and citrus green mould control. Poster presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.
11. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.
12. Erasmus, A., Lennox, C.L., Jordaan, H., Smilanick, J.L., Lesar, K., Fourie, P.H., 2013. Optimising imazalil application in citrus packhouses. Post-harvest Innovation Programme: Symposium, Spier Estate, South Africa, 19 November, p 27

Other CRI extension fora:

- CRI Postharvest Technical Forum regional workshops
  - 2009
    - Arno Erasmus, Jan-Cor Brink, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. Current status and optimisation of imazalil application in citrus packhouses.
  - 2010
    - Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. Optimisation of imazalil application in citrus packhouses.
  - 2011
    - Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. The efficacy of imazalil sulphate dip applications for the control of citrus green mould.
    - Ncumisa Njombolwana, Arno Erasmus, Wilma du Plooy, Paul Fourie. Evaluation of protective and curative control of *Penicillium digitatum* following imazalil application in wax.
  - 2012
    - Arno Erasmus, Ncumisa Njombolwana, Elbie Liebenberg, Mareli Kellerman, Keith Lesar and Paul H. Fourie. Fungicide application and residue loading.
    - Arno Erasmus, Ncumisa Njombolwana, Elbie Liebenberg, Mareli Kellerman, Keith Lesar and Paul H. Fourie. Management of critical control points.
    - Arno Erasmus, Ncumisa Njombolwana, Elbie Liebenberg, Mareli Kellerman, Keith Lesar and Paul H. Fourie. Fungicide options and resistance management.
  - 2013
    - N. Njombolwana, A. Erasmus, M. Kellerman, C. Mcaleer, S.B. Coetzee, D. Joubert, L. Korsten, P. Fourie. Evaluation of a commercial drench application system.
    - Arno Erasmus, Ncumisa Njombolwana, Keith Lesar and Paul Fourie. The fungicide dip tank.
  - 2014
    - Arno Erasmus, Ncumisa Njombolwana, Keith Lesar and Paul Fourie. The fungicide dip tank.
    - Mareli Kellerman, Arno Erasmus, Paul Fourie. Drench research feedback.
    - Mareli Kellerman, Arno Erasmus, Ilze Beukes, Lindy Rose, Geert de Wever, Adele McLeod and Paul Fourie. Fungicide resistance management.
- SA Fruit Jourtnal articles
  - Hannes Bester, Hennie le Roux, Keith Lesar, Arno Erasmus en Paul Fourie. 2011. Na-oes bederf van sitrusvrugte: duur lesse uit die 2010 pakseisoen. *SA Fruit Journal* April/May 2011.
- Cutting Edge
  - No. 63: Responsible use of fungicides in citrus packhouses - Paul Fourie and Keith Lesar
  - No. 69: Philabuster Recommendations - Keith Lesar and Paul Fourie
  - No. 80: pH Adjustment in the imazalil hot water bath - Keith Lesar and Paul Fourie
  - No. 177: Pre-Packhouse and Packhouse Treatment Recommendations for 2014 - Keith Lesar and Arno Erasmus

4.5.4 FINAL REPORT: Practical impact of imazalil resistance on control of postharvest citrus green and blue mould

Project 1034 (June 2011-March 2013) by Arno Erasmus (CRI-Nelspruit) and Paul Fourie (CRI-USPP)

Summary

Citrus green and blue mould, caused by *Penicillium digitatum* (PD) and *P. italicum* (PI), respectively, are mostly controlled by means of postharvest fungicide applications. Currently, IMZ is regarded as the most effective

fungicide in use. Effective IMZ concentrations that inhibit 50% (EC_{50}) growth of nine PD and five PI isolates were assessed *in vitro* and the various isolates categorized according to their resistance (R) factors. Effective residue levels that provided 50% curative ($ER_{50}C$) and protective ($ER_{50}P$) control of these isolates were determined *in vivo*. All the PI isolates were sensitive, having EC_{50} values of 0.005 - 0.050 µg.mL^{-1}. Three PD isolates were sensitive (0.027 – 0.038 µg.mL^{-1}), while one resistant isolate was categorized as low resistant (R-factor of 19), one as moderately resistant (R-factor of 33.2), three as resistant (R-factor of 50 - 57.6) and one as highly resistant (R-factor of 70.7). Sensitive PD isolates had mean $ER_{50}C$ and $ER_{50}P$ values on Valencia orange fruit of 0.29 and 0.20 µg.g^{-1}, and 0.33 and 0.32 µg.g^{-1} on navel fruit, respectively. ER_{50} values for resistant isolates did not always correlate with EC_{50} values and ranged from 1.22 – 4.56 µg.g^{-1} for $ER_{50}C$ and 1.00 – 6.62 µg.g^{-1} for $ER_{50}P$ values. $ER_{50}P$ values for resistant isolates could not be obtained on navel orange fruit, but $ER_{50}C$ values (1.42 – 1.65 µg.g^{-1}) were similar to those obtained on Valencia fruit. The PI isolates all behaved similar to the sensitive PD isolates with $ER_{50}C$ and $ER_{50}P$ values on navel and Valencia fruit < 0.38 µg.g^{-1}. Alternative fungicides were assessed for the control of an IMZ sensitive, resistant and highly resistant PD isolate; these included sodium *ortho*-phenylpenate (SOPP), thiabendazole (TBZ), guazatine (GZT), imazalil (IMZ), pyrimethanil (PYR) and Philabuster® (PLB; a combination of IMZ and PYR), fludioxonil (FLU), azoxystrobin (AZO), Graduate®A^{+} (a combination of FLU and AZO) and propiconazole (PPZ). Multiple fungicide resistance was shown to IMZ, GZT, TBZ and PPZ in both resistant isolates. For the sensitive isolates, IMZ, SOPP, TBZ, GZT and PLB provided best curative control, while IMZ, GZT and PLB provided best protective control. For the IMZ-resistant isolates, SOPP, PYR and PLB gave the best curative control, while none of the fungicides provided adequate protective control.

Opsomming

Sitrus groen- en blouskimmel, veroorsaak deur onderskeidelik *Penicillium digitatum* (PD) en *P. italicum* (PI), word meestal beheer deur middel van naoes swamdoder programme. Tans word imazalil (IMZ) beskou as die mees doeltreffende swamdoder in gebruik. Effektiewe IMZ konsentrasies wat 50% (EK_{50}) groei inhibeer is *in vitro* vir nege PD en vyf PI isolate bepaal waarna die verskillende isolate volgens hul weerstands (W) faktore geklassifiseer is. Effektiewe residuvlakke wat 50% kuratiewe ($ER_{50}K$) en beskermende ($ER_{50}B$) beheer bied van hierdie isolate is *in vivo* bepaal. Al die PI isolate was sensitief, met EK_{50} waardes van 0.005-0.050 µg.mL^{-1}. Drie PD isolate was sensitief (0.027-0.038 µg.mL^{-1}), terwyl een bestande isolaat geklassifiseer was as ŉ laag weerstandbiedend (R-faktor van 19), een as matig weerstandbiedend (R-faktor van 33.2), drie as weerstandbiedend (R-faktor van 50-57.6) en een as hoogs weerstandbiedend (R-faktor van 70.7). Sensitiewe PD isolate het gemiddelde $ER_{50}K$ en $ER_{50}B$ waardes op Valencia lemoen vrugte van onderskeidelik 0.29 en 0.20 µg.g^{-1}, en 0.33 en 0.32 µg.g^{-1} op nawel lemoen vrugte gehad. ER_{50} waardes vir weerstandbiedende isolate het nie altyd ooreenstem met EK_{50} waardes nie en het gewissel tussen 1.22 – 4.56 µg.g^{-1} vir $ER_{50}K$ en 1.00-6.62 µg.g^{-1} vir $ER_{50}B$. $ER_{50}B$ waardes vir die weerstandbiedende isolate kon nie bereken word op nawel lemoen vrugte nie, maar $ER_{50}K$ waardes (1.42 – 1.65 µg.g^{-1}) was soortgelyk aan dié op Valencia lemoen vrugte. Die PI isolate was soortgelyk aan die sensitiewe PD isolate met $ER_{50}K$ en $ER_{50}B$ waardes op nawel en Valencia lemoen vrugte van < 0.38 µg.g^{-1}. Alternatiewe swamdoders is vir die beheer van ŉ IMZ sensitiewe, ŉ weerstandbiedende en ŉ hoogs weerstandbiedende PD isolaat ondersoek; bogenoemde het ingesluit natrium orto - phenylpenate (SOPP), tiabendasool (TBZ), guazatine (GZT), imasalil (IMZ), pyrimethanil (PYR) en Philabuster® (PLB, ŉ kombinasie van IMZ en PYR), fludioksonil (FLU), azoxystrobin (AZO), Graduate®A^{+} (ŉ kombinasie van FLU en AZO) en propikonasool (PPZ). Veelvuldige weerstandbiedendheid teen swamdoders is vir IMZ , GZT , TBZ en PPZ getoon in beide weerstandbiedende isolate. Vir die sensitiewe isolaat het IMZ , SOPP , TBZ , GZT en PLB die beste kuratiewe beheer getoon, terwyl IMZ, GZT en PLB die beste beskermende beheer getoon het. Vir die IMZ -weerstandbiedende isolate het SOPP, PYR en PLB die beste kuratiewe beheer getoon, terwyl geeneen van die swamdoders voldoende beskermende beheer kon gee nie.

Introduction

See Addendum A

Objectives

1. Determining the benchmark residue levels for control of *P. digitatum* and *P. italicum*
2. Determing the EC50 and EC95 values of 9 *P. digitatum* and 7 *P. italicum* isolates
3. Evaluate the efficacy of other registered fungicides on IMZ resistant isolates of *P. digitatum* and *P. italicum*

Materials and methods

Please see Addendum A.

Results and discussion
Please see Addendum A

Objective / Milestone	Achievement
1. Determining the benchmark residue levels for control of *P. digitatum* and *P. italicum*	This has been determined as effective residue levels that will give 50% curative ($ER_{50}C$) and 50% protective ($ER_{50}P$) control. This was done on Valencia and navel oranges.
2. Determing the EC50 and EC95 values of 9 *P. digitatum* and 7 *P. italicum* isolates	The EC50 values for the mentioned isolates have been determined.
3. Evaluate the efficacy of other registered fungicides on IMZ resistant isolates of *P. digitatum* and *P. italicum*	This has been done with guazatine, thiabendazole, sodium-orthophenylphenol, pyrimethanil, fludioxonil, azoxystrobin, Philabuster®, Graduate® A^+, propiconazole, on Valencia and Navel oranges.

Future research

This research has been concluded and is presented as a chapter in a PhD study and will also soon be submitted for publication in a peer reviewed scientific journal. Research gaps have been identified a project proposal has been accepted for CRI funding from April 2014:

- Project 1102 - Quantification of imazalil resistance in *Penicillium digitatum* populations in citrus packhouses.

Technology transfer

Research paper to be submitted for publication in 2014:

1. Arno Erasmus, Cheryl L. Lennox, Keith Lesar, Paul H. Fourie. 20XX. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Postharvest Biology and Technology.

Presentations at international congresses:

1. Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.

Presentations at local congresses:

1. Arno Erasmus, Vongani Rikhotso, Cheryl L. Lennox, Keith Lesar and Paul H Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.

Other CRI extension fora:

- CRI Postharvest Technical Forum regional workshops
  2012. Arno Erasmus, Ncumisa Njombolwana, Elbie Liebenberg, Mareli Kellerman, Keith Lesar and Paul H. Fourie. Fungicide options and resistance management.

Addendum A

Chapter 5 in PhD dissertation of Arno Erasmus

PRACTICAL IMPACT OF IMAZALIL RESISTANCE ON CONTROL OF POSTHARVEST CITRUS GREEN AND BLUE MOULD

1. Introduction

South Africa is the largest exporter of shipped fresh citrus fruit worldwide (Edmonds, 2013). Due to harvest and postharvest handling processes fruit can incur injuries and these make the fruit susceptible to green and blue mould (Rose et al., 1951). Green mould is caused by the pathogen *Penicillium digitatum* (Pers.:Fr.) Sacc (PD) (Smith, 1897; Eckert and Eaks, 1989). It is regarded as one of the major causes of losses due to decay on South African export fruit (Pelser, 1977). *Penicillium italicum* Wehmer (PI), which causes blue mould, is often neglected in literature due to the main focus mostly being on PD. *Penicillium italicum* is of economic importance due to being more adapted for growth and development at lower temperatures (< 4°C) than PD (Wyatt and Parish, 1995; Plaza et al., 2003) Shipping and storage temperature protocols require temperatures <10°C for the majority of exported citrus cultivars. Both these pathogens require a fresh wound as shallow as 0.25 mm for successful infection (Kavanagh and Wood, 1971). It takes as short as 4 h from germination to establishment of infection (Plaza et al., 2003) and a water-soaked lesion should be visible around the infection site 3 days after infection. Seven to 10 days after inoculation the whole fruit rind could be covered with sporulating green or blue mould (Smilanick et al., 2006). Except for the losses due to the actual decayed fruit, the sporulating infections also soil the neighbouring fruit and this has a further economic impact.

Due to long-distance export routes, the South African citrus industry has to rely on fungicides for the control of postharvest diseases. Currently there are six registered postharvest fungicides for the control of green mould in the South African citrus industry. Prochloraz, which is not favoured commercially, due to apparent reduced efficacy (Keith Lesar, pers. comm.). Sodium ortho-phenyl-phenol (SOPP) has been in use for > 50 years (Johnson et al., 2001) but is not commonly used in South Africa, mostly due to the possible risk of phytotoxicity if not managed well. Resistance to SOPP has already been reported in the early 1960s (Harding, 1962). Thiabendazole is widely used in South Africa and world-wide, especially in the drench and wax applications. It has been in use since the late 1960s, soon after which resistance development in green and blue mould populations were reported (Harding, 1972). Guazatine (GZT) is the only green mould fungicide that also has good curative action against sour rot (*Geotricum citri-aurantii*), but is only allowed in member countries of Codex Alimentarius (www.codexalimentarius.org). GZT has been in use since the early 1980s (Brown, 1988). Wild (1983) reported GZT resistance already in 1983. Imazalil (IMZ) is the most effective and reliable green mould fungicide currently in use. Laville (1977) reported first on IMZ's efficacy in the late 1970s and registered use started in the early 1980s (Bus et al., 1991); soon after the case of IMZ resistance was reported (Eckert, 1987). Pyrimethanil (PYR) has more recently been introduced as green mould fungicide. It became available for citrus use in California more than two decades after the registration of IMZ (Kanetis et al., 2007; Kanetis et al., 2008). The combination product, Philabuster® (PLB, which contains IMZ and PYR; Janssen PMP, Belgium) was developed and registered for use on citrus against green mould. Resistance to PYR has already been reported in field populations, but not yet from the packhouse environment (Kinay et al., 2007; Kanetis et al., 2008). Other active ingredients being evaluated for postharvest citrus use are fludioxonil (FLU) and azoxystrobin (AZO), and were found to have some activity against green mould (Kanetis et al., 2007; Kanetis et al., 2008). These researchers also showed that the combination of the two actives (FLU and AZO) had potential. Graduate®A$^+$ [GRA); Syngenta, USA] has been developed and may have some potential for the control of green mould. Fludioxonil is still new and being registered in certain countries (D'Aquino et al., 2013); no resistance have been reported yet to our knowledge. Finally, propiconazole (PPZ) was reported to have an action against green mould (McKay et al., 2012). The fungicides FLU, AZO, GRA and PPZ are not registered in South Africa for postharvest use on fresh citrus fruit.

The majority (> 78%) of South African packhouses apply IMZ in a sulphate formulation by means of a fungicide dip tank (Erasmus et al., 2011 / Chapter 2). This application gives variable results in terms of residue loading due to differences in exposure time, solution pH and concentration. A survey conducted by Erasmus et al. (2011; Chapter 2) indicated that the median residue level loaded was 1.02 µg.g^{-1} with the lowest level at 0.24 µg.g^{-1} and the highest level at 3.85 µg.g^{-1}. The ideal IMZ residue level for control and sporulation inhibition is regarded as 2 µg.g^{-1} (Kaplan and Dave, 1979; Brown et al., 1983; Brown and Dezman, 1990; Smilanick et al.,

1997; Erasmus et al., 2011 / Chapter 2). The maximum residue level (MRL) is 5 $\mu g.g^{-1}$, but certain markets demand even lower levels (Cranney, 2012). In previous studies, an IMZ residue level of ≈ 1 $\mu g.mg^{-1}$ was shown to give adequate control of an IMZ sensitive (S) isolate of PD and ≥ 2 $\mu g.mg^{-1}$ was needed to control 80 - 95% infections caused by an IMZ resistant (R) isolate (Erasmus et al., 2011, 2013 / Chapter 2 & 3). However, the previous studies were conducted using one S and one R isolate only and with 4 – 6 h incubation time. To our knowledge information on effective IMZ residue levels for control of PI is not documented.

The mechanism of IMZ resistance development is described as the over-expression of the gene P*d*CYP51, which will increase the amount of P405-dependant sterol 14α-demethylase, which will in its turn affect IMZ-sensitivity (Hamamoto et al., 2000b; Ghosoph et al., 2007a; Kiralj and Ferreira, 2008). Resistance to IMZ is polygenic and involves 21 genes on 8 loci and is linked with 6 groups; hence, it is theoretically more difficult for *Penicillium* to develop resistance to this fungicide (Laville et al., 1977). IMZ-resistant isolates of *P. digitatum* are generally less fit on fruit not treated with IMZ when compared to IMZ-sensitive isolates (Dave, Sales & Walia 1989; Holmes, Eckert 1995, Kinay et al. 2007). This loss of fitness was only evident when the IMZ-resistant isolate is in competition with the IMZ-sensitive isolate and is not well understood (Holmes, Eckert 1995).

In this study, a number of PD and PI isolates with various levels of sensitivity to IMZ were used to determine effective IMZ residue levels for the curative and protective control of both *Penicillium* species following IMZ application in fungicide dip tanks. Additionally, this study determined the efficacy of 9 active ingredients or combinations thereof as alternatives for IMZ, specifically against resistant isolates to understand their potential role in an anti-resistance fungicide program.

2. Materials and methods

2.1. Penicillium *isolates and inoculum preparation*

Nine PD and five PI isolates were used in trials done for this project. Seven PD and all five PI isolates were obtained from University Pretoria (UP; South Africa). The sensitive and resistant PD isolates used in previous work (Erasmus et al., 2011, 2013 / Chapter 2 & 3) were included in the study and were coded PD3 and PD5, respectively. The UP isolates were coded PD1, PD2, PD4, PD6, PD7, PD8 and PD9. The five PI isolates were coded PI1, PI2, PI3, PI4 and PI7.

In order to obtain inoculum for biological efficacy tests, the isolates were grown at ambient temperature on potato dextrose agar medium (PDA; Difco™ Potato dextrose agar, Becton, Dickinson and company, Sparks, USA) in Petri dishes and were re-plated in 2-week cycles. Conidia were harvested from 10- to 14-day-old cultures approximately 12 hours before trials commenced and stored at 4°C. The surface of a culture was washed with sterile deionised water amended with Tween 20 (Sigma-Aldrich, St Louis, Missouri, USA) at a concentration of 0.01 $mL.L^{-1}$. Spore suspensions were adjusted with a spectrophotometer (Cecil 1011, Cecil Instruments Limited, Peterborough, UK) to an absorbance of 0.1 at 420 nm, which correlates with a concentration of 1×10^6 $spores.ml^{-1}$ (Morris and Nicholls, 1978; Eckert and Brown, 1986). The conidial suspensions were placed on magnetic stirrers to maintain a uniform suspension of spores during inoculation.

2.2. IMZ sensitivity of the nine PD and five PI isolates

The effective concentration that inhibits 50% mycelial growth (EC_{50}) of the various isolates was determined *in vitro*. Potato dextrose agar was amended with IMZ (Imazacure, 750 $g.kg^{-1}$ SG, ICA International Chemicals, Stellenbosch, South Africa), which ranged in specific concentrations levels. Isolates were pre-screened for sensitivity and depending on the results, a specific range was used for each isolate. The ranges were 0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.05, 0.1, 0.5 and 1 $\mu g.mL^{-1}$ or 0, 1,1.5, 2, 2.5, 3, 4 and 5 $\mu g.mL^{-1}$. Spore suspensions with a concentration of 1×10^6 $spores.mL^{-1}$ were prepared for each isolate. A droplet of 20 µL spore suspension was placed in the middle of each specific amended PDA plate. The plates were incubated at 22°C for 5 days after which the colony diameters were measured twice perpendicularly. The percentage inhibition was calculated relative to colony diameters on unamended control plates for each specific isolate. Five replicate plates were prepared for each concentration per isolate and the trial was repeated three times. Percentage inhibition data of each isolate was regressed against IMZ concentration using non-linear regression with the function, Y = C/(1+Exp(-A-B*X)). The coefficient of determination (R^2) was used to demonstrate goodness of fit. EC_{50} value was calculated from the model for each isolate. This was done for each trial. The EC_{50} data from the three trials were subjected to analyses of variance and significant differences between the EC_{50} values of different isolates were compared by means of Fisher's test at a 95% confidence interval. All analyses were done using XLSTAT® (Addinsoft, www.xlstat.com). The resistance (R) factor for resistance isolates was determined by dividing the EC_{50} value of a resistant isolate by the mean EC_{50} value of the sensitive PD. From these results the isolates were arbitrarily categorised in terms of IMZ sensitivity as sensitive, low resistant, moderate resistant, resistant and highly resistant.

2.3. Effective IMZ residue levels for curative and protective control

2.3.1. Fruit

Untreated export quality Valencia sweet orange ('McClean' and 'Valencia Late') and navel orange ('Palmer' and 'Washington') fruit were obtained from various citrus packhouses in the Mpumalanga and Limpopo provinces of South Africa during the seasons of 2011 and 2012. Fruit were washed with an aqueous solution of 125 $\mu g.mL^{-1}$ didecyl dimethyl ammonium chloride (Sprorekill, ICA International Chemicals, Stellenbosch, South Africa) and left to dry before it was stored at 3.5 – 7°C for ±3 days. A day before a trial, fruit were transferred from cold storage to ambient in order for fruit temperature to reach ambient and to allow any condensation to evaporate.

2.3.2. Imazalil treatment

Fruit were treated with an IMZ sulphate formulation (Imazacure, 750 $g.kg^{-1}$ SG). A pilot trial was conducted first to determine the various IMZ concentrations needed to load a range of residue levels from very low (< 0.1 $\mu g.g^{-1}$) to very high (> 10 $\mu g.g^{-1}$) on the fruit following a 60-s dip at 22°C. From the pilot trial IMZ concentrations of 5, 10, 20, 40, 80, 160, 320, 640, 1280 and 2560 $\mu g.mL^{-1}$ were selected as the range that was used for treatments. The trial was repeated twice with all the PD isolates (PD1 – PD9) and three times with the PI isolates (PI1 – PI4 and PI7) on Valencia orange fruit. For comparative purposes, two isolates of PD (PD5 and PD9) were included with the PI isolates. Two similar trials were conducted on navel oranges with four PD isolates (PD3, PD5, PD6 and PD7) and two PI isolates (PI2 and PI4). Within each trial three replicate groups consisting of 12 fruit (six for curative control and six for protective control) were dipped at a time. Six extra fruit were added to two replicates for each treatment concentration per trial for IMZ residue analyses.

2.3.3. Residue analyses

Within 48 hours after each trial the fruit destined for residue analyses were prepared. For each replicate treatment, 6 fruit were cut into 4 equally sized pieces of which 3 were discarded and the rest weighed and macerated to a fine pulp by using a blender (Salton Elite Blender, Almalgamated Appliance Holdings Limited, Reuven, South Africa). The samples were frozen until analysis. Imazalil (chloramizol) residue analyses were conducted by Hearshaw and Kinnes Analytical Laboratory (Cape Town, South Africa). The samples were extracted using acetonitrile followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography tandem mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, California, USA). Residue values analysed from the different trials were subjected to an analyses of variance and Fisher's test at a 95% confidence interval using XLSTAT®.

2.3.4. Inoculation, incubation and evaluation

Fruit were treated protectively and curatively. Those destined for curative treatments were inoculated with the various isolates 24 h before treatment and those destined for protective treatment were first dip-treated, left to dry and then inoculated within ± 4 h after treatment. Fruit were simultaneously wounded and inoculated by dipping a stainless steel rod with a narrow, concave tip (2 mm long; 1mm diameter) into the spore suspension and then wounding the fruit rind through the flavedo into the top layer of the albedo. Four inoculated wounds were induced equal distances apart surrounding the calyx. After treatment and inoculation the fruit were incubated at 22°C. The fruit were incubated in lock back table grape cartons (APL cartons, Worcester, South Africa) on count SFT13 nectarine trays (Huhtamaki South Africa (Pty) Ltd, Atlantis, South Africa) and enclosed in transparent polyethylene bags.

Fruit were rated for infection and sporulation 5 and 10 days after inoculation, respectively. The number of infected wounds per fruit were evaluated using an ultra-violet light source (UV-A at 365 nm, Labino Mid-light; www.labino.com). Infected wounds could be identified as yellow fluorescence under UV light (Erasmus et al., 2011 / Chapter 2). Infection data were normalised to percentage control in relation to the untreated control. Sporulation was evaluated for each fruit as described by Erasmus *et* al. (2011; Chapter 2). Infected fruit were given a rating from 1 to 6 relating to the fruit area covered with green sporulation. Where 1 = infection with no sporulation, 2 = sporulation area less than 100 mm^2, 3 = sporulation area less than 50% of the fruit and more than 100 mm^2, 4 = sporulation area more than 50% of fruit and less than 75%, 5 = sporulation area more than 75% of the fruit and less 100%; and 6 = 100% covered with sporulating green mould. Infected fruit rated ≥ 4 were regarded as sporulating. Sporulation incidence per replicate (%) was calculated.

2.3.5. Calculating the effective residues for curative and protective control

Percentage control data for each replicate were regressed against residue levels of each specific trial using the non-linear function, Y = C/(1+Exp(-A-B*X)), in XLSTAT®. The coefficient of determination (R^2) was used to demonstrate goodness of fit. The effective residue levels for 50% curative or protective control ($ER_{50}C$ and $ER_{50}P$, respectively) were calculated from the model for each isolate. The ER_{50} levels determined for each isolate in each trial were subjected to analysis of variance and the differences compared by means of Fisher's test at a 95% confidence interval. Data were analysed separately for the PD isolates on Valencia orange fruit, the PI isolates on Valencia orange fruit and for the selected PD and PI isolates on Navel orange fruit.

2.4. Efficacy of alternative fungicides against IMZ resistant isolates of P. digitatum

Three PD isolates, an IMZ sensitive, resistant and highly resistant (as determined in 2.2), were used and inoculated as described in 2.3.4. Fruit were treated curatively and protectively with 9 fungicides: SOPP (SOPP Super 20%, Advantage Agri Products, Paarl, South Africa), TBZ (ICA TBZ, ICA International Chemicals), GZT (Citricure, ICA International Chemicals), IMZ (Imazacure SG, ICA International Chemicals), PYR (Protector, ICA International Chemicals), FLU (Scholar, Syngenta, Midrand, South Africa), AZO (Ortiva, Syngenta), PLB (Janssen PMP, Belgium), GRA (Syngenta) and PPZ (Tilt, Syngenta) were applied in at concentrations registered in South Africa or at experimental concentrations, as 60 s dip treatments (Table 8). Fruit were incubated and evaluated as described in 2.1.3. Trials consisted of three replicates with six fruit and each trial was repeated three times each on navel and Valencia oranges. Percentage control data were subjected to analysis of variance using XLSTAT® and Fisher's test to compare differences at a 95% confidence interval.

3. Results

3.1. IMZ sensitivity of nine PD and five PI isolates

Non-linear regression with the function Y = C/(1+Exp(-A-B*X)) resulted in very good fits for all isolates; R^2 values of > 0.84 (Table 1). There were significant differences between EC_{50} values of the various isolates ($P < 0.0001$; ANOVA not shown). PD5 had the highest level of resistance with an EC_{50} of 2.29 $\mu g.mL^{-1}$. PD1, PD4 and PD7 all had similar EC_{50} values (1.63, 1.87 and 1.62 $\mu g.mL^{-1}$, respectively; Table 1), but significantly lower than PD5. PD8 and PD2 had significantly lower EC_{50} levels than PD1, PD4 and PD7 and also differed significantly from each other (1.08 and 0.62 $\mu g.mL^{-1}$, respectively). PD3, PD6, PD9 and all the PI isolates had similar and significantly lower EC_{50} levels compared to the resistant isolates which ranged from 0.05 to 0.01 $\mu g.mL^{-1}$. Based on the Fisher's LSD test on EC_{50} values and their R factors the isolates were characterised as sensitive (PD3, PD6, PD9 and all the PI isolates), low resistant (PD2), moderately resistant (PD8), resistant (PD1, PD4 and PD7) and highly resistant (PD5).

3.2. Effective IMZ residue levels for curative and protective control

3.2.1. Valencia orange fruit - Penicillium digitatum

3.2.1.1. IMZ residue levels

Statistically similar residues were loaded following the 5 – 640 $\mu g.mL^{-1}$ treatments (0.18 – 1.26 $\mu g.g^{-1}$; results not shown), and significantly higher residue levels loaded following dips in 1280 $\mu g.mL^{-1}$ (5.43 $\mu g.g^{-1}$), and 2560 treatment (10.60 $\mu g.g^{-1}$).

3.2.1.2. ER_{50} levels

The R^2 values for the function, Y = C/(1+Exp(-A-B*X)), were > 0.75 indicating very good fits (Table 2). Analysis of variance for the IMZ $ER_{50}C$ and $ER_{50}P$ values for the nine isolates showed a meaningful action × isolate interaction ($P = 0.069$; ANOVA not shown). The three sensitive isolates clustered together having the lowest $ER_{50}C$ and $ER_{50}P$ values (ranging from 0.26 – 0.29 $\mu g.g^{-1}$ and 0.13 – 0.27 $\mu g.g^{-1}$, respectively; Table 2). For curative control, the low resistant, moderately resistant, one resistant (PD1) and the highly resistant isolates had similar $ER_{50}C$ values (ranging from 1.22 – 1.91 $\mu g.g^{-1}$); these values were 4- to 7-fold higher than those for the sensitive isolates but were not significantly different. The other two resistant isolates (PD4 and PD7) had significantly higher $EC_{50}C$ values (4.37 and 4.56 $\mu g.g^{-1}$, respectively) than all the isolates, but similar to the lower level of the highly resistant isolate (1.91 $\mu g.g^{-1}$). Protectively, the low resistant isolate clustered with two resistant isolates (PD4 and PD7) having significantly higher $ER_{50}P$ values (2.94 – 4.46 $\mu g.g^{-1}$) compared to the moderately resistant (PD8) and one of the resistant isolates (PD1) with values of 1.08 and 1.00 $\mu g.g^{-1}$, respectively. The highly resistant isolate (PD5) had the highest $ER_{50}P$ value (6.62 $\mu g.g^{-1}$), but not significantly higher than PD2 and PD7.

3.2.1.3. Sporulation

As certain treatments had zero infected fruit, data for the various sensitive and resistant isolates were pooled for statistical analyses. Analysis of variance for percentage sporulating fruit showed a significant isolate x concentration interaction (P = 0.032; ANOVA not shown). For the sensitive isolates there was generally no significant difference in sporulation levels on infected fruit between the untreated fruit and the IMZ treated fruit regardless of residue level (Table 3). Similarly, there was generally no statistical difference for the resistant isolates between the untreated and IMZ treated fruit. Interestingly, on fruit loaded with 0.18 and 0.24 $\mu g.g^{-1}$ significantly lower sporulation incidence (33.3 and 27.8%, respectively) was observed than on fruit loaded with 5.43 and 10.60 $\mu g.g^{-1}$ (80.6 and 79.6, respectively). A lower sporulation incidence was also evident for sensitive isolates on 0.18 $\mu g.g^{-1}$ loaded fruit. A meaningful effect for curative or protective action was also observed (P = 0.112). The curative treatments had generally higher levels (> 15% overall) of sporulating fruit compared to the protective treatments.

3.2.2. Valencia orange fruit - Penicillium italicum *and two comparative PD isolates*

3.2.2.1. Residue levels

IMZ residue levels loaded following dips in concentrations of 5 – 320 $\mu g.mL^{-1}$ increased from 0.07 – 0.61 $\mu g.g^{-1}$ (results not shown). The highest three dip concentrations loaded significantly higher residue levels and differed significantly from each other (1.15, 2.31 and 5.53 $\mu g.g^{-1}$ for 640, 1280 and 2560 $\mu g.mL^{-1}$, respectively).

3.2.2.2. ER_{50} levels

Data fitted the function, Y = C/(1+Exp(-A-B*X)), relatively well (R^2 > 0.68; Table 4). Analysis of variance for the IMZ ER_{50} values showed a significant action × isolate interaction (P = 0.0001; ANOVA not shown). The $ER_{50}P$ level for the highly resistant isolate, PD5 (4.38 $\mu g.g^{-1}$; Table 4), was significantly higher than its $ER_{50}C$ level (1.47 $\mu g.g^{-1}$). Both these values were significantly higher than the rest of the sensitive PD and PI isolates' $ER_{50}P$ and $ER_{50}C$ levels (0.09 - 0.20 $\mu g.g^{-1}$ and 0.11 – 0.26 $\mu g.g^{-1}$, respectively).

3.2.3. Navel orange fruit - Penicillium digitatum and P. italicum

3.2.3.1. Residue levels

IMZ residue levels loaded on navel orange fruit increased with treatment concentration: means of 0.13, 0.19, 0.22, 0.36, 0.56, 0.87, 1.37, 1.72, 3.09 and 5.64 $\mu g.g^{-1}$ were loaded for the 5, 10, 20, 40, 80, 160, 320, 640, 1280 and 2560 $\mu g.mL^{-1}$ treatments, respectively.

3.2.3.2. ER_{50} levels

The $ER_{50}P$ values for PD5 and PD7 could not be determined, due to low levels of control (≈4%; result not shown). Therefore, ER_{50} data were analysed separately for curative and protective action. The R^2 values were > 0.74 for lines that were fitted by means of the function, Y = C/(1+Exp(-A-B*X)) (Table 5 and 6). Analysis of variance for the $ER_{50}C$ levels for green and blue mould caused by the 4 PD and 2 PI isolates indicated significant differences between isolates (P = 0.001; ANOVA not shown). The highly resistant and resistant isolate, PD5 and PD7, had significantly higher $ER_{50}C$ levels (1.42 and 1.65 $\mu g.g^{-1}$, respectively) than the sensitive PD and PI isolates (0.21 – 0.37 $\mu g.g^{-1}$; Table 5); $ER_{50}C$ values for the sensitive PD and PI isolates did not differ significantly. Analyses of variance for the $ER_{50}P$ levels showed no significant difference (P = 0.213; ANOVA's not shown) between the $ER_{50}P$ levels for IMZ sensitive PD and PI isolates, which were 0.28 - 0.35 and 0.15 – 0.29 $\mu g.g^{-1}$, respectively (Table 6).

3.2.3.3. Sporulation inhibition

Similar to section 1.1.1. data for the sensitive PD, resistant PD and sensitive PI isolates were pooled for statistical analyses. Analyses of variance for percentage sporulation incidence data showed a significant isolate x concentration interaction (P < 0.0001; ANOVA not shown). For sensitive PD isolates, there was no significant difference in sporulation incidence between the untreated fruit and IMZ treated fruit with residues of 0.13 – 0.56 $\mu g.g^{-1}$, where these levels ranged from 94.4 - 100.0% (Table 7). Treatments that loaded higher residue levels (0.87 – 5.64 $\mu g.g^{-1}$) had significantly lower levels of sporulating fruit (76.9 – 26.4%). For the resistant PD isolates, no significant differences were found regardless of residue level and the sporulation incidence was > 90.0%. The sensitive PI isolates infected fruit showed a similar trend to the sensitive PD infected fruit, and significant reduction in sporulation incidence was observed from 0.19 – 1.72 $\mu g.g^{-1}$ (63.7 – 16.9%) compared with the untreated control (95.8%). The ANOVA showed a significant isolate x action (curative and protective) interaction (P = 0.006; ANOVA not shown). Sporulation incidence on infected fruit levels did not differ between

the curative and protective treatments when inoculated with resistant PD isolates (> 95.0%; results not shown), but curative and protective treatments of the sensitive PD isolates showed significantly lower sporulation levels (85.6 and 74.6%, respectively) and differed significantly from each other. The sensitive PI isolates showed significantly lower sporulation incidence for curative and protective treatments (46.9 and 42.3%, respectively).

3.3. Efficacy of alternative fungicides against IMZ resistant isolates of P. digitatum

3.3.1. Green mould control

Analysis of variance for percentage green mould control data showed a significant four factor interaction for citrus kind (navel and Valencia) × action (curative and protective) × isolate [sensitive (PD3), resistant (PD4) and highly resistant (PD5)] × fungicide (AZO, GRA, FLU, GZT, PLB, IMZ, PPZ, PYR, SOPP and TBZ) (P = 0.0002; ANOVA not shown). This difference could be mostly ascribed to Valencia trials having lower control levels compared to the navel trials. To simplify the interpretation the significant action x isolate x fungicide interaction was discussed (P < 0.0001) Curatively, SOPP, TBZ, GZT and PLB gave similar control levels to IMZ (> 90.0%; Table 8) of the sensitive isolate. PYR gave weaker control (78.0%) than these fungicides, but significantly higher than FLU, AZO, GRA and PPZ (< 39.0%). IMZ exhibited poor control of the two resistant isolates (35.3 and 30.3% for the resistant and highly resistant isolate, respectively). PPZ, TBZ and GZT treatment also resulted in very low control levels of these two isolates (< 39.0%). SOPP and PYR controlled the resistant isolates the best (> 90%). PLB gave ≈ 81.0% control of the resistant isolates, which was markedly lower than PYR and SOPP control of the resistant isolates, as well as PLB control of the sensitive isolate. The rest of the fungicides showed low control levels of < 48.0%.

Protectively, IMZ gave slightly poorer control (88.2%), but not significant poorer than its curative control of the IMZ sensitive isolate. GZT gave similar protective control (81.6%), while PLB gave the best protective control at 97.5%. SOPP gave much weaker protective control (19.6%) than curative, which could be ascribed to the rinsing of fruit immediately after SOPP treatment. TBZ and PYR gave significantly lower protective control (46.0 and 46.4%, respectively), compared to its curative treatments. FLU, AZO, GRA and PPZ gave slightly better protective control levels than curative, but even though it was significant in some cases the control levels were relatively low (<54.0%). IMZ, PPZ, TBZ and GZT failed to protectively control the IMZ resistant isolates (< 18.1%), while PLB also gave relative poor protective control (33.3 – 41.9%). GRA, PYR and AZO showed some protective control potential against the IMZ resistant isolates, but at varying levels (45.1 - 73.6%). Interestingly, control of the IMZ resistant isolates by these fungicides was markedly to significantly better than their control of the sensitive isolate (30.8 - 53.9%).

3.3.2. Sporulation

Sporulation data of one navel and three Valencia trials were combined. Analyses of variance for percentage sporulation incidence of infected orange fruit showed a significant action x fungicide and isolate x fungicide interaction were shown (P = 0.015 and < 0.0001, respectively; ANOVA not shown). However, the three factor isolate x action x fungicide interaction (P = 0.571) results are shown in Table 9. For the sensitive isolate, the levels of sporulation incidence on infected fruit were generally high (> 80.0), regardless of curative or protective treatment with most fungicides. However, IMZ treatment resulted in significant lower levels (62.8 and 69.4%, for curative and protective treatments, respectively), as well as curative and protective PLB treatment (23.6 and 2.8%, respectively). IMZ and PLB did not inhibit sporulation of the resistant isolates (>74.3%) and low levels of sporulation incidence were recorded only for SOPP (33.3%), but only in the curative treatments. For the rest of the fungicides the majority of levels were > 72.9 %, regardless of curative or protective treatment.

4. Discussion

Fungicide resistance monitoring and characterisation is generally conducted by means of *in vitro* growth studies (Staub and Sozzi, 1984; Russell, 2004). From these studies the baseline sensitivity for the given fungicide can be determined that forms the basis from where resistance development can be followed. In many citrus production regions including California (Holmes and Eckert, 1999), Florida (Brown, 1989), Uruguay (Pérez et al., 2011) and Morocco (Boubaker et al., 2009) the baseline sensitivity for IMZ has been established. All these studied determined the baseline sensitivity of IMZ to be < 0.05 $\mu g.g^{-1}$. To our knowledge no IMZ baseline has been determined for South Africa. *In* vitro fungicide sensitivity characterisation is rarely corroborated with *in vivo* characterisation (Wild, 1994; Kinay et al., 2007). Peréz et al. (2011) showed that isolates that would grow on 1 $\mu g.mL^{-1}$ IMZ *in vitro* would be able to overcome an IMZ residue of 3 $\mu g.g^{-1}$ loaded on citrus fruit; this was an attempt to establish the *in vitro* concentration by which practical resistance can be detected. This study is a first attempt to quantitatively categorise different PD and PI isolates based on *in vitro* as well as *in vivo* studies. It is

clearly shown that the practical impact of a specific resistant isolate may differ significantly from what could be predicted or expected from *in vitro* categorisation.

Only one of the sensitive PD isolates (PD3) originated from a secluded orchard that has never been exposed to any postharvest fungicides. More isolates that have never been exposed to imazalil are required to establish a proper IMZ baseline sensitivity level (Russell, 2004; Kinay et al., 2007). Nonetheless, the average EC_{50} value of the sensitive PD isolates in this study (0.03 $\mu g.g^{-1}$) relates well to baselines determined in other work (Kinay et al., 2007; Holmes and Eckert, 1999; Brown, 1989; Pérez et al., 2011).

Hamamoto et al. (2000) suggested that the *PdCYP51* gene can be expressed in increasing levels that will render higher levels of DMI resistance. Ghosoph et al. (2007) confirmed these findings and alluded to the same conclusion that the level of *PdCYP51* expression could be related to the level of IMZ resistance. So far three *CYP51* genes that contributes to IMZ resistance have been characterised: IMZ-R1 (Hamamoto et al., 2000), IMZ-R2 (Ghosoph et al., 2007b) and IMZ-R3 (Sun et al., 2013). Through molecular characterisation, all the resistant isolates in this study were characterised as the IMZ-R3 genotype (Mareli Kellerman, pers. comm.). From the EC_{50} values it was shown that the IMZ resistant isolates in our study had different levels of IMZ resistance with R-factors of 19 to 71. Theoretically, an isolate with an R-factor of >2 can be considered resistant (Delp and Dekker, 1985). R-factors typically vary from as low as 5 to 100 (Brent and Hollomon, 2007). R-factor values obtained in our study may be considered to be small when compared to those found with other fungicides, where the differences in terms of sensitivity levels are much wider (Staub, 1991). Even though the difference between the sensitive and resistant PD isolates in this study could be considered relatively low, the effect on disease control on fruit was quite substantial. Furthermore, the ER_{50} values, which were determined *in vivo* on fruit, did not fall into these distinct categories as determined *in vitro*.

The sensitive PD isolates had similar ER_{50} values regardless of action (curative of protective) treatment or citrus type (< 0.37 $\mu g.g^{-1}$). However, the $ER_{50}C$ and $ER_{50}P$ values of the resistant isolates could not be predicted from their EC_{50} values. On Valencia, two resistant isolates, PD4 and PD7, showed the highest level of resistance in curative treatment ($ER_{50}C$ levels of 4.37- 4.56 $\mu g.g^{-1}$), while the highly resistant isolate, PD5, had a significantly lower $ER_{50}C$ level of 1.91 $\mu g.g^{-1}$, which was similar to the low and moderately resistant isolates. For protective control, however, this highly resistant isolate, as well as the low resistant isolate had the highest $ER_{50}P$ values. The low resistant and highly resistant isolates behaved similarly on Valencia with lower $ER_{50}C$ (1.65 and 1.91 $\mu g.g^{-1}$, respectively) and higher $ER_{50}P$ values (4.46 and 6.62 $\mu g.g^{-1}$, respectively). The other resistant isolates had similar respective values for curative and protective ER_{50} values.

In vitro EC_{50} values did not correlate with *in vivo* ER_{50} values, and this was further complicated when different ER_{50} values were obtained from different citrus types. Infection levels of the resistant isolates on navel oranges were so severe that $ER_{50}P$ values could not be determined. The highly resistant isolate behaved similarly in curative treatments on navel and Valencia ($ER_{50}C$ values of 1.91 and 1.42 $\mu g.g^{-1}$, respectively), but the resistant isolate had lower ER_{50} values on navel compared with Valencia (1.65 and 4.56 $\mu g.g^{-1}$, respectively). In cases with high ER_{50} values, it could be assumed that a residue level of > 5 $\mu g.g^{-1}$ (exceeding the IMZ MRL) would be required to completely control the resistant isolates. Also the isolate that were categorised as low resistant proved to be "highly resistant" with an $ER_{50}P$ value of 4.46 $\mu g.g^{-1}$. If isolates with this level of resistance prevail in an environment where fruit are stored in a long term protocol and repacked; failure of control can be expected (Holmes and Eckert, 1999).

In vitro categorisation of fungal populations are commonly used as a diagnostic measure of fungicide resistance. For PD, a discriminatory concentration of 1.0 $\mu g.mL^{-1}$ IMZ amended PDA was suggested for commercial resistance monitoring (Pérez et al., 2011). Our work indicates that this level could be reduced to 0.5 $\mu g.mL^{-1}$. While none of the sensitive isolates in our study was able to grow at 0.5 $\mu g.mL^{-1}$, the low resistant PD isolate had an EC_{50} of 0.62 $\mu g.mL^{-1}$ and EC_{95} of 1.05 $\mu g.mL^{-1}$ (results not shown). As this isolate will be substantially inhibited at 1.0 $\mu g.mL^{-1}$, it might be regarded as sensitive in a discriminatory *in vitro* assay. Importantly, this isolate had ER_{50} values of 1.65 and 4.46 $\mu g.g^{-1}$ (for curative and protective treatment, respectively), which will cause loss of control in a packhouse as demonstrated in this study.

At the onset of the study, a collection of sensitive and resistant PI isolates was sourced for inclusion in the study. However, following *in vitro* and *in vivo* characterisation, all the PI isolates proved to be sensitive. Imazalil resistant isolates of PI are usually less prevalent than *P. digitatum* (Eckert, 1990; Bus et al., 1991; Holmes and Eckert, 1999). One reason for this could be due to the fact that PD grows much faster than PI at temperatures 15°C - 28°C (Eckert and Eaks, 1989). These temperatures are predominant during harvest time in most citrus regions. It was shown that resistant PD isolates were not less virulent compared to sensitive PD isolates, but less competitive (Holmes and Eckert, 1995).

Residue levels varied between the different trials. Imazalil residue levels on Valencia orange fruit were approximately double in the trial where all nine PD isolates were used compared to the trial where the PI isolates were involved (0.24 -10.6 $\mu g.g^{-1}$ compared to 0.07 – 5.53 $\mu g.g^{-1}$ in dip treatments that ranged from 5 – 2560

µg.mL^{-1} for the respective trials). The IMZ residue levels on navel oranges were similar to those on the second Valencia orange trial mentioned above (0.13 – 5.64 µg.g^{-1}). It is suspected that the fluctuation in residue loading can be ascribed to fluctuating municipal water quality, where an increase in the pH level of water can have an increasing effect on residue loading in an IMZ sulphate solution (Erasmus et al., 2011, 2013 / Chapter 2 & 3). Regrettably, the pH levels of IMZ sulphate solutions used in this study were not monitored. Despite variation in residue loading between fruit types and batches, the sensitive and highly resistant isolates (PD9 and PD5, respectively) were used as reference isolates and relatively comparable ER_{50} values were determined in the different trials.

This project was not specifically designed to study sporulation or its inhibition as was done in previous studies (Brown et al., 1983; Brown and Dezman, 1990); however, trends in sporulation inhibition were observed between treatments. On Valencia, no differences were found between curative and protective treatments, but there was a tendency for the resistant PD isolates to have lower sporulation incidences on infected fruit with lower residue levels (27.8 – 67.6% on 0.18 – 0.89 µg.g^{-1}), compared to the sensitive PD isolates (all > 66.2%). In contrast, sensitive PD infections showed lower levels of sporulation on navel fruit with higher residue levels (26.4 – 68.1% on 1.37 – 5.64 µg.g^{-1}), while resistant PD isolates had levels of > 90% sporulation regardless of residue level. The sensitive PI isolates had the lowest level of sporulation; on fruit with residues of 1.37 and 1.72 µg.g^{-1}, sporulation incidences were 12.0 and 16.9%, respectively. This apparently enhanced sporulation inhibition effect by IMZ could in part explain why resistant PI isolates are less prevalent than those of PD. The poor inhibition of sporulation on fruit infected with resistant isolates, regardless of residue level is problematic and shows that increasing the IMZ residue level in order to combat resistance is not an effective practice. This was also shown in previous studies where a residue of ≈ 5 µg.g^{-1} could not lead to sporulation inhibition of a resistant isolate (Erasmus et al., 2011, 2013 / Chapter 2 & 3; Eckert, 1990).

The conventional green mould fungicides (IMZ, SOPP, TBZ, GZT, PYR and PLB) gave excellent curative control (≥ 90.0%) against sensitive PD isolates. PYR and SOPP equalled this level of curative control of the resistant isolates. PLB also performed well with > 80% curative control of the resistant isolates. Protectively, only PLB, IMZ and GZT stood out giving levels of > 80.0% control of the sensitive isolate infections, while none of the fungicides were as effective against the resistant isolate infections. The highest protective control levels on these isolates resulted from PYR and GRA (≈ 60 and ≈ 70%, respectively). The protective action of green mould fungicide can possibly be improved by an alternative application method. This was the case for IMZ, where applicationin wax coatings resulted in better protective control compared to dip treatments (Njombolwana et al., 2013a, 2013b).

Multiple resistance was observed in two IMZ resistant isolates (PD4 and PD5). GZT, TBZ and PPZ were ineffective in controlling the IMZ resistant isolates, while providing significantly better control of the sensitive isolate. These fungicides have been in use longer than IMZ and resistance in the PD population is well known. Resistance to GZT and TBZ was reported in 1983 in Australia (WILD, 1983). To our knowledge, this is the first report of multiple resistance to IMZ, GZT, PPZ and TBZ in PD. Multiple resistance have been reported for IMZ, TBZ and SOPP (Holmes and Eckert, 1999). Imazalil and PPZ fall in the same DMI class, and cross resistance has been shown between these two fungicides (Mckay et al., 2012). Some indication of potential negative cross resistance was also observed where the IMZ resistant isolates were markedly better controlled with GRA, PYR and AZO than their respective control of the sensitive isolates. This will, however, have to be investigated further.

The poor control of the IMZ sensitive isolate with PPZ in our study could be due to the 24 h incubation period being too long. Good results were obtained on infection of up to16 h (McKay et al., 2012).

The older fungicide SOPP proved to be a very good alternative for curative control, but cannot be considered for protective control as fruit needs to be rinsed after treatment to prevent phytotoxicity. If the pH is controlled the risk of phytotoxicity may be reduced. The pH should be managed at a level of 12, and lowering the pH will increase residue loading (Dezman et al., 1986). Protective control by SOPP in our trials was relatively poor, but the pH was not adjusted to 12.

The excellent curative control by PYR reported in this work confirm other work showing this fungicide as a favourable IMZ alternative (Adaskaveg et al., 2005; D'Aquino et al., 2006; Smilanick et al., 2006). Unfortunately, relatively poor protective control was achieved.

PLB provided excellent curative and protective control of IMZ sensitive isolates and relatively good curative control of IMZ resistant isolates. However, the significantly weaker control of IMZ resistant isolates, especially protectively, was most probably due to IMZ resistance, as well as the lower PYR concentration in at the formulated product (500 µg.mL^{-1}), which is 50% lower that PYR is recommended as stand-alone product. Moreover, Lado et al. (2010) recommended 750 µg.mL^{-1} PLB as an effective concentration, while 500 µg.mL^{-1} (the registered concentration) was evaluated in this study. Schirra et al. (2010) got very good results with a combination of 600 µg.g^{-1} each of IMZ and PYR.

AZO, FLU and GRA gave relatively poor curative control (< 62%). Kanetis et al. (2007) showed that these actives showed very good potential on infections of 21 h and younger. In our work the infections was 24 h old, which is realistically comparable to industry situations where fruit can stand for longer than a day after harvest before the first fungicide application. D'Aquino et al. (2013) showed excellent curative control (≤ 15% infection) with 600 $\mu g.mL^{-1}$ FLU on 24 h old green mould infections. The differences between our work and that of D'Aquino et al. (2013) might be ascribed to different inoculation methods, as they induced 2 mm wide by 2 mm deep wounds before dipping the fruit in a spore suspension of 1×10^5 $spores.mL^{-1}$. In our study, fruit were wound-inoculated simultaneously by dipping the wound-inducer in a suspension of 1×10^6 $spores.mL^{-1}$ prior to wounding; this resulted in approximately 4×10^4 spores deposited at each wound site, which might be more severe than found in the field. Schirra et al. (2010) used a similar inoculation method to D'Aquino et al. (2013), but their spore suspension was 1×10^6 $spores.mL^{-1}$. They could only achieve ≈ 12 – 13% control with 30 and 60 s dip treatments in 600 $\mu g.g^{-1}$ FLU and AZO. Other fungicides, however, were able to control infections that originate from this type of inoculation. Protectively these actives showed some potential, especially GRA giving 73.6 and 63.1% control of infections from the two respective resistant isolates in this study. Interestingly, protective control of the sensitive isolate was markedly to significantly poorer than that of the resistant isolates following treatment with GRA, FLU, AZO and PYR. One of the attributes of FLU and AZO is the inhibition of conidium germination (; Bushong and Timmer, 2000; Rosslenbroich and Stuebler, 2000 Kanetis et al., 2007), which might explain the protective ability of these fungicides.

The sporulation inhibition effect of IMZ is better expressed when it is applied in wax coatings than when applied in an aqueous solution (Erasmus et al., 2011, 2013 / Chapter 2 & 3; Njombolwana et al., 2013a, 2013b). Brown and Dezman (1990) showed that fruit with an intact wax layer and treated with IMZ had lower levels of sporulation compared to fruit with the wax layer removed. Residues levels of > 2 $\mu g.g^{-1}$ are required for the inhibition of sporulation where the IMZ EC formulation was used (Brown and Dezman, 1990; Smilanick et al., 1997). So far no consistent trends could be observed in terms of sporulation control with aqueous IMZ sulphate treatments. PYR has also been shown to have the ability to inhibit or reduce green mould sporulation, but not as well as IMZ (Smilanick et al., 2006). In their study, the combination of IMZ and PYR showed sporulation inhibition of an IMZ sensitive isolate of *P. digitatum* applied within wax or aqueous solution. Our work confirms this as PLB was able to control sporulation the best on the sensitive isolate infections (23.6 and 2.8% sporulation for curative and protective, respectively). This might be ascribed to a synergistic effect between IMZ and PYR, as sporulation inhibition for these actives individually was relatively poor. However, sporulation inhibition by PLB was relatively poor for the resistant isolate infections, and comparable to PYR alone. Kanetis et al. (2007) also found that PLB was unable to inhibit sporulation on green mould caused by IMZ resistant isolates. The only fungicide that showed some level of sporulation inhibition of the resistant isolates was SOPP, but for curative treatment only (33.3%).

In Chapter 4, it has been shown that post dip-treatment brushing can reduce > 90% of the potential residue to levels of < 0.5 $\mu g.g^{-1}$. This level gave good curative control of the sensitive isolate (PD3), but poor inhibition of sporulation due to the reduced residue levels. The protective ability of the reduced residue loads was also questioned. In this study, the $ER_{50}C$ and $ER_{50}P$ values for sensitive PD isolates from 0.20 - 0.33 $\mu g.g^{-1}$ confirms the good curative control at low residue levels, but also indicate that good protective control of IMZ sensitive isolates could also be achieved. In the absence of IMZ resistance, green mould can therefore be effectively controlled if fruit is dip-treated within 24 h after harvest with relatively low residue levels. However, it can be anticipated that older infections will escape control (Chapter 4) and their sporulation will not be inhibited.

This work shows the importance of loading an effective IMZ residue to combat the green and blue mould. Although sensitive isolates could be controlled with < 0.5 $\mu g.g^{-1}$, sporulation inhibition was mostly observed at higher residue loads (> 2 $\mu g.g^{-1}$). The ideal IMZ residue level of 2 – 3 $\mu g.g^{-1}$ was, however, not effective against all resistant isolates, but should reduce infection and inoculum buildup in packhouse environments. Our work showed that further increasing IMZ residue levels will not be effective in improving control of resistance PD isolates, especially considering the MRL restriction. In packhouses where IMZ resistance is prevalent, fungicides with alternative modes of action could be applied; although none of these fungicides equalled IMZ in its combined curative, protective and sporulation control abilities of IMZ sensitive PD isolates, they would be effective when applied in an integrated programme. Each fungicide's optimal application needs to be specifically determined, as the profound effect of application on residue loading and control has been demonstrated in previous studies (Erasmus et al., 2011, 2013 / Chapter 2 & 3; Njombolwana et al., 2013a, 2013b).

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Table 4.5.4.1. Effective imazalil (IMZ) concentration that inhibits 50% mycelia growth (EC_{50}) and resistance factors (R factor) of nine *Penicillium digitatum* (PD) and five *P. italicum* (PI) isolates that were determined following mycelium growth measurements on potato dextrose agar amended with IMZ at concentrations ranging from 0 to 5 $\mu g.mL^{-1}$ from which percentage growth inhibition data for each isolate and IMZ concentrations were subjected to non-linear regression using the function, Y = C/(1+Exp(-A-B*X.

Sensitivity category[a] and isolate	Function variables and standard deviations[b]				EC_{50} ($\mu g.mL^{-1}$)[c]	R factor[d]
	A	B	C	R^2		
Sensitive						
PI4	-4.5 (2.0)	1132.7 (602.4)	84.5 (5.2)	> 0.85	0.005 e	
PI1	-4.8 (2.5)	1167.9 (801.2)	93.2 (1.6)	> 0.95	0.005 e	
PI7	-6.7 (4.4)	3338.9 (4124.6)	87.1 (2.8)	> 0.90	0.007 e	
PI2	-4.0 (1.8)	174.1 (124.2)	98.0 (1.3)	> 0.88	0.027 e	
PD6	-23.7 (13.9)	991.7 (643.3)	100.9 (1.2)	> 0.99	0.027 e	
PD3	-5.1 (1.1)	135.1 (28.9)	87.6 (36.6)	> 0.98	0.032 e	
PD9	-7.8 (4.5)	192.2 (74.7)	101.4 (1.5)	> 0.84	0.038 e	
PI3	-4.6 (1.6)	91.2 (30.6)	100.8 (1.0)	> 0.99	0.050 e	
Low resistant						
PD2	-4.2 (1.0)	6.7 (1.5)	101.3 (1.4)	> 0.97	0.615 d	19.0
Moderately resistant						
PD8	-17.6 (10.2)	17.4 (10.5)	100.5 (0.7)	> 0.98	1.075 c	33.2
Resistant						
PD7	-7.6 (0.7)	4.7 (0.1)	99.9 (0.1)	> 0.95	1.618 b	50.0
PD1	-15.4 (1.3)	9.5 (1.2)	100.0 (0.2)	> 0.99	1.634 b	50.5
PD4	-6.6 (1.2)	3.5 (0.5)	101.4 (0.2)	> 0.90	1.865 b	57.6
Highly resistant						
PD5	-26.2 (25.2)	9.5 (7.0)	100.8 (0.6)	> 0.99	2.290 a	70.7

[a]The resistant isolates were categorized using the R factor and the Fisher's LSD test.
[b]Mean values of three trials, where lines were fitted on the data from 5 replicates per trial.
[c] Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)
[d]The R factor was determined by dividing the EC_{50} value of a resistant isolate by the mean EC_{50} value of the three sensitive PD isolates (PD3, PD6 and PD9).

Table 4.5.4.2. Effective imazalil (IMZ) residue values for predicted 50% curative ($ER_{50}C$) and protective ($ER_{50}P$) control of green mould caused by nine *Penicilium digitatum* (PD) isolates respectively inoculated 24 h prior to or ± 4 h after treatment on Valencia orange fruit dipped in a range of IMZ concentrations (0 – 2560 $\mu g.mL^{-1}$) at 22°C for 60 s. The ER_{50} values were calculated from the function, Y = C/(1+Exp(-A-B*X)), of which the parameters were determined from regression of percentage control data for each isolate on IMZ residue derived from the inoculation trials.

Isolate[a]	Function variables and standard deviances[b]			R^2	ER_{50} ($\mu g.g^{-1}$)[c]
	A	B	C		
Curative					
Sensitive					
PD3	-4.8 (0.9)	19.4 (1.5)	88.5 (1.7)	> 0.92	0.26 ab
PD6	-3.0 (0.8)	11.5 (4.6)	94.8 (0.8)	> 0.89	0.29 ab
PD9	-4.5 (0.2)	11.8 (8.4)	100.6 (10.9)	> 0.90	0.26 ab
Low resistant					
PD2	-4.4 (2.3)	4.8 (3.8)	88.8 (3.5)	> 0.81	1.65 abc
Moderately resistant					
PD8	-4.7 (0.7)	9.4 (4.3)	94.3 (3.5)	> 0.92	1.22 ab
Resistant					
PD1	-4.0 (0.7)	3.7 (2.0)	99.6 (0.4)	> 0.94	1.40 abc
PD4	-2.5 (0.1)	0.7 (0.2)	85.3 (14.8)	> 0.82	4.37 de
PD7	-2.5 (0.2)	0.8 (0.4)	84.7 (7.0)	> 0.79	4.56 de
Highly resistant					
PD5	-5.5 (2.5)	2.2 (0.7)	1252.9 (1173.9)	> 0.87	1.91 abcd
Protective					
Sensitive					
PD3	-3.7 (1.6)	19.8 (4.7)	93.7 (2.4)	> 0.84	0.19 a
PD6	-3.4 (0.5)	14.2 (3.4)	95.2 (6.4)	> 0.91	0.27 ab
PD9	-7.7 (4.2)	91.7 (71.6)	92.3 (3.6)	> 0.82	0.13 a
Low resistant					
PD2	-2.2 (0.2)	0.7 (0.4)	85.9 (0.2)	> 0.75	4.46 de
Moderately resistant					
PD8	-4.7 (0.5)	6.0 (1.6)	65.8 (8.9)	> 0.81	1.08 ab
Resistant					
PD1	-8.0 (0.9)	8.1 (1.4)	98.8 (0.0)	> 0.95	1.00 ab
PD4	-5.5 (2.9)	2.2 (0.9)	69.6 (2.5)	> 0.90	2.94 bcd
PD7	-2.7 (0.3)	0.8 (0.3)	85.0 (0.9)	> 0.84	4.00 cde
Highly resistant					
PD5	18.4 (20.8)	-6.5 (7.0)	52.3 (47.6)	> 0.79	6.62 e

[a]Each isolate was categorized in terms of IMZ sensitivity as indicated by their EC_{50} values.
[b]Mean values of two trials, where lines were fitted on the data from three replicates per trial.
[c]Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.3. Mean sporulation incidence (%) on infected Valencia orange fruit that were inoculated with three imazalil (IMZ) sensitive and six IMZ resistant *Pencillium digitatum* isolates, and curatively and protectively dip-treated in IMZ solutions ranging from 0 to 2560 µg.mL^{-1} to effect a range of IMZ residues on fruit.

IMZ residue (µg.g^{-1})	Sporulation incidence on infected fruit (%)[a]	
	Sensitive isolates	Resistant isolates
0.00	83.1 abc	70.8 bcde
0.18	66.2 cde	33.3 de
0.20	92.5 ab	63.8 cde
0.24	88.1 abc	27.8 e
0.30	85.2 abc	57.4 cde
0.57	100.0 a	62.3 cde
0.89	98.6 a	67.6 cde
1.20	89.6 abc	72.3 bcd
1.26	80.0 abc	71.5 bcde
5.43		80.6 abc
10.60		79.6 abc

[a] Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.4. Effective imazalil (IMZ) residue values for predicted 50% curative ($ER_{50}C$) and protective ($ER_{50}P$) control of green mould caused by two *Penicilium digitatum* (PD) and five *P. italicum* (PI) isolates respectively inoculated 24 h prior to or ± 4 h after treatment on Valencia orange fruit dipped in a range of IMZ concentrations (0 – 2560 µg.mL^{-1}) at 22°C for 60 s. The ER_{50} values were calculated from the function, Y = C/(1+Exp(-A-B*X)), of which the parameters were determined from regression of percentage control data for each isolate on IMZ residue derived from the inoculation trials.

Isolate[a]	Function variables and standard deviances[b]			R^2	ER_{50} (µg.g^{-1})[c]
	A	B	C		
Curative					
Sensitive					
PD9	-2.6 (1.2)	22.3 (15.1)	92.7 (3.5)	> 0.88	0.15 a
PI1	-2.0 (0.2)	12.3 (2.4)	96.4 (2.1)	> 0.83	0.18 a
PI2	-2.8 (0.4)	11.3 (1.0)	93.2 (1.6)	> 0.87	0.26 a
PI3	-3.1 (0.9)	23.9 (9.9)	95.8 (2.0)	> 0.81	0.14 a
PI4	-3.0 (1.8)	31.2 (19.3)	95.5 (3.8)	> 0.76	0.11 a
PI7	-13.5 (15.8)	137.1 (176.7)	89.6 (1.7)	> 0.77	0.17 a
Highly resistant					
PD5	-4.0 (0.6)	5.2 (3.4)	75.8 (9.5)	> 0.78	1.47 b
Protective					
Sensitive					
PD9	-3.0 (1.4)	41.0 (26.3)	93.0 (2.0)	> 0.81	0.11 a
PI1	-3.0 (0.7)	31.5 (19.8)	93.1 (0.6)	> 0.84	0.12 a
PI2	-3.1 (0.6)	17.1 (3.2)	88.0 (4.6)	> 0.88	0.20 a
PI3	-2.2 (0.4)	13.8 (3.9)	97.3 (1.8)	> 0.90	0.17 a
PI4	-3.4 (0.7)	36.2 (8.2)	95.8 (1.1)	> 0.69	0.10 a
PI7	-7.3 (4.3)	86.7 (57.0)	96.1 (1.3)	> 0.85	0.09 a
Highly resistant					
PD5	-4.2 (1.3)	1.4 (0.2)	47.6 (14.0)	> 0.68	4.38 c

[a]Each isolate was categorized in terms of IMZ sensitivity as indicated by their EC_{50} values.
[b]Mean values of three trials, where lines were fitted on the data from three replicates per trial.
[c]Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.5. Effective imazalil (IMZ) residue values for predicted 50% curative ($ER_{50}C$) control of green mould caused by four *Penicilium digitatum* (PD) and two *P. italicum* (PI) isolates respectively inoculated 24 h prior to treatment on navel orange fruit dipped in a range of IMZ concentrations (0 – 2560 µg.mL^{-1}) at 22°C for 60 s. The ER_{50} values were calculated from the function, Y = C/(1+Exp(-A-B*X)), of which the parameters were determined from regression of percentage control data for each isolate on IMZ residue derived from the inoculation trials.

Sensitivity category[a]	Isolate	Function variables and standard deviances[b]			R^2	$ER_{50}C$ (µg.g^{-1})[c]
		A	B	C		
Sensitive	PD3	-3.9 (0.7)	12.3 (3.3)	94.2 (3.8)	> 0.89	0.37 A
	PD6	-2.2 (0.7)	9.3 (4.8)	98.7 (0.5)	> 0.85	0.28 A
	PI2	-3.5 (0.9)	12.0 (3.9)	91.3 (4.2)	> 0.93	0.37 A
	PI4	-17.6 (15.3)	106.1 (97.5)	97.5 (0.0)	> 0.94	0.21 A
Resistant	PD7	-25.9 (22.2)	15.3 (12.5)	68.1 (12.6)	> 0.93	1.65 B
Highly resistant	PD5	-5.1 (0.4)	4.0 (0.6)	78.6 (3.5)	> 0.88	1.42 B

[a]Each isolate was categorized in terms of IMZ sensitivity as indicated by their EC_{50} values.
[b]Mean values of two trials, where lines were fitted on the data from three replicates per trial.
[c]Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.6. Effective imazalil (IMZ) residue values for predicted 50% protective ($ER_{50}P$) control of green mould caused by two *Penicilium digitatum* (PD) and two *P. italicum* (PI) isolates respectively inoculated ± 4 h after treatment on navel orange fruit dipped in a range of IMZ concentrations (0 – 2560 µg.mL^{-1}) at 22°C for 60 s. The ER_{50} values were calculated from the function, Y = C/(1+Exp(-A-B*X)), of which the parameters were determined from regression of percentage control data for each isolate on IMZ residue derived from the inoculation trials.

Sensitivity category[a]	Isolate	Function variables and standard deviances[b]			R^2	$ER_{50}P$ (µg.g^{-1})[c]
		A	B	C		
Sensitive	PD3	-3.1 (1.0)	12.3 (5.5)	96.3 (2.1)	> 0.90	0.28 A
	PD6	-2.3 (0.2)	6.7 (0.4)	96.9 (0.4)	> 0.91	0.35 A
	PI2	-7.7 (5.3)	23.5 (10.3)	94.1 (2.6)	> 0.74	0.29 A
	PI4	-5.8 (2.2)	42.2 (19.0)	96.3 (1.2)	> 0.85	0.15 A

[a]Each isolate was categorized in terms of IMZ sensitivity as indicated by their EC_{50} values.
[b]Mean values of two trials, where lines were fitted on the data from three replicates per trial.
[c]Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.7. Mean sporulation incidence (%) on infected Navel orange fruit that were inoculated with 2 imazalil (IMZ) sensitive *Penicillium digitatum* (PD), two IMZ resistant PD and two IMZ sensitive *P. italicum* (PI) isolates, and curatively and protectively dip-treated in IMZ solutions ranging from 0 to 2560 µg.mL^{-1} to effect a range of IMZ residues on fruit.

IMZ residue (µg.g^{-1})	Sporulation incidence on infected fruit (%)[a]		
	Sensitive PD isolates	Resistant PD isolates	Sensitive PI isolates
0.00	100.0 a	100.0 a	95.8 ab
0.13	100.0 a	99.3 a	89.5 ab
0.19	99.3 a	100.0 a	63.7 c
0.22	99.3 a	100.0 a	60.3 cd
0.36	95.0 ab	94.4 ab	42.7 de
0.56	94.4 ab	91.7 ab	36.7 ef
0.87	76.9 bc	99.3 a	24.0 efg
1.37	68.1 c	90.1 ab	12.0 g
1.72	57.4 cd	98.3 a	16.9 fg
3.09	26.4 efg	96.9 a	
5.64	64.4 c	95.4 ab	

[a] Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.8. Mean percentage green mould control on orange fruit inoculated with an imazalil (IMZ) sensitive, resistant or highly resistant *Pencillium digitatum* isolate 24 h prior to (curative) or ± 4 h after (protective) 60 s aqueous dip treatment with 10 different fungicides at registered or experimental concentrations.

Action	Product	Concentration ($\mu g.g^{-1}$)	Green mould control (%)[a]		
			IMZ sensitive isolate	IMZ resistant isolate	IMZ highly resistant isolate
Curative	Imazalil (IMZ)	500	92.1 abc	35.3 lmnop	30.3 opq
	Sodium o-phenylphenate	20000	97.9 a	98.2 a	99.1 a
	Thiabendazole	1000	92.5 ab	3.1 wxy	2.3 xy
	Guazatine	1000	90.0 abc	38.6 klmno	24.0 pqrs
	Pyrimethanil	1000	78.0 de	90.4 abc	93.9 a
	Philabuster®	500	93.8 a	81.0 cde	81.2 bcde
	Fludioxonil	500	12.2 tuvwxy	29.5 opqr	18.5 rstu
	Azoxystrobin	500	8.9 uvwxy	19.4 qrstu	12.4 tuvwxy
	Graduate®A+	500	30.7 nopq	47.5 ijk	37.7 klmno
	Propiconazole	500	38.9 klmno	14.2 stuvw	10.0 tuvwxy
Protective	Imazalil	500	88.2 abcd	1.7 xy	1.1 y
	Sodium o-phenylphenate	20000	19.6 qrstu	13.0 stuvwx	15.9 stuv
	Thiabendazole	1000	46.0 ijkl	3.0 wxy	3.2 wxy
	Guazatine	1000	81.6 bcde	18.1 rstu	5.1 vwxy
	Pyrimethanil	1000	46.4 ijkl	59.7 gh	57.2 ghi
	Philabuster®	500	97.5 a	41.9 klmn	33.3 nop
	Fludioxonil	500	15.0 stuv	34.2 mnop	20.5 qrst
	Azoxystrobin	500	30.8 nopq	55.9 ghij	45.1 jklm
	Graduate®A+	500	53.9 ghij	73.6 ef	63.1 fg
	Propiconazole	500	48.8 hijk	1.6 xy	3.2 wxy

[a] Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

Table 4.5.4.9. Mean sporulation incidence (%) on infected orange fruit inoculated with an imazalil (IMZ) sensitive, resistant or highly resistant *Pencillium digitatum* isolate 24 h prior to (curative) or ± 4 h after (protective) 60 s aqueous dip treatment with 10 different fungicides at registered or experimental concentrations.

Action	Product	Concentration ($\mu g.mL^{-1}$)	Sporulation incidence oninfected fruit (%)[a]		
			IMZ sensitve isolate	IMZ resistant isolate	IMZ highly resistant isolate
Curative	Imazalil	500	62.8 def	98.6 l	100.0 l
	Sodium o-phenylphenate	20000	100.0 l	33.3 cd	33.3 abc
	Thiabendazole	1000	80.7 fghijkl	98.6 l	100.0 l
	Guazatine	1000	83.3 fghijkl	91.4 ghijkl	100.0 l
	Pyrimethanil	1000	84.7 fghijkl	53.9 cde	72.9 defghi
	Philabuster®	500	23.6 ab	74.3 defghij	93.3 hijkl
	Fludioxonil	500	100.0 l	88.9 ghijkl	100.0 l
	Azoxystrobin	500	100.0 l	84.4 fghijkl	100.0 l
	Graduate®A+	500	84.0 fghijkl	71.5 defgh	98.6 l
	Propiconazole	500	94.4 hijkl	98.6 l	100.0 l
	Water control		100.0 l	100.0 l	100.0 l
	Untreated control		100.0 l	100.0 l	100.0 l
Protective	Imazalil	500	69.4 defg	100.0 l	100.0 l
	Sodium o-phenylphenate	20000	93.7 hijkl	79.2 fghijkl	89.4 ghijkl
	Thiabendazole	1000	93.3 hijkl	100.0 l	100.0 l
	Guazatine	1000	92.8 ghijkl	98.3 kl	100.0 l
	Pyrimethanil	1000	98.6 l	85.7 fghijkl	96.9 jkl
	Philabuster®	500	2.8 a	95.8 ijkl	100.0 l
	Fludioxonil	500	87.5 ghijkl	74.7 efghijk	100.0 l
	Azoxystrobin	500	100.0 l	79.2 fghijkl	95.3 hijkl
	Graduate®A+	500	89.4 ghijkl	50.6 cd	92.1 ghijkl
	Propiconazole	500	95.8 ijkl	98.3 kl	100.0 l
	Water control		100.0 l	100.0 l	100.0 l
	Untreated control		100.0 l	100.0 l	100.0 l

[a]Each value followed by the same letter is not significantly different according to Fisher's LSD test ($P \leq 0.05$)

4.5.5 PROGRESS REPORT: The JBT heated flooder as an alternative application method for fungicides in citrus packhouses

Project 1050 (April 2012 - March 2013) by Arno Erasmus (CRI-Nelspruit), Paul Fourie (CRI--USPP), Wilma du Plooy (JBT South Africa) and Charlene Jewell (JBT California)

Summary

The majority of South African packhouses use a dip tank to apply imazalil (IMZ) for the control of green mould (caused by *Penicillium digitatum*). John Bean Technologies' (JBT) division in California developed an alternative to the dip tank that has been in use for the past decade. The JBT heated flooder (JHF) applies fungicide in an aqueous solution by means of a number of weirs that creates a seamless laminar flow that falls onto the fruit over rotating brushes. This type of application gives more consistency in terms of residue loading and disease control. JBT's division in South Africa build and installed an experimental flooder unit at CRI in Nelspruit to compare this new technology to current technology in use (dip application). Due to technical problems the unit was only installed during July 2012 in the CRI Nelspruit postharvest laboratory. Results on work conducted during 2012 qnd 2013 are presented in this report. The dip was compared to the flooder application, the effect of flooder solution temperature on IMZ residue loading and green mould control was investigated and the effect of number of weirs was investigated. Results show that the flooder is an effective alternative IMZ application to the dip tank. Similar results were obtained for fruit treated at solution

temperatures 25 and 35°C in terms of residue loading and curative control. In addition the flooder gave better protective control levels compared to the dip application. Higher temperatures tended to give better residue loading and curative control. Application using three to five weirs gave more consistent curative control compared to one and two weirs. Lemon and soft citrus fruit should not be treated at temperatures higher than 45°C to prevent rind injury. Further work with the flooder will be incorporated with a new project in 2014. The final report which should include the manuscript of a scientific article will be submitted in 2015.

Opsomming

Die meerderheid pakhuise in die Suid-Afrikaanse sitrusbedryf gebruik ŉ dompelbad vir die aanwending van imazalil (IMZ) om groenskimmel (veroorsaak deur *Penicillium digitatum*) te beheer. John Bean Technologies (JBT) se afdeling in Kalifornië het ŉ alternatief vir die dompelbad ontwikkel wat reeds die afgelope dekade in gebruik is. Die vloedtoediener wend swamdoder in ŉ water oplossing aan deur middel van ŉ aantal watervalle met ŉ soomlose laminêre vloei wat op die vrugte val oor roterende borsels. Hierdie tipe van aanwending gee meer betroubaarheid in terme van residu-lading en siektebeheer. JBT se afdeling in Suid-Afrika het ŉ eksperimentele vloedtoediener-eenheid by CRI in Nelspruit geïnstalleer. Die doel was om hierdie nuwe tegnologie te vergelyk met die huidige tegnologie in gebruik (dompelbad). Weens tegniese probleme is die eenheid slegs gedurende Julie 2012 geïnstalleer in die CRI naoes laboratorium. Resultate op werk wat tydens 2012 en 2013 voltooi is word in hierdie verslag bespreek. Die dompelbad is met die vloedtoediener vergelyk, die effek van vloedtoediener oplossingstemperatuur en die effek van aantal watervalle is ook ondersoek. Alle proewe is in terme van residu-lading en groenskimmel-beheer geëvalueer. Resultate toon dat die vloedtoediener ŉ doeltreffende alternatiewe IMZ aanwendingsmetode vir die dompelbad is. Soortgelyke resultate in terme van residue lading en kuratiewe beheer is met die vloedtoediener en dompelbad verkry vir vrugte behandel in oplossingstemperature van 25 en 35°C. Benewens dit het die vloedtoediener ook beter beskermende beheer in vergelyking met die dompelbad getoon. Hoër temperature in die vloedtoediener het geneig om beter residu-lading en kuratiewe beheer te gee. Aanwending met drie tot vyf watervalle het meer betroubare kuratiewe beheer in vergelyking met een en twee watervalle gegee. Suurlemoen en sagte sitrus vrugte moet nie by temperature hoër as 45°C behandel word nie om skil beserings te voorkom. Verdere werk met die vloedtoediener sal met ŉ nuwe projek in 2014 geïnkorporeer word. Die finale verslag wat die manuskrip van ŉ wetenskaplike artikel moet insluit, sal in 2015 voorgelê word.

4.5.6 PROGRESS REPORT: Identification and modelling of postharvest decay risk indicators

Project 1073 (2013/14–2014/15) by Arno Erasmus, Catherine Savage, Mareli Kellerman, Paul Cronjé and Paul H. Fourie (CRI)

Summary

Pre-harvest risk indicators have been identified and measured during the navel orange harvest season of 2013. Deadwood, fruit count, decay (in the orchard and postharvest), inoculum load, skirt height and wounding during harvest have been measured on six different orchards throughout Mpumalanga. Despite meaningful measurements and significant differences between the orchards, these did not correlate with the postharvest decay measured. Further analyses will still be conducted and a final report submitted in 2015. Due to limited funds, this project was not funded for 2014/15. The results obtained to date will be used to inform on changes to the methodology, should the project be resubmitted for funding.

Opsomming

Voor-oes risiko-aanwysers is geïdentifiseer en gemeet tydens die nawel lemoen oes-seisoen van 2013. Dooie hout, vrugte lading, bederf (in die boord en naoes), inokulum lading in die boord, romp hoogte en wonde tydens oes is gemeet op ses verskillende plotte versprei oor Mpumalanga. Ten spyte van betekenisvolle verskille tussen boorde, het geen van die aanwysers met na-oes verrotting gekorreleer. Verdere analise word gedoen en ŉ finale verslag sal in 2015 ingedien word. Weens beperkte befondsing is hierdie projek nie in 2014/15 befonds. Resultate tot op hede sal gebruik word om veranderinge in metodes te maak, indien die projek weer vir befondsing voorgelê word.

4.5.7 **PROGRESS REPORT: Use of hot water, potassium silicate and biological control agents to reduce postharvest disease and chilling injury in citrus fruit**

Project UKZN1 (2010/10-2014/4) by Mark Laing & Iona Basdew (UKZN)

Summary

The aim of this research was to investigate the integration of potassium silicate fertilisation, hot water treatment and biocontrol agents for postharvest disease and chilling injury control in citrus. Objectives were to (1) determine the optimal temperature x duration treatments for *Penicillium digitatum* and *Phyllosticta citricarpa* infections in various citrus cultivars and to translate this to use of the JBT heated flooder; (2) optimise the preventative effects of the yeast biocontrol agent B13, combined with the best curative hot water treatment (i.e., integrated curative + preventative treatment); and (3) evaluate the buffering effects of pre-harvest applications of potassium silicate to citrus trees on the chilling injuries suffered by lemons (will be completed in 2014). During the past season, hot water treatments were conducted on three different types of citrus (navel and 2 cultivars of Valencia oranges). The most effective treatment combinations were in the ranges of 62-68°C for 10 and 15 seconds for both navel and Valencia oranges. These can be tested on the JBT flooder for large volume treatments. Hot water treatments were also carried out on fruit with both visible and latent infections of citrus black spot (CBS) on navel oranges and Eureka lemons. From preliminary trials, the best treatments were able to suppress latent infections of CBS as demonstrated by a significant reduction in lesion development in treated fruit relative to the untreated controls. For navels this was a combination of 56°C x 15 seconds to 58°C x 10 seconds. For lemons this was 66°C x 20 seconds to 68°C x 20 seconds. In preloiminary trials with the Yeast B13, it only delivered preventative protection against infection of *P. digitatum*, hence the need for a curative hot water treatment first. Preliminary trials of Yeast B13 were successful on navel and two cultivars of Valencia, showing that it was as effective as imazalil (not significantly different) at preventing further infections by *P. digitatum*. A pilot trial on the treatment of navels with HWT + Yeast B13 showed that the combination could provide 100% protection of inoculated fruit versus 100% infection of untreated control fruit.

Opsomming

Die doel van hierdie navorsing was om die integrasie van kaliumsilikaat bemesting, warm water behandeling (WWB) en biologiese agente vir na-oes siektes en koueskade beheer in sitrus te ondersoek. Doelwitte was om (1) die optimale temperatuur x blootstellingstyd van behandelings vir *Penicillium digitatum* en *Phyllosticta citricarpa* infeksies in verskillende sitrus kultivars te bepaal en dan oor te dra vir gebruik op die JBT vloedtoediener, (2) om die voorkomende effek van die B13 gis as biologiese beheermiddel gekombineer met die beste genesende warm water behandeling (geïntegreerde genesende + voorkomende behandeling) te optimiseer; en (3) om die buffer effek van voor-oes toedienings van kaliumsilikaat op sitrusbome op die koue skade op suurlemoene te evalueer (sal in 2014 voltooi word). Gedurende die afgelope seisoen, was warm water behandelings op drie verskillende tipes sitrus (nawel, 2 kultivars van Valencia) uitgevoer. Die mees doeltreffende behandeling kombinasies was in die reeks van 62-68°C vir 10 en 15 sekondes vir onderskeidelik nawel en Valencia lemoene. Dit kan nou getoets word deur groter volumes met die JBT vloedtoediener te behandel. Warm water behandelings is ook uitgevoer op nawel en Eureka suurlemoen vrugte met beide sigbare en latente infeksies van sitrus swartvlek (CBS). Die beste behandelings in loodsproewe kon latente infeksies van CBS onderdruk, soos gedemonstreer deur ʼn aansienlike vermindering in die letsel ontwikkeling in behandelde vrugte relatief tot die onbehandelde kontrole. Vir nawels, was dit ʼn kombinasie van 56°C x 15 sekondes tot 58°C x 10 sekondes. Vir suurlemoene was dit 66°C x 20 sekondes tot 68°C x 20 sekondes. Met betrekking tot die B13 gis loodsproewe, het B13 slegs voorkomende beskerming teen infeksie van *P. digitatum* gelewer; daarom die nodigheid vir ʼn genesende warm water behandeling. Voorlopige proewe van B13 was suksesvol op nawel en twee kultivars van Valencia lemoene. Resultate wys dat dit net so effektief soos imazalil (nie beduidende verskil nie) op die voorkoming van verdere infeksies deur *P. digitatum* was. ʼn Loodsproef met die behandeling van nawels met WWB + B13 het gedemonstreer dat die kombinasie tot ʼn 100% beskerming van geïnokuleerde vrugte kan bied teenoor 100% infeksie van onbehandelde vrugte.

4.6 PROGRAMME: CITRUS BLACK SPOT

Programme coordinator: G.C. Schutte (CRI)

4.6.1 Programme summary

Uncharacteristic and highly suitable climatic conditions for CBS with early rains (October 2012 onwards) resulted in favourable conditions at an early stage of fruit susceptibility and making it difficult to ensure effective fruit protection. Repeated rainfall periods were recorded during the fruit susceptibility period. Low levels of ascospore release occurred during the 2013-2014 season with the first ascospore release occurring only in December and a second one in January at Hermitage and February at Kirkwood (4.6.2).

No results could be obtained from a field trial where various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of black spot. This is because the grower hosting the trial site applied a commercial spray consisting of Cabrio and mancozeb over our whole trial site during January 2013 (4.6.3).

Microsatellite markers are widely used in population genetic studies showing high levels of allelic variation enabling the quantification of genetic diversity within and amongst pathogen populations and provide insights into the evolutionary history of pathogens and their epidemiology. Genomic sequence data of *Phyllosticta citricarpa* was generated with the Ion Torrent Personal Genome Machine and assembled into 123 contigs, which was used to mine for microsatellites and for primer design. Of the 32 primers designed, eleven were shown to be polymorphic. Five South African populations that were genotyped were shown to be similar in genetic structure, which could be an indication of a recent introduction (founder population) that would be consistent with the first report of CBS in South Africa only being in 1929. Although the markers were not highly polymorphic in the South African populations, loci that were monomorphic in these populations were polymorphic in seven populations representing four different countries around the world namely USA, China, Brazil and Australia. Populations from China showed the highest genetic diversity. A total of five national populations (n = 138) and seven international populations (n = 402) have been collected and genotyped with the 11 SSR markers (4.6.4).

Using different backgrounds to determine the amount of spray volume that gets lost during foliar spray operations onto which various pigments were sprayed, were not successful. Fighter was selected as standard registered treatment and sprayed as low (5 L/tree), medium (10 L/tree) and high (20 L/tree) volume applications on Navel orange trees before harvest to determine their ambimobile movement in fruit for the control of brown rot. A fluorescent pigment was also added to each spray tank to determine spray coverage on fruit samples that were drawn on a weekly basis for five weeks. Low, medium and high volume spray applications of Fighter at ½x and 1x rates reacted like contact algaecides for the first two to three weeks after application whereupon their systemic action kicks in to protect the fruit from there on (4.6.5).

A project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway to develop various steps in a model, assigning probabilities to these steps and identifying research gaps. Data were collected to improve the models and to distinguish between the CBS pathogen and endophytic *Guignardia* sp., new modelling approaches and surveys and experiments to determine efficacy of CBS orchard and packhouse control measures (4.6.6).

Programopsomming

Die aanvang van die reënseisoen (Oktober 2012) was ongekend vroeg was met swaar reën wat tot 'n gunstige toestande vir vroeë infeksie gelei het en moeilik was om vrugte te beskerm. Gedurende die seisoen is herhaaldelike reënvalperiodes gemeet gedurende die vatbaarheidsperiode vir die vrugte. Aan die ander kant het laer vlakke van askospoorvrystelling gedurende die 2013-2014 seisoen voorgekom met die eerste askospoorvrystelling gedurende Desember en 'n tweede een gedurende Januarie by Hermitage en Februarie by Kirkwood (4.6.2).

Verskeie nuwe sistemiese- en kontakswamdoders asook adjuvante in kombinasies met geregistreerde swamdoders is op 'Valencia' lemoene beproef vir die beheer van swartvlek volgens vooropgestelde protokolle van die onderskeie maatskappye. Geen proefresultate is verkry nie omrede die plaas onwetend 'n kommersiële bespuiting bestaande uit Cabrio en mancozeb oor die hele proefperseel toegedien het (4.6.3).

Mikrosateliet merkers word baie in genetiese studies gebruik, omdat hulle hoë vlakke van alleliese variasie toon wat die kwantifisering van genetiese diversiteit binne en tussen patogeen populasies moontlik maak en

insig gee oor die evolusionêre geskiedenis van patogene en hul epidemiologie verskaf. Data van genoomvolgordes van *Phyllosticta citricarpa* is gegenereer met die "Ion Torrent Personal Genome" Masjien en in 123 contigs saamgevoeg, wat gebruik is om vir mikrosateliete en vir "primer" ontwikkeling te myn. Van die 32 primers wat ontwikkel is, het elf geblyk om polimorfies te wees. Vyf Suid-Afrikaanse populasies wat ontleed is, het getoon om van dieselfde genetiese struktuur te wees, wat 'n aanduiding van 'n onlangse inkoms kan wees en wat ooreenstem met die eerste aantekening van SSV in Suid-Afrika in 1929. Alhoewel die merkers nie hoogs polimorfies in die Suid-Afrikaanse populasies was nie, was loci wat monomorfies in hierdie populasies was, polimorfies in sewe populasies van vier ander lande naamlik China, die Verenigde State van Amerika, Brasilië en Australië. Populasies van China het die meeste genetiese diversiteit getoon. 'n Totaal van vyf plaaslike populasies (n = 138) en sewe internasionale populasies (n = 402) is versamel en met hierdie 11 SSR merkers genotipeer (4.6.4).

Verskillende tipes agtergronde waarop verskillende pigmente gespuit is om die spuitvolume wat verlore gaan tydens bespuitings te bepaal, was nie suksesvol nie. Fighter was geselekteer as 'n standard behandeling en gespuit as lae (5 L/boom), medium (10 L/boom) en hoë (20 L/boom) volume toedienings op Navel lemoene voor oes om hulle ambimobiliteit in vrugte te toets vir die beheer van Phytophthora bruinvrot. Fluoriserende pigment is ook tot elke spuittenk toegevoeg om die bedekking op vrugte te bepaal wat op 'n weeklikse basis getrek is vir vyf weke. Vir die eerste twee tot drie weke het die lae, medium en hoë volume toediening van Fighter teen ½x en 1x soos kontak-algisiedes gereageer waarna die sistemiese aksie eers ingeskop het en vrugte van daar af beskerm het (4.6.5).

Sitrusswartvlek is die belangrikste sitrus-siekte in Suid-Afrika, veral gegewe sy impak op marktoegang word geformaliseer deur *ad hoc* navorsing wat fokus op die ontwikkeling en verbetering van 'n model vir *Guignardia* pseudotesium rypwording en spoorvrystelling gebasseer op meso- en mikroklimaat data. 'n Samewerkingsprojek wat deur die Florida sitrusbedryf in VSA befonds word, beoog om 'n kwantitatiewe pesrisiko-analiese-model vir *Guignardia citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Gapings is in die model geidentifiseer wat aangespreek word (4.6.6).

4.6.2 PROGRESS REPORT: Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection

Project 919 (September 2008 – June 2014) by G.C. Schutte (CRI) and S. Serfontein (QMS)

Summary

Uncharacteristic and highly suitable climatic conditions for CBS were experienced in 2012-2013 season in the Eastern Cape. The onset of rains (October 2012 onwards) was unusually early, with heavy rain in October, resulting in favourable conditions at an early stage of fruit susceptibility and making it difficult to ensure effective fruit protection. Throughout the season, repeated rainfall periods were recorded during the fruit susceptibility period. On the other hand, much lower levels of ascospore release occurred during the 2013-2014 season with the first ascospore release occurring only in December and a second one in January at Hermitage and February at Kirkwood.

Opsomming

Ongewone en hoë geskikte klimatologiese toestande was ondervind gedurende die 2012-2013 seisoen in die Oos Kaap. Die aanvang van die reënseisoen (Oktober 2012) was ongekend vroeg was met swaar reën in Oktober wat tot 'n gunstige kondisie vir vroeë vrugvatbaarheid gelei het en moeilik was om te beskerm. Gedurende die seisoen is herhaaldelike reënvalperiodes gemeet gedurende die vatbaarheidsperiode vir die vrugte. Aan die ander kant het laer vlakke van askospoorvrystelling gedurende die 2013-2014 seisoen voorgekom met die eerste askospoorvrystelling gedurende Desember en 'n tweede een gedurende Januarie by Hermitage en Februarie by Kirkwood.

4.6.3 **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. No results could be obtained because Crocodile Valley did a commercial spray application over the whole trial site. The trial will be repeated.

Opsomming

Verskeie nuwe sistemiese en kontakswamdoders asook adjuvante in kombinasies met geregistreerde swamdoders is op 'Valencia' lemoene beproef vir die beheer van swartvlek volgens vooropgestelde protokolle van die onderskeie maatskappye. Geen proefresultate is verkry nie omrede Crocodile Valley 'n kommersiële bespuiting in Januarie 2013 oor die hele proefperseel toegedien het. Die proef word herhaal.

4.6.4 **PROGRESS REPORT: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely**
Project 977 (2010/11 – 2015/16) by E. Carstens (CRI)

Summary

Genetic markers such as microsatellite markers are very useful and widely used in population genetic studies, since they show high levels of allelic variation. This enables the quantification of genetic diversity within and amongst pathogen populations. These markers can also provide insights into the evolutionary history of pathogens and their epidemiology. Genomic sequence data of *Phyllosticta citricarpa* was generated with the Ion Torrent Personal Genome Machine and assembled into 123 contigs, which was used to mine for microsatellites and for primer design. Of the 32 primers designed, eleven were shown to be polymorphic. Five South African populations that were genotyped were shown to be similar in genetic structure, which could be an indication of a recent introduction (founder population) that would be consistent with the first report of CBS in South Africa only being in 1929. Although the markers were not highly polymorphic in the South African populations, loci that were monomorphic in these populations were polymorphic in seven populations representing four different countries around the world namely USA, China, Brazil and Australia. Populations from China showed the highest genetic diversity. A total of five national populations (n = 138) and seven international populations (n = 402) have been collected and genotyped with the 11 SSR markers.

Opsomming

Genetiese merkers soos mikrosateliete is geskik en word baie in genetiese studies gebruik omdat hulle hoë vlakke van alleliese variasie toon. Dit maak die kwantifisering van genetiese diversiteit binne en tussen patogeen populasies moontlik. Hierdie merkers kan insig oor die evolusionêre geskiedenis van patogene en hul epidemiologie verskaf. Data van genoomvolgordes van *Phyllosticta citricarpa* is gegenereer met die "Ion Torrent Personal Genome" masjien en in 123 contigs saamgevoeg, wat gebruik is om vir mikrosateliete en vir "primer" ontwikkeling te myn. Van die 32 primers wat ontwikkel is, het elf geblyk om polimorfies te wees. Vyf Suid-Afrikaanse populasies wat hiermee ontleed is, het getoon om van dieselfde genetiese struktuur te wees, wat 'n aanduiding van 'n onlangse inkoms kan wees en wat ooreenstem met die eerste aantekening van SSV in Suid-Afrika in 1929. Alhoewel die merkers nie hoogs polimorfies in die Suid-Afrikaanse populasies was nie, was loci wat monomorfies in hierdie populasies was, polimorfies in sewe populasies van vier ander lande, naamlik China, die Verenigde State van Amerika, Brasilië en Australië. Populasies van China het die meeste genetiese diversiteit getoon. 'n Totaal van vyf plaaslike populasies (n = 138) en sewe internasionale populasies (n = 402) is versamel en met hierdie 11 SSR merkers genotipeer.

4.6.5 **PROGRESS 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 & M.C. Pretorius (CRI)

Summary

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.

Opsomming

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.

4.6.6 **PROGRESS REPORT: Epidemiology and pest risk assessment of *Guignardia citricarpa***
Project 1026 (April 2011 - March 2015) by Paul Fourie, Vaughan Hattingh & Tian Schutte (CRI)

Summary

Citrus Black Spot is the most important citrus disease in South Africa, especially 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 will focus on developing and improving a model for *Guignardia* 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. 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 *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway. Two workshops were held in Florida and further progress was made by developing the various steps in the model, assigning probabilities to these steps and identifying research gaps. These research gaps are currently being addressed in this project, and for the past period included data collection to improve the models and to distinguish between the CBS pathogen and endophytic *Guignardia* sp., new modelling approaches and surveys and experiments to determine efficacy of CBS orchard and packhouse control measures.

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 sal fokus op die ontwikkeling en verbetering van ʼn model vir *Guignardia* 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. 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 *Guignardia citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Twee werkswinkels is gehou, en noemenswaardige vordering is gemaak, spesifiek deur die verskillende stappe in die model verder te ontwikkel en identifiseer, moontlikhede van die stappe te kwantifiseer, en gapings in die beskikbare kennis te identifiseer. Navorsing is tans onder weg om nuwe data in te samel ter verbetering van die modelle en om tussen die swartvlek patogeen en ʼn endofitiese *Guignardia* sp. te onderskei, nuwe modellering-strategië te beproef, asook ondersoeke om die sukses van swartvlek beheer in boorde en pakhuise te kwantifiseer.

4.7 **CRI Diagnostic Centre** (Elaine Basson, M.C. Pretorius, Timothy Zulu and Bhekisisa Cele, CRI)

Analysis	**Citrus nurseries**	**Commercial samples**	**Other crops**	**Research samples**
Nematode: Roots	15	712	28	1122
Nematode: Soil	3	23	36	1122
Phytophthora spp.	3070	764	226	1289
Water spore trap	193	6	12	0
Black spot identification (PCR)	0	103	0	559
Black spot benzimidazole resistance	0	156	0	7
Citrus greening (PCR)	0	5	0	9
Fungal Isolation (other)	0	13	9	1
Fruit & Foliar identification	0	21	2	0
Soil dilution plating	0	54	11	0
TOTAL	**3281**	**1857**	**324**	**4109**

Citrus Accredited 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, 3070 nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, which include re-submission samples, 7.04% tested positive, compared with the 12.06% in 2012/13. In addition to soil and water samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans.* For the nematode root samples, 0% tested positive and for the nematode soil samples 0% tested positive.

Commercial samples

Samples were received from the following citrus growing areas: Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, Swaziland and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Forty-two percent of the 712 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10 g of roots, and nematicide treatments were recommended. Sixty three percent of the 764 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of apricot, banana, grape, guava, kikuju, macadamia, pea, peppadews, protea, sorghum, tobacco and tomato. Nematodes found present on these crops included: *Scutellonema, Meloidogyne, Pratylenchus, Rotelynchulus, Tylenchus, Hemicycliophora, Paratylenchus, Criconema, Hemicriconemoides, Hoplolaimus, Helicotylenchulus, Paratrichodorus, Xiphinema, Tylenchorhynchus* and *Tylenchulus semipenetrans. Phytophthora* and *Pythium* analyses were done on apricot, avo, banana, dryland, grape, guava, kikuju, macadamia, ornamentals, pea, peppadews, protea, and sorghum. The diagnostic centre analysed 115 soil samples from macadamia nurseries and ten from avocado nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 4109 samples from experimental trials. The Diagnostic Centre assisted in trials to identify 559 possible citrus black spot lesions for *Phyllosticta citricarpa* and *P. capitalensis* using PCR protocol.

CRI Diagnostiese Sentrum (Elaine Basson, M.C. Pretorius, Timothy Zulu & Bhekisisa Cele)

Ontleding	**Sitrus kwekerye**	**Kommersiële monsters**	**Ander gewasse**	**Navorsings-monsters**
Aalwurms: Wortels	15	712	28	1122
Aalwurms: Grond	3	23	36	1122
Phytophthora	3070	764	226	1289
Water spoorlokval	193	6	12	0
Swartvlek (PKR)	0	103	0	559
Swartvlek benzimidazole bestandheid	0	156	0	7
Sitrusvergroeningsiekte (PKR)	0	5	0	9
Swamisolasie (ander)	0	13	9	1
Vrug- en blaar identifikasie	0	21	2	0
Grondverdunningsplate	0	54	11	0
TOTAAL	**3281**	**1857**	**324**	**4109**

Sitrus Geakkrediteerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalliks monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 3070 monsters, wat her-monsters insluit, is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 7.04% positief getoets het; in 2012/13 het 12.06% monsters positief getoets. Benewens die water en grondmonsters, moet kwekerye een keer per jaar ŉ wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die 15

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, Swaziland en Wes-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Twee-en-veertig persent van die 712 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10 g wortels gehad. Aalwurmdoderbehandelings is in daardie gevalle aanbeveel. Drie-en-sestig persent van die 764 monsters wat vir *Phytophthora* ontleed is het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van appelkose, piesangs, druiwe, koejawel, kikuju, makadamias, ertjies, peppadews, proteas, sorghum, tabak en tamatie gedoen. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Scutellonema, Meloidogyne, Pratylenchus, Rotelynchulus, Tylenchus, Hemicycliophora, Paratylenchus, Criconema, Hemicriconemoides, Hoplolaimus, Helicotylenchulus, Paratrichodorus, Xiphinema, Tylenchorhynchus* en *Tylenchulus semipenetrans.* Monsters van appelkose, avokados, piesangs, droogland gewasse, druiwe, koejawel, kikuju, makadamias, 'ornamentals', ertjies, peppadews, proteas en sorghum is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 115 monsters vanaf macadamia kwekerye en tien monsters vanaf avokado kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 4109 monsters afkomstig uit navorsingsprojekte gedoen. Die Diagnostiese Sentrum het ook hulp verleen aan navorsingsprojekte in die identifikasie van 559 moontlike sitrus swartvlek letsels vir *Phyllosticta citricarpa* en *P. capitalensis* deur middle van PKR.

5 PORTFOLIO: HORTICULTURE

5.1 PORTFOLIO SUMMARY

By Tim G Grout (Manager: Research & Technical)

Horticultural research results are often incremental in nature because it takes time to determine which of the many factors that influence citrus trees have a significant impact on production, fruit quality, appearance or rind integrity. When the role of such factors is elucidated, the resultant improvement in horticultural practices can be worth millions of Rand to the industry but this is soon forgotten as the new practice becomes the norm and research focuses on the next challenge. Some significant steps that were made during this last report period include confirmation that thiabendazole does reduce chilling injury and that lycopene in the rind of red grapefruit also reduces chilling injury. Increased levels of ethylene synthesis in lemon fruit reduce the incidence of peteca spot and prevention of water loss between harvest and waxing reduces the likelihood of pitting in Valencias and mandarins. It is now also clear that humates and fulvate, when applied with N, P or K, can increase microbial activity in some soils and reduce leaching of N. Frequent applications of silicon may also improve resistance to fungal diseases but as with other micronutrients, the type of formulation can have a significant influence on uptake by the plant. Leaf cuticle thickness was found to vary between citrus cultivars and apart from influencing uptake of micronutrients this may play a significant role in adaptation to different climates. One of the more obvious responses to our severe climate is sunburn and this is being seen quite frequently in the evaluation of some new mandarin hybrids in hot areas. Even the choice of rootstock can have an effect on susceptibility to sunburn by changing the shape of the tree canopy. A 14-year-old trial of Delta Valencia on 42 different rootstocks showed that a new rootstock, Citrange 32, produced double the crop found on trees with the commonly-used rootstocks, but six years earlier this rootstock gave similar results to many of the others. An endeavour to test the loading of warmer fruit destined for Japan to save on pre-cooling costs failed because shipping companies were not prepared to take the risk of atypical protocols, or chilling injury. On a positive note, we did manage to appoint a research horticulturist, Jakkie Stander, in Stellenbosch so he can start addressing some of the outstanding horticultural research requirements.

PORTEFEULJEOPSOMMING

Die resultate van tuinboukundige navorsing bou dikwels op mekaar omdat dit tyd neem om te bepaal watter van die verskeie faktore wat sitrusbome affekteer, ŉ beduidende impak op produksie, vrugkwaliteit, voorkoms of skil-integriteit het. Wanneer die rol van sulke faktore uitgeklaar word, kan die gevolglike verbetering in tuinboukundige praktyke miljoene rande vir die bedryf werd wees, maar dit is gou vergete wanneer die nuwe praktyke die norm word en navorsing op die volgende uitdaging fokus. ŉ Paar belangrike bevindings wat gedurende die laaste verslagperiode gemaak is, sluit die bevestiging in dat thiabendazole wel koueskade verminder, en dat die likopeen in die skil van rooi pomelo's ook koueskade verminder. Verhoogde vlakke van etileen-sintese in die skille van suurlemoene verminder die voorkoms van peteka kol, en so kan ook die voorkoming van waterverlies tussen oes en waksbehandeling die waarskynlikheid van gepokte skil in Valencias en mandaryne verminder. Dit is ook nou duidelik dat "humates" en "fulvate", wanneer saam met N, P of K toegedien word, die mikrobiese aktiwiteit in sommige gronde kan verhoog en die loging van N kan verminder. Gereelde toedienings van silikon kan ook die weerstand teen swamsiektes verbeter, maar soos met ander mikro-voedingstowwe, kan die tipe formulasie ŉ beduidende invloed op die opname deur die plant hê. Daar is gevind dat die dikte van die kutikula van die blaar tussen sitruskultivars verskil en, afgesien van die invloed op die opname van mikro-voedingstowwe, ook ŉ betekenisvolle rol in die aanpassing in verskillende klimate mag speel. Een van die meer ooglopende reaksies tot ons uiterste klimaat, is sonbrand, en dit word dikwels in die evaluering van sommige van die nuwe mandaryn-kruisings in die warm areas gesien. Selfs die keuse van ŉ onderstok kan ŉ effek op die vatbaarheid vir sonbrand hê deur die vorm van die boom se blaardak te verander. ŉ Veertien-jaar oue proef van Delta Valencia op 42 verskillende onderstokke het getoon dat ŉ nuwe onderstok, Citrange 32, dubbel die oes gelewer het as bome met die onderstokke wat algemeen gebruik word, maar ses jaar vroeër het hierdie onderstok soortgelyke resultate as baie ander gegee. ŉ Poging om die laai van warmer vrugte na Japan te toets om sodoende op voorverkoelingskostes te bespaar, het nie geslaag nie omdat die skeepsrederye nie bereid was om die risiko van a-tipiese protokolle of koueskade te dra nie. Op ŉ positiewe noot, ons het daarin geslaag om ŉ tuinboukundige navorser, Jakkie Stander, in Stellenbosch aan te stel waar hy kan begin om van die uitstaande tuinboukundige navorsing aan te spreek.

5.2 PROGRAMME: RIND CONDITION

Programme coordinator: Paul Cronjé (CRI-SU)

5.2.1 Programme summary

Two research projects were successfully completed during 2013 and reported on in addition to two ongoing research reports on postharvest pitting and peteca of lemon. The first project that was completed was on strategies to reduce the incidence of chilling injury and is a culmination of various experiments over several years (5.2.2). The findings in this project have been incorporated in handling practise and will serve as a starting point for further projects on this topic. In this report the importance of identifying and manipulating factors such as cultivar and harvest date was indicated as they affect CI incidence. In addition, preharvest colour development as seen in 'Star Ruby' grapefruit, as well as the use of TBZ and wax in the packhouse was seen to significantly influence CI incidence. In the other completed project the viability of using Non-destructive monitoring (near infra-red technology) to predict postharvest rind quality of 'Nules Clementine' Mandarin and Valencia orange fruit was studied (5.2.5). This powerful technology was able to separate fruit according to canopy position but was not able to reliably predict the incidence of rind disorders. This was probably due to the biochemical complexity of the citrus fruit rind that results in a vast amount of information masking the chemical signal picked up by the NIR technology. This non-invasive technology, however, currently offers the only cost-effective way to determine biochemical changes in a packhouse environment. Very low incidence of peteca spot of lemon occurred in the commercial situation in the various lemon production areas. This was mirrored in the results in the projects in which the ethylene metabolism was manipulated (5.2.3). However, the results confirmed that by increasing fruit ethylene synthesis pre-harvest, no increase in peteca would occur. In the postharvest pitting experiment (5.2.4) the fluctuations in rind water balance, as influenced by ambient conditions during handling have been shown to play a part in inducing rind disorders. By altering the VPD (vapour pressure deficit) between harvest and wax application of Valencia and mandarin fruit a higher incidence of pitting was recorded supporting previous results. The progress made in the various projects on rind condition and rind disorders during 2013 season has been significant and has not only yielded new scientific understanding of the fruit rind physiology but also practical actions that could be incorporated in the production or packing of citrus fruit.

Programopsomming

Gedurende die 2013 seisoen is twee projekte in die program suksesvol afgehandel en die resultaat word in die verslag saamgevat. Die vordering wat gemaak was gedurende 2013 in twee projekte maar wat nog oor die volgende paar seisoene sal sterk word bespreek. Die eerste projek wat afgehandel was, het bepaal watter maatreëls gebruik kan word om die voorkoms van koueskade te verminder (5.2.2). Dit was duidelik dat faktore wat beheerbaar is soos kultivars keuse en oes datum gebruik moet word om die waarskynlikheid van die voorkoms van koueskade te beperk. Daar moet ook aan aspekte soos kleurvorming, veral by 'Star Ruby' pomoloë en die gebruik van TBZ en waks wat in die pakhuis aangewend kan word om koueskade te beheer. Die ander afgehandelde projek het gepoog om deur die gebruik van nie-destruktiewe tegnologie (*Near Infra Red*) te voorspel of ŉ vrug skilafbraak sal ontwikkel (5.2.5). Die tegnologie kon suksesvol seker voorspellings maak soos vrug posisie in ŉ boom, maar kon nie skildefekte voorspel nie. Die rede kan moontlik gevind word in die komplekse biochemie van die skil wat die sein ontvang deur die NIR "kamoefleer". Hierdie tegnologie is egter tans nog die enigste koste-effektiewe manier om in ŉ pakhuis omgewing biochemies verandering in vrugte op te spoor. Gedurende die seisoen was baie lae voorkoms van peteka by pakhuise en uitvoerders gesien en die tendens is ook in die proewe gesien (5.2.3). Wat egter bevestig was in die jaar was dat om die vrug etileen sintese te verhoog deur voor oes spuite van Ethephon nie negatiewe resultate lewer soos hoër peteka voorkoms nie. Verandering in die skil se waterbalans, soos beïnvloed deur hoe en lae dampdrukverskille tussen pluk en waks aanwending, verhoog die kans om gepokteskile te ontwikkel (5.2.4). Die vordering gemaak gedurende die afgelope seisoen het nie net voorheen onbekende fisiologiese aspekte van die skil uitgelig nie maar ook gelei tot praktiese implementeerbare stappe in die voor en na-oes produksie van sitrusvrugte.

5.2.2 FINAL REPORT: Development of postharvest treatments to prevent chilling injury in various citrus species

Project 832 (2005/6-2013/4) by P.J.R. Cronje (CRI)

Summary

Citrus fruit exported from South Africa to markets such as the USA and China undergo a mandatory exposure of -0.6°C (22-24 days) during shipment to kill any insect larvae in the fruit, however, this protocol causes chilling injury (CI). In this project aspects of fruit production, handling and shipping were addressed in order to reduce CI. The current research strategy to address chilling injury differs from other rind disorders

in that we do not try to elucidate the mechanism of the disorders or causal factors, as these are known for CI. Therefore, the focus is on finding treatments that could be developed in practise that will either improve rind condition or protect the rind against low temperature damage. In the first part of the report the focus was on finding proof of variation in CI due to cultivar, harvest date and climate differences. In this study it was found that 'Washington' was more susceptible to CI compared to 'Navelina' navel orange. Fruit from the coldest part of Citrusdal (Tharakama) had the highest incidence of CI. The incidence of CI was overall less when fruit were harvested in the middle of the commercial harvest window; however, the internal maturity at harvest does not appear to be related to the sensitivity of orange fruit to CI. The second part looked at supplying a proof of concept regarding the use of Near-Infrared (NIR) spectroscopy as a potential management tool to identify variation in CI susceptibility and to predict fruit quality parameters in relation to CI. When analysing the NIR data with principal components analysis (PCA), score plots were obtained that separated fruit in clusters from the inside and outside of the canopy as well as different sizes and rind colours (green vs. orange). However, analysing data with partial least square regression (PLS) using fruit quality parameters (firmness, rind colour and mass) the NIR spectra obtained with the integrated sphere did not provide a good prediction model for CI index. The third section focused on the efficacy of thiabendazole (TBZ) application in the packline to reduce CI. The results of the application of different fungicides from the TBZ chemical group indicated that the TBZ dip treatments had the highest efficacy in reducing both the incidence and severity of CI and in addition were more effective when applied in warm (40°C) than cold (10°C) water. Applications at the commercial recommended rate (20 $mL.L^{-1}$) and half of the commercial recommended rate were both effective in reducing the incidence of CI. In an additional study, application of CO_2, Ethephon, 1-MCP and wax were tested for their impact on CI. Of all these treatments it was only TBZ and wax that was consistently effective in reducing the incidence of CI. In a novel study, previously unknown in Citriculture, it was ascertained that the amount of pink pigment (lycopene) accumulated in the flavedo of the 'Star Ruby' grapefruit has a protective action and if adequate concentrations are present in the flavedo they result in a significant reduction of CI. However, no postharvest treatments proved to enhance lycopene accumulation. It was only by increasing the amount of shade on the fruit from stage II (Oct-Dec) onwards that higher levels were realised. It is concluded that 'Star Ruby' grapefruit growers should focus on developing a leafy tree canopy with adequate fruiting position towards the main trunk. In addition by sorting fruit during packaging on the distribution of visible pigment the probability of economically devastating chilling incidence can be reduced. Freeze damage of fruit exported in containers to cold sterilisation markets is an increasingly worrisome occurrence. The impact of freeze damage was compared to high CO_2 exposure to differentiate between these types of postharvest stress. It was seen that freeze damage resulted in a totally changed fruit composition and was not similar to high CO_2 (closed ventilation scenario) under cold sterilisation conditions. For the successful reduction of CI incidence in commercial shipments of citrus fruit the focus should not be on a single factor but rather a strategy that encompasses pre-harvest factors that would influence rind quality as well as specific postharvest technologies known to decrease the impact of CI.

Opsomming

Sitrusvrugte ondergaan 'n verpligte blootstelling (22-24 dae) aan -0.6°C om moontlike insek-larwes te dood gedurende die uitvoer na markte soos die VSA en China, maar hierdie protokol veroorsaak koueskade. Die doel van hierdie projek oor verskeie seisoene was eerstens om die invloed van produksie, na-oes hantering en verskepings aksies op koueskade-sensitiwiteit van sitrus te bepaal. Die huidige navorsing strategie in koueskade is anders as ander skildefekte in dat daar nie gekyk word na wat die skildefek veroorsaak of die meganisme nie, want dit is bekend, maar slegs na strategie om die voorkoms te verminder. Verskillende faktore soos kultivar, mikroklimaat, oes-datum, vruggrootte en skilkleur beïnvloed die koueskade-sensitiwiteit van sitrus. In die projek was bevind dat die 'Washington' meer sensitief is vir koueskade as die 'navelina' nawels. Vrugte afkomstig uit die koudste deel van Citrusdal (Tharakama) het die hoogste voorkoms van koueskade. In die algemeen was vrugte ge-oes in die middel van die kommersiële-venster die minste koueskade-sensitief, maar interne rypheid hou nie verband met koueskade-sensitiwiteit nie. Naby-Infrarooi (NIR) spektroskopie is getoets as 'n potensiële instrument om vrugkwaliteit parameters te voorspel met betrekking tot koueskade. Deur ontleding van die NIR data met behulp van 'PCA' kon vrugte groepeer word volgens posisie (binne vs. buite blaredak), groottes en skilkleur. Deur 'PLS Regression' was data verder ontleed met inagneming van vrugkwaliteit parameters (fermheid, skil kleur en massa). Die NIR spektra wat verkry was kon egter nie 'n goeie voorspelling model vir koueskade verskaf nie. Die effektiwiteit van thiabendazole (TBZ) toediening in die verpakkings lyn, om koueskade te verminder was ondersoek en TBZ verminder koueskade na dit toegedien was in die waks, 'drench' of baddens. Die toediening van verskillende swamdoders van die TBZ chemiese groep in baddens, het aangedui dat die TBZ doop behandeling effektief was om die voorkoms van koueskade te verminder. Daarbenewens was TBZ meer effektief as dit toegedien word in warm (40°C) as koue (10°C) water, asook teen die volle (20 $ml.L^{-1}$) en die helfte van die aanbevole kommersiële dosis. In 'n addisionele eksperiment was die effektiwiteit van hoe CO_2 konsentrasie, 1-MCP, Ethephon en waks getoets. Slegs die waks toedings het konstant die koueskade voorkoms verhinder. Verder meer was wakstoediening effektief in die vermindering van die voorkoms van koueskade en byvoeging van

TBZ in die waks het die effektiwiteit verhoog. In ʼn studie wat vir die eerste keer die positiewe impak van lycopene op kouebestandheid van 'Star Ruby' pomelo aangetoon het, was bevind dat die lycopene sintese in 'Star Ruby' skil nog nie te volle verstaan is nie. Geen na-oes behandelings kon egter die lycopene in die skil verhoog nie en dus slegs deur beskaduing voor-oes (vanaf Oct-Des) wat genoegsame lycopene in die skil kan vorm. Die word aanbeveel dat 'Star Ruby' produsente poog om deur genoegsame vegetatiewe groei meer skadu in die boom te ontwikkel vir genoegsame beskading van die vrugte. Vriesskade gedure verskeping in houers na kouesterilisasie markte toon ʼn jaarlikse toename. Die impak van die vries van vrugte was vergelyk met hoë CO_2 blootstelling (soos ondervind in die toe ventilasie-gate-scenario in houers). Daar was egter geen verband tussen die twee na-oes stres toestande nie. Die suksesvolle vermindering van koueskade tydens kommersiële verskeping van sitrusvrugte kan nie fokus op ʼn enkele faktor nie, maar op ʼn strategie wat bestaan uit voor-oes faktore wat die vrugskil kwaliteit beïnvloed, sowel as spesifieke na-oes tegnologieë en hanteringsprotokolle wat bekend is vir die vermindering van koueskade. Soortgelyke navorsing word vir komende seisoen beplan.

General introduction: Chilling injury

The South African citrus industry exports citrus fruit to different countries including UK, Europe, Middle East, Russia, China, Japan, Far East and the United States. Citrus fruit exported from South Africa to the USA, Korea, Thailand and China undergoes a cold treatment for 24 days at -0.6°C preceded by precooling at the same temperature, and fruit to Japan for 12 days at -0.6˚C. This temperature treatment is used to disinfest fruit of insect larvae of major pests in citrus such as false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) and Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (White and Elson, 2004; EPPO, 2007). *T. leucotreta* is a major phytosanitary threat, and thus, regulatory procedures such as cold treatment and strict inspection before and after harvest are enforced by several countries such as the USA and China (Hofmeyr et al., 2005). However, this cold treatment may cause chilling injury (CI) which is a physiological disorder induced by low temperatures (<4°C). CI is an external quality defect on affected fruit, and symptoms vary including scalding, watery breakdown, rind pitting, soft glazed continuing lesions of mandarins, sunken tissue and damage to the stylar end of lemons (Reuther et al., 1989). Certain cultivars are more susceptible to CI; for example, mandarin is less susceptible than navel and Valencia orange fruit). Commercial experience in South Africa shows lemons and grapefruit are even more susceptible than mandarins.

This final project report will be presented in three sections that focus on differ aspects of cold injury. The results and discussions of the first section will be further divided into four separate sections to accommodate different experiments but will be concluded with a general discussion and conclusion section.

A. Strategies to reduce chilling injury of citrus fruit

A.1. Influence of cultivar, location and harvest date on chilling injury of Navel orange fruit.

A.2. Preliminary study on the use of Near-Infrared Spectroscopy to predict chilling injury susceptibility of Navel orange fruit.

A.3. Postharvest application of thiabendazole reduces Chilling injury of Navel Orange Fruit.

A.4. Various postharvest treatments to reduce CI of citrus

A.5. General discussion and conclusion

B. Increased lycopene content in the fruit flavedo to reduce chilling injury of grapefruit during cold sterilization shipments

C. The influence of postharvest storage on citrus fruit quality under high carbon dioxide and freezing conditions

Introduction

The increased volumes of citrus fruit in the market place and the ensuing competition have resulted in a focus on external quality which is the sum of the colour development as well as lack of blemishes due to physical or physiological stresses. Whereas horticultural research in Citriculture has primarily focused in the past on obtaining high yield and fruit sugar content the understanding of preharvest aspects on postharvest performance, as seen by fruit quality during shelf life and the lack of disorder or decay development, is limited. The major factors that influence the physiological processes, i.e. photosynthesis, assimilate transport

and accumulation, are also suspected of directly, or more often indirectly, influencing external or rind quality and determine fruit susceptibility to disorder development such as chilling injury.

Significant differences exist between cultivar groups in CI along the following general pattern: limes > lemons > white grapefruit > pink grapefruit > Navel orange > 'Valencia orange > mandarin. It is suspected that within a cultivar group, such as the Navel orange, large variation could occur but details are lacking. Rootstock choice is influential in yield, vegetative growth and soil borne disease resistance but Reynaldo (1999) reported that 'Ruby Red' grapefruit budded on a rough lemon rootstock had significantly lower losses due to CI and decay at a temperature range of 4–12°C compared to those grafted on *C. amblycarpa*. Canopy position is important for internal fruit quality and insufficient photosynthetic active radiation (PAR) contributes to the reduced TSS concentration of inside fruit (Sites and Reitz, 1949). PAR levels in a canopy have also been shown to influence rind condition and high exposure to PAR positively influenced rind carotenoid (colour) and carbohydrate content (Cronje, 2013).

Postharvest treatments that could decrease fruit sensitivity or protect fruit against chilling damage have received increased research attention over the last 15 years and the influence of antioxidant levels and strategies to increase these compounds in the flavedo have been the focus. Meir et al, (1996) observed a reduction in incidence of CI when fruit, such as avocado, grapefruit and bell pepper, was treated with methyl jasmonate (MA) which is a volatile methyl ester of JA, especially at low dosages. Droby et al. (1999) reported that postharvest application of JA (10 $\mu mol.L^{-1}$) on grapefruit stored for 6 weeks at 2°C was the most effective in reducing incidence of CI and green mould decay. When JA is applied at very low concentrations, it will enhance the resistance of grapefruit and thus reduce the incidence of CI. These are naturally occurring compounds used in very low doses and are more environmental friendly; it may therefore be possible to use them to reduce the application of fungicides to control decay of fruit (Droby et al., 1999). In addition, the use of Salicylic acid (SA) is known for resistance against certain pathogen attacks and also regulates the activity of alternative oxidase proteins which will alter the alternative oxidase pathway (Rhoads and McIntosh, 1992).

Postharvest temperature treatments have resulted in the most consistent impact on CI in citrus. Intermittent warming during storage reduces the occurrence of CI if done before any visible CI damage (Schirra and Cohen, 1999). Hot water baths (dip and brushing) reduce the incidence of CI with 'Star Ruby' grapefruit (Porat et al. 2000) and Ghasemnezhad et al. (2008) reported a reduction in CI with 'Fortune' mandarin especially when it was treated with a temperature of 47.5°C for 2 or 5 min and at 50°C for 2 min before storage for 8 weeks at 2°C. The implementation of this technology is hampered by the danger of heat damage and temperatures between 47.5°C and 50°C for 2 or 5 min cause rind damage. It is thought that the warm water treatments induce heat shock proteins (HSP), in addition to the activation of other defence pathways. Sala and Lafuente, (1999) reported an increase in CAT, an antioxidant which is one of the most important enzymes involved in heat induced chilling tolerance of 'Fortune' mandarin. Furthermore there was an increase in the activity of other antioxidants such as SOD and APX.

Certain postharvest fungicides, such as thiabendazole (TBZ) have been shown to influence the incidence of CI. Application of thiabendazole was effective in reducing the incidence of CI with 'Tarocco' oranges (Schirra and Mulas, 1995) and 'Star Ruby' grapefruit (Schirra et al., 2000). Schirra et al. (2000) reported that higher concentrations of 1200 $mg.L^{-1}$ TBZ (15°C) as a dip did not alleviate incidence of CI but a lower concentrations i.e. 200 $mg.L^{-1}$ (50°C) was beneficial in reducing the incidence of CI. Limited research was done on the mechanism of how this specific fungicide reduces the incidence of CI. CI susceptibility of citrus fruit is thought to be related to the efficiency of the antioxidant system in the plant and TBZ treatment may be related to the antioxidants' properties of fruit (Lindhout, 2007).

Postharvest wax application of citrus fruit is a way to seal fruit and to reduce moisture loss which also occurs during the incidence of CI. Gas exchange and transpiration of fresh produce are restricted by waxing fruit but waxing also reduces the incidence of CI and oleocellosis (Wang 1993, Wild 1993, 1998). Lindhout, 2007 reported that the application of wax was effective in reducing the incidence of CI in navel orange fruit.

The aim of this study was to investigate those pre and postharvest factors that could be manipulated to reduce the incidence of CI of citrus fruit specifically to determine the effect of harvest date, cultivar and microclimate on CI of navel orange fruit. Secondly the possible use of near infra-red spectroscopy to predict CI susceptibility of citrus fruit as influenced by fruit characteristics was studied. Thirdly the effectiveness of postharvest treatments *viz.* TBZ and wax applications in a commercial packline system on the CI incidence of navel orange fruit was evaluated.

A.1. Influence of cultivar, location and harvest date on chilling injury of Navel orange fruit.

Materials and methods

Plant material

Three areas which were separated by an average of 20 km in the Citrusdal valley were chosen as the source for sampling 'Washington' and 'Navelina' navel orange fruit. The three areas represent climatically different areas in the same valley and therefore served to create potential contrast in CI susceptibility due to climate difference i.e. Hexrivier and Brakfontein, (warmest with the lowest rainfall), Tharakama, (top of the valley highest rainfall, lowest temperature) and whereas Ouwerf farm situated in the middle of the valley fell between these two ambient climates (Table 5.2.2.1).

Fruit were harvested at three different sample dates in 2011, early (2 weeks before commercial harvest, 11 May), commercial harvest (25 May) and two weeks later (9 June). For each treatment 60 fruit were harvested to ensure enough fruit for four evaluation dates during cold storage and for each evaluation date three replicates of five fruit were used. Fruit were stored at -0.6°C for 35 days and were evaluated on day 1, 7, 21 and 35 for CI symptoms and ethylene production. The flavedo was peeled for the estimation of lipid peroxidation by using thin fruit peelers and frozen in liquid nitrogen whereafter it was stored at -80°C and freeze dried before it was finely ground prior to the estimation of the peroxidation of lipids. In 2012, fruit were harvested at the same sites and trees, and the same harvest dates were used. Fruit were stored for longer, i.e. 40 d, to ensure higher levels of CI to create greater contrast between treatments.

Data collection

Rind colour attribute, lightness (L*), chroma (C), and hue angle (H) were measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan).

Fruit size (height and diameter), Internal quality, (Total soluble solids, TSS °Brix) Titratable acidity (TA)

CI incidence was determined in 2011 after 7, 21 and 35 d of storage at -0.6 °C and after 1 week at shelf life (20°C) the fruit was evaluated for any CI symptoms. In 2012 the fruit were stored for 40 d. The CI data were collected to illustrate incidence and expressed as percentage CI, as well as severity of the injury expressed as CI index (Fig. 5.2.2.1). The incidence data were collected as yes/no whereas the severity fruit was scored from 0 to 3 and calculated with the following equation:

$$\text{Chilling injury index (0-3)} = \sum \frac{[\text{Chilling injury (scale 0-3)} \times \text{no. of fruit in each class}]}{\text{Total number of fruit in replicate}}$$

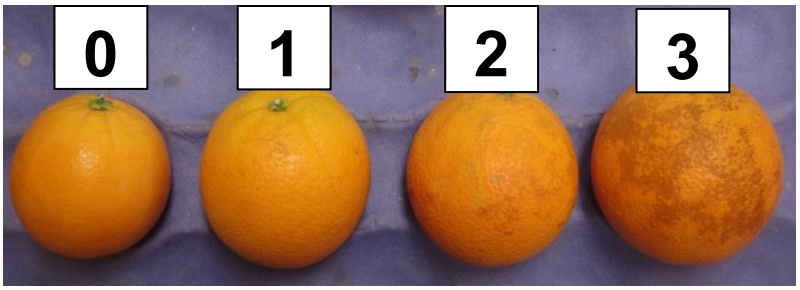

Figure 5.2.2.1. Chilling injury (scalding) scoring of navel orange according to severity (0 to 3)

Lipid peroxidation was determined with the modified method of Heath and Packer (1968). The complete method is in dissertation (http://hdl.handle.net/10019.1/80254).

Statistical analysis

Data of the CI index (ranked data) was analysed by using a one-way Anova and two-way Anova (test interaction) using SAS (SAS v.6.12; SAS Institute, Cary, VC, USA). Each treatment was compared with the other using Fisher least significance differences (LSD). The *p*-values illustrate the significance differences in the Figures presented. If the *p*-value is smaller than 0.05 there are significant differences between treatments. For data not analysed by Anova the standard deviation was given.

Table 5.2.2.1. Detailed information of the different sites in the Citrusdal valley that were used in 2011 and 2012 to sample Navel orange fruit

Location in valley	Farm and climate (avg. values over year)	Cultivar	Row orienta-tion	Plant density (m)	Year planted	Rootstock	Irrigation	Avg. yield (ton/ha)
Top	Tharakama 32°85'S, 19°09'E Avg. rainfall: 550 mm Avg. max: 27 °C Avg. min: 9 °C Elev. 233 m	Washington	North-South	7.6 × 3.8	1950	Rough lemon (*C. jambhiri* Lush)	Micro	30-35
		Navelina	North-South	6 × 3	1950	Rangpur lime (*C. limonia* Osbeck)	Drip	35
Middle	Ouwerf 32°65'S 19°05'E Avg. rainfall: 300 mm Avg. max: 28.1 °C Avg. min: 10.2 °C Elev. 180 m	Washington	North-South	6 × 3	1976	Rough lemon	Micro	50
		Navelina	North-South	6 × 4	1995	Rough lemon	Drip	38
Bottom	Brakfontein 32°.51'S, 18°99' E Hexrivier 32°.47' S, 18°97'E Avg. rainfall: 250 mm Avg. max: 29.2 °C Avg. min: 11 °C Elev. 151 m	Washington	East-West	5.7 × 3	1982	Rough lemon	Drip	40
		Washington	North-South	6.1 × 6.1	1931	Rough lemon	Micro	24
		Navelina	North-South	5.5 × 5.2	1996	Rough lemon	Drip	25

Results

No significant difference occurred in CI incidence between 'Washington' and 'Navelina' navel oranges harvested in 2011, however, there was 10% higher CI in the 'Washington' orange fruit (Fig. 5.2.2.2). No significant difference was found in CI incidence and CI index for 'Navelina' oranges between the various sites in 2011 and 2012 (Fig. 5.2.2.3). However, the same pattern is evident from both seasons with 'Navelina' orange fruit from Tharakama (cold area), being more susceptible compared to lower down in the valley.

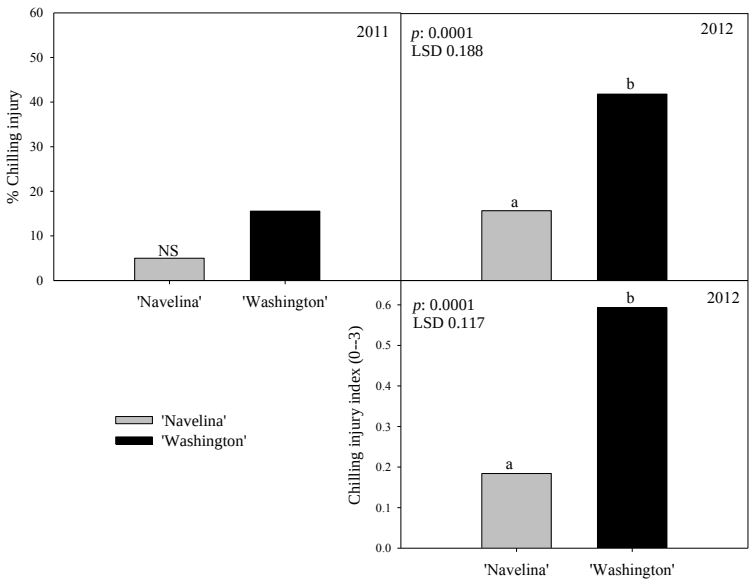

Figure 5.2.2.2. Incidence (% CI) and chilling injury severity index (0-3) of 'Washington' and 'Navelina' navel orange fruit harvested during 2011 and 2012 and stored at -0.6 °C for 35 d (in 2011) and for 40 d (in 2012), followed by one week at 20°C. Different letters indicate significant differences at the 95% level.

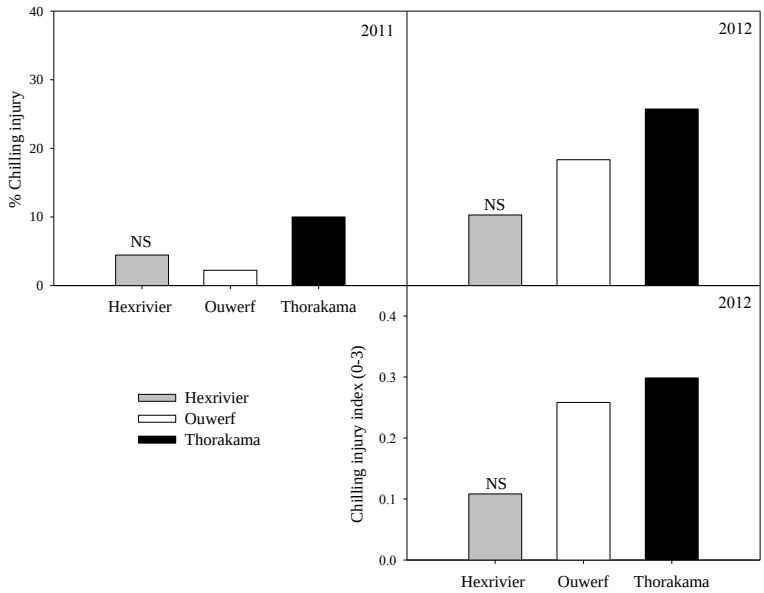

Figure 5.2.2.3. Incidence (% CI) and chilling injury severity index (0-3) of 'Navelina' navel orange fruit harvested during 2011 and 2012 at different sites in Citrusdal *viz.* Hexrivier (bottom of the valley) Ouwerf (middle of the valley) and Tharakama (top of the valley) and stored at -0.6°C for 35 d (in 2011) and for 40 d (in 2012), followed by one week shelf life at 20°C. Different letters indicate significant differences at the 95% level.

There was no significant difference between the different sites for 'Washington' navel oranges in 2011 even though the highest incidence of CI per region was recorded at Tharakama (Fig. 5.2.2.4). In 2012 there was a difference between fruit from Hexrivier and Brakfontein (both at the bottom of the valley). However, the same trend of higher CI susceptibility higher up in the valley was seen with a significant difference in CI incidence between Hexrivier and Tharakama.

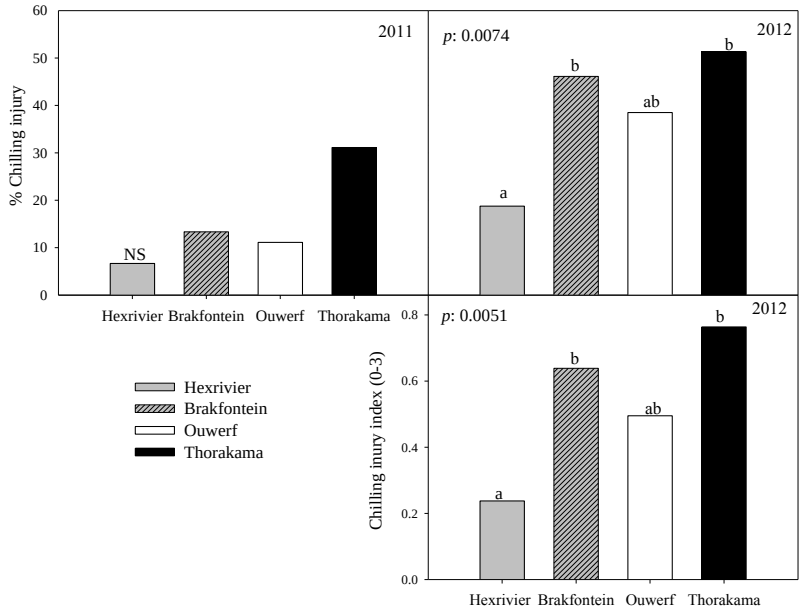

Figure 5.2.2.4. Incidence (% CI) and chilling injury severity index (0-3) of 'Washington' navel orange fruit harvested during 2011 and 2012 at different sites in Citrusdal *viz.* Hexrivier and Brakfontein (bottom of the valley), Ouwerf (middle of the valley), Tharakama (top of the valley) fruit was stored at -0.6°C for 35 d (in 2011) and for 40 d (in 2012), followed by one week shelf life at 20°C. Different letters indicate significant differences at the 95% level.

Analysing the CI index from 2012 for different harvest dates at each farm separately, indicate significant differences between different harvests dates (Fig. 5.2.2.5). CI incidence and the CI index follow the same trend for each site and harvest date. 'Washington' navel orange fruit from Tharakama had a difference in CI between early and late harvested fruit (late fruit lowest and early fruit highest) and at Hexrivier significant difference in CI occurred between all the harvest dates with the lowest incidence of CI at the commercial harvest date. At Brakfontein and Ouwerf there were no significant differences between the different harvest dates; however, the same trend occurred with the lowest incidence of chilling injury at the commercial harvest date.

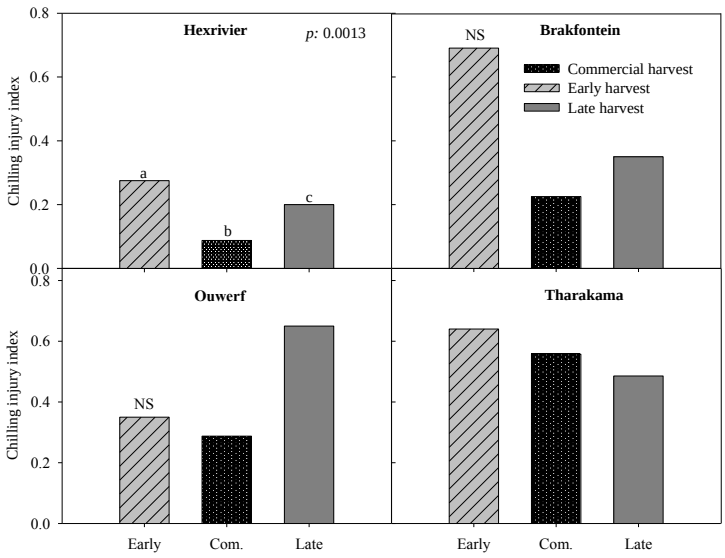

Figure 5.2.2.5. Chilling injury index (0-3) of 'Washington' navel orange fruit harvested at four different sites in Citrusdal during 2012: Hexrivier, Brakfontein (bottom of the valley) Ouwerf (middle of the valley) and Tharakama (top of the valley) and at 3 different dates i.e. early (11 May), commercial (25 May) and late (9 June) which was stored for 40 d at -0.6°C, followed by one week at 20°C. Different letters indicate significant differences at the 95% level.

The ratio (TSS:TA) of 'Washington' navel fruit between the different harvest dates did not differ significantly between Hexrivier, Ouwerf and Tharakama, however, at Brakfontein the late harvested fruit significantly differed from the early and commercial harvested fruit (Table 5.2.2.2). The internal quality of 'Navelina' navel didn't differ significantly at Hexrivier and Tharakama between the different harvest dates, however, at Ouwerf the late harvested fruit had a significantly higher ratio compared to the early and commercial harvested fruit.

There was no specific trend for the MDA estimation with CI, however, lipid peroxidation seemed to be linked to maturity rather than CI. Some of the graphs have an increase in the MDA concentration at day 35 and some of the graphs a decrease in the concentration. The different harvest dates also vary at the different sites. However, at Hexrivier the late harvested 'Washington' navel fruit had a higher MDA concentration from day 1 to 21 and Ouwerf and Brakfontein also showed a higher MDA concentration at day 7 and 21 when fruit were harvested late. At Tharakama the late and commercial harvested fruit had a higher MDA concentration at day 1 and 7 compared to early harvested fruit however at day 35 the early and the commercial harvest date has a higher MDA concentration (Fig. 5.2.2.6). The 'Navelina' navel fruit at Hexrivier show a higher MDA concentration when harvested late and commercially from day 1-21 and at Ouwerf the late and commercial harvested fruit had a higher MDA concentration compared to the early harvested fruit (Fig. 5.2.2.7).

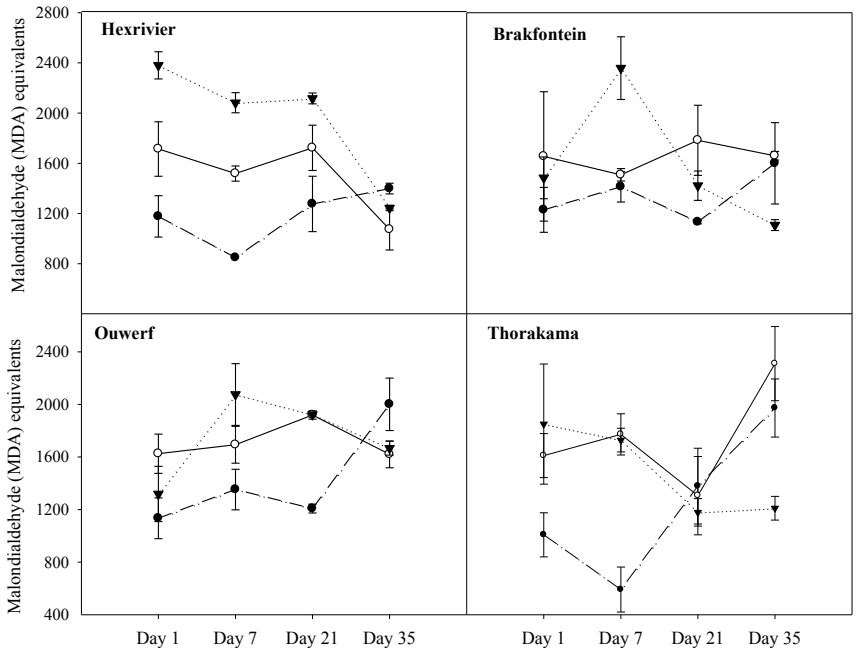

Figure 5.2.2.6. Lipid peroxidation indicated by malondialdehyde concentration (nmol.g DW^{-1}) in flavedo of 'Washington' navel orange fruit rind harvested early (11 May 2011), commercial (25 May 2011) and late (9 June 2011) at four different sites in Citrusdal i.e. Hexrivier, Brakfontein, Ouwerf and Tharakama. Fruit was stored for 35 d at -0.6°C, followed by one week at 20°C.

Table 5.2.2.2. TSS:TA average of 'Navelina' and 'Washington' navel orange fruit harvested at 3 harvest dates early (11 May 2011) commercial (25 May 2011) and late (9 June 2011) at 3 different sites Hexrivier (the bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley).

		Navelina				**Washington**		
	Hexrivier	**Brakfontein**	**Ouwerf**	**Tharakama**		**Hexrivier**	**Ouwerf**	**Tharakama**
Early	10.96	9.53a	10.09	12.12		11.32	9.74b	11.29a
Comm.	11.24	10.08a	10.50	12.16		11.16	13.04a	10.07a
Late	11.59	13.74b	11.00	12.23		12.17	13.83a	
p-value	*0.102*	*0.0001*	*0.208*	*0.987*		*0.06*	*0.004*	*0.102*

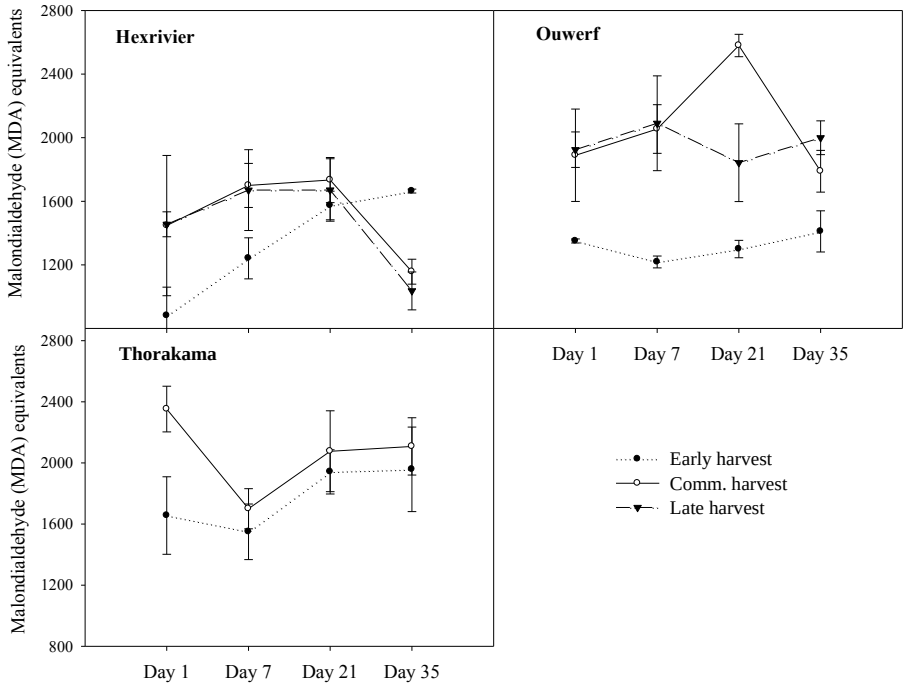

Figure 5.2.2.7. Lipid peroxidation indicated by malondialdehyde concentration (µmol.g DW^{-1}) in flavedo of 'Navelina' navel orange fruit rind harvested early (11 May 2011), commercial (25 May 2011) and late (9 June 2011) at three different sites in Citrusdal i.e. Hexrivier, Ouwerf and Tharakama. Fruit was stored for 35 d at -0.6°C, followed by one week at 20°C.

Discussion

Susceptibility to CI varies between navel orange cultivars with 'Washington' being more susceptible than 'Navelina'. Similar results were reported by Lindhout (2007), where 'Navelina' navel demonstrated low CI severity compared to 'Thomson' navel (moderately sensitive) and 'Roberts' navel orange fruit with a very high CI sensitivity. The variation in CI susceptibility of 'Washington' navel between the different sites showed that the top of the valley (cold area) had the highest incidence of CI for fruit harvested in 2011 and 2012 even though Brakfontein at the bottom of the valley had a high incidence of CI in 2012. Gonzalez-Aguilar et al. (2000) reported that 'Fortune' mandarin harvested in mid-season, the cooler months of the year, had a higher incidence of CI. The difference in CI susceptibility between harvest dates could be influenced by two factors i.e. ambient temperature and fruit maturity. In Florida, grapefruit that were harvested in the midseason had the lowest incidence of CI compared to early or late harvested fruit (Purvis et al., 1979) and were thought to be a result of immaturity and over maturity early and late in the season. In contrast, Gonzalez-Aguilar et al. (2000) reported that in Spain 'Fortune' mandarin fruit harvested early and late in the season had similar, although low incidence of CI. The fruit that were harvested in the midwinter had the highest incidence of CI. This increase in CI was thought to be a result of low temperature stress while fruit was still on the tree. CI in this study at the bottom of the valley Hexrivier, Brakfontein and Ouwerf follows in general a similar pattern to Florida, with the early and late fruit being more susceptible, however, Tharakama showed similarity to Purvis et al. 1979 where fruit harvested at the coldest site had the highest incidence of CI.

Fruit that are harvested late could be over mature and more susceptible to CI. Coggins (1969) reported that during maturation in citrus fruit, the albedo and flavedo cells enlarge and can become very vacuolated, the tissue can become structurally weak and spongy and the epicuticular wax could have more micro cracks consequently resulting in less resistance to the escape of water vapour from the rind. Schirra and D'hallewin (1997) reported that 'Fortune' mandarin harvested in the mid-season and dipped in warm water (53°C) reduced the incidence of CI and no heat damage occurred, but early and late harvested fruit were susceptible to heat damage, indicating the effect of fruit maturity on the rind's physiological condition. The immature fruit was also more susceptible to CI which could be related to rind pigments not synthesising to a threshold level needed for oxidative stress protection. Dou (2004) reported that 'Rio Red' grapefruit has a higher lycopene and B-carotene concentration with a consistently lower incidence of CI compared to the other cultivars. Lower temperature is needed for Gibberellin (GA) to be inhibited which influences chloroplast breakdown and the carotenoid production where there is conversion of chloroplast into a chromoplast.

Lipid peroxidation indicated by malondialdehyde did not show specific trends in relation to CI development and cold storage duration. It is thought, however, from this study that duration of cold storage could increase MDA due to an increase in the lipid peroxidation with incidence of CI. Certain fruit harvested early, late or commercial at different sites show an increase in MDA concentration but some show a decrease when stored for 35 days. An explanation could be that fruit variation could have contributed to the high amount of variation between the measurements. However, some of the sites did show that the late harvested fruit had a higher lipid peroxidation compared to the early harvested fruit. Late harvested fruit rind is older and more prone to lipid peroxidation. It is also possible that there are other compounds in citrus rind that interfered with the measurements which are not excluded. For future experiments it is advisable that high performance liquid chromatography (HPLC) should be used to measure the specific lipid peroxidation in the rind of the orange.

The internal maturity at harvest does not appear to be correlated to the development of CI in navel orange fruit. The TSS of the different sites increased or did not change between successive harvests for the different sites while CI incidence decreased towards commercial harvest and increased at late harvest in 2011. Similarly Lindhout (2007) also reported that maturity does not appear to be related to CI. The TSS and the maturity index increased and TA decreased between two sites in Australia (Dareton and Iraak) but the CI only increased significantly in fruit harvested from the Dareton site. This result reinforced the concept of the rind and the pulp being independent physiologically.

To conclude, there is a distinction in CI susceptibility between the two different cultivars with 'Navelina' being more susceptible than 'Washington' navel orange. In commercial handling, particular attention should be given to 'Washington' navel because of its high susceptibility to CI. For the different sites in Citrusdal the highest incidence of CI was at Tharakama (coldest part) at the top of the valley with the lowest average temperature during the year. The incidence of CI was overall less when fruit were harvested at the commercial harvest date. For future prospects the estimation of lipid peroxidation of the citrus rind could be done using high performance liquid chromatography (HPLC). The identification of a biochemical product that could be related to rind maturity could be useful in detecting optimum harvest date to decrease CI.

A.2. Preliminary study on the use of Near-Infrared Spectroscopy to predict chilling injury susceptibility of Navel orange fruit

Materials and methods

Plant material

'Washington' navel orange (*Citrus sinensis* L. Osb) planted in 1982 on 'Rough Lemon' rootstock (*Citrus jambhiri* Lush) were harvested on 25 July 2011 at Brakfontein (32°51'S 18°99' E) in Citrusdal. The tree density was 5.7 m by 3 m and the crop yield 40 ton.ha^{-1}. For the second season, on 25 July 2012, 'Washington' navel oranges planted in 1950 on 'Rough Lemon' rootstock (*Citrus jambhiri* Lush) were harvested at Tharakama (32°85'S 19°09'E) in Citrusdal. For this experiment 240 'Washington' navel orange fruit were harvested in the following manner for the trial: 40 fruit harvested at the outside of the canopy (10-30 cm inside the canopy) and 40 fruit inside the canopy (> 30 cm inside the canopy) with the same average size and colour. The second set of fruit comprised 40 large (± 82 mm) and 40 small (± 65 mm) fruit from the outside of the canopy with the same colour and lastly 40 orange coloured fruit with average hue angle 66 and 40 green with average hue angle 73 of the same size (Fig. 5.2.2.10). In 2011 all samples' fruit mass, colour and firmness were determined after harvest before NIR spectra were acquired from fruit harvested. After measurements, the fruit was stored for 35 d at -0.6°C and 1 week on the shelf before measuring mass, colour, firmness and determination of the CI index. Only the CI index, weight loss and colour parameters were determined for navel orange fruit harvested in 2012.

Data collection

Fruit mass was determined before and after storage using an Elec checking scale NBK-30(Model NWH 10422, UWE South Africa). The colour measured and chilling injury scores were as described above. Texture properties were measured using a TA.XT plus Texture Analyser (stable Micro systems, Surrey, England) with extra software (Exponent Version 5.0.9.0). The load cell of texture analyser contains a force sensor which moves down to the sample at a constant velocity. The analyser was calibrated with a calibration weight of 10 kg. A cylindrical flat head aluminium probe which was 39 mm in length and 35 mm in diameter was used for the compression load cell. The load cell moved at a constant velocity to the sample at 2 mm per second, when the probe come in contact with the fruit the fruit was compressed for 6 mm. The sensor in the load cell measured the force that was needed to compress the fruit (Hanssens, 2011).

NIR spectra were taken using the integrated sphere which is an accessory on the Brüker Multi Purpose Analyser (MPA) FT-NIR spectrometer (Brüker Optik GmbH, Etlingen, Gremany) and is used to measure diffuse reflectance of highly scattering solid media. The integrating sphere consists of a 50 mm wide sample cup holder for the measurements of homogenous samples. Fruit was placed on top of the holder cup and the NIR beam is directed into the sphere from where it is directed in through the optical window to the sample. The beam scatters off the sample and the light that is reflected enters the sphere and is directed to the detector. The sphere is gold coated to ensure that all light beams are collected and directed to the detector. The wavelength region which was scanned was from 780 nm to 2500 nm with a resolution of $8.cm^{-1}$. For each fruit 64 scans were taken per spectrum with a resolution of 16 (Hanssens, 2011). Each fruit was evaluated from 6 different positions. The first spectrum was taken at the top and the second at the bottom, the third to sixth were taken with equatorial positions (with 90º difference between consecutive positions) were directed toward the source.

Statistical analysis

Data of the CI index (ranked data) were analysed by using the one-way Anova of SAS (SAS v.6.12; SAS Institute, Cary, VC, USA). Each treatment was compared with the other using Fisher least significance differences (LSD). The *p*-values illustrate the significance differences in the Figures presented. If *p*-value is smaller than 0.05 there is significant differences between treatments. Correlations were done between the CI index and the physical parameters (colour, firmness and mass) using Statistica V.10 (Statsoft Inc. Tulsa, UK). To determine if it is possible to measure the physical properties with NIR before storage and to classify the Navel orange according to susceptibility to CI the following analyses were done.

Principal component analyses (PCA) were obtained using Unscrambler 9.7 (CAMO software AS, Norway) which transforms the data to a new coordinate system such that the greatest variance by any projection comes to lie on the first coordinate. This is a manipulation of the data where the aim is to present the variation in different variables using a small number of factors. With PCA the variables are transformed into principal components (Cozzolino et al., 2011). The use of principal components allows viewing the nature of data in small number of dimensions, which consist of matrices with many variables (Beebe et al., 1998). Partial least squares regression (PLS) method is commonly used in quantitative spectroscopy to correlate spectroscopy data X with related chemical measured data Y. Partial least square regression was applied to spectral data to develop prediction models for firmness, hue angle, Chroma, lightness and mass. Partial least squares were obtained from Unscrambler 9.7 (CAMO software AS, Norway) and OPUS version 6.5 (Bruker Optik GmbH, Ettlingen, Germany). Before the Calibrations the reflectance data were transformed to absorbance, mean normalised and optionally treated by scatter correction using Unscrambler 9.7. Pre-processing or scatter correction is a mathematical manipulation of the data before the primary analysis and is used to remove or reduce irrelevant sources of variation (Beebe et al., 1998). For the validation of PLS a test set validation was done where 40% of the data was used for validation. To assess the accuracy of the calibration performance root mean square of calibration (RMSEE), root mean square error of prediction (RMSEP), and the residual predictive deviation (RPD) were calculated (Williams and Sobering, 1996). A good model should have a lower RMSEE, RMSEP and a higher correlation coefficient (r) or coefficient of determination (r^2) and RPD value. An RPD between 1.5 and 2 indicates that the model can discriminate low from high values; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy (Nicolaï et al., 2007).

Results

Chilling injury and external quality aspects

The results showed significant differences between the CI index obtained directly after storage compared to CI index after a week of shelf life (Fig. 5.2.2.8). Fruit from the outside had a significantly higher CI index compared to fruit from the inside in 2011; however, in 2012, there was no significant difference between the treatments. Smaller fruit were more susceptible to incidence of CI compared to large fruit (Fig. 5.2.2.9). There were significant differences between orange and orange/green 'Washington' navel oranges. Fruit with better rind colour (low hue angle) were more susceptible to CI and had a 41.2 % higher CI index compared to the orange /green fruit in 2011 (Fig. 5.2.2.10).

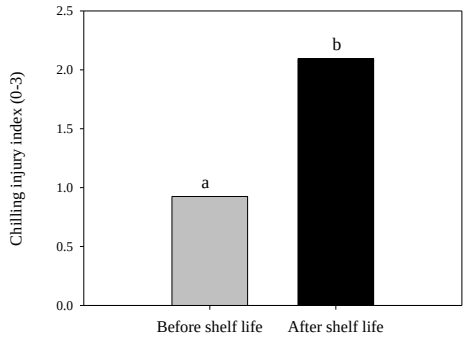

Figure 5.2.2.8. Chilling injury index (0-3) between 'Washington' navel orange fruit stored at -0.6 °C for 35 d determined before and after a week of shelf life (20°C) ($p \leq 0.05$).

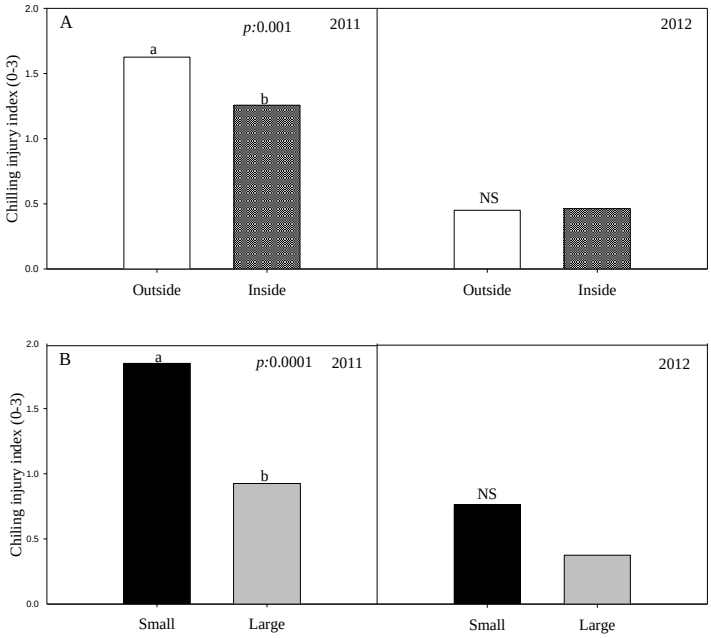

Figure 5.2.2.9. Chilling injury index (0-3) between 'Washington' navel orange fruit classified according to position (A) and fruit size (B) large (±82 mm) and small (±62 mm) in diameter. The fruit was stored at -0.6°C for 35 d and 7 d of shelf life. Different letters indicate significant differences at the 95% level.

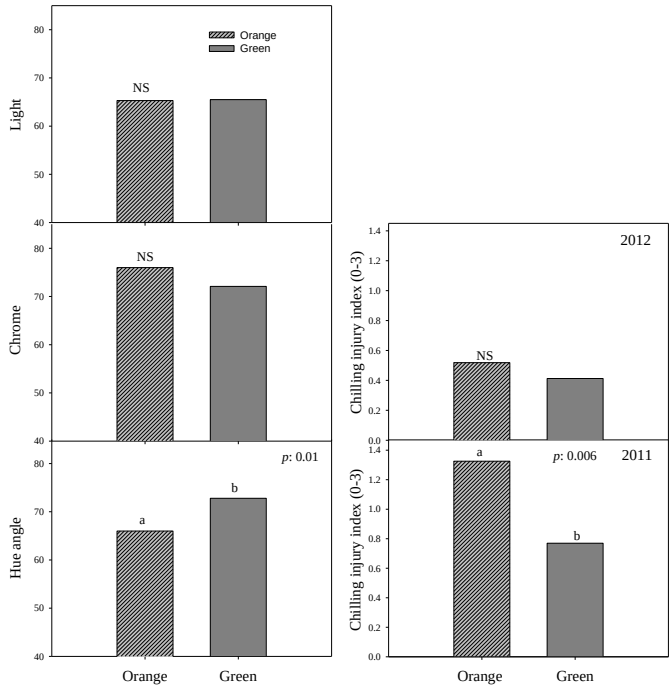

Figure 5.2.2.10. Colour attributes (L*, C*, H*) for 2011 and Chilling injury index (0-3) in 2011 and 2012 of green and orange colour 'Washington' navel orange fruit stored at -0.6°C for 35 d. Different letters indicate significant differences at the 95% level.

Negative correlations existed between CI and the different physical parameters *viz.* mass (before & after cold storage), diameter and length of the fruit which indicated that smaller fruit has a higher susceptibility to CI than larger fruit. Fruit firmness, before and after storage, had no correlation with the incidence of CI (Table 5.2.2.3).

The hue angle (before & after cold storage) had a negative correlation which indicates that greener fruit (with a higher hue angle) had a lower susceptibility to CI than orange coloured fruit. Orange fruit with an average of 66° hue had a 42% higher incidence in CI than green fruit with a higher average hue angle of 72.8°. The other colour attributes i.e. Chroma (C) also had negative correlations with CI, indicating that fruit with more vivid colour had lower incidence of CI.

Fruit NIR spectra

Typical diffuse absorbance spectra of fruit acquired by the FT-NIR spectrometer NIR are shown in Figure 5.2.2.11. The wavelength range of 800 nm to 2500 nm was applied to develop the calibration models. In the wavelength from 800 nm to 1800 nm the spectra curves begin to ascend until 1450 nm and then reduces up to 1670 nm, and rise again. There are two large absorption peaks at 1210 nm and 1450 nm, which are associated with bonds of C-H and overtones of bond O-H in H_2O, respectively.

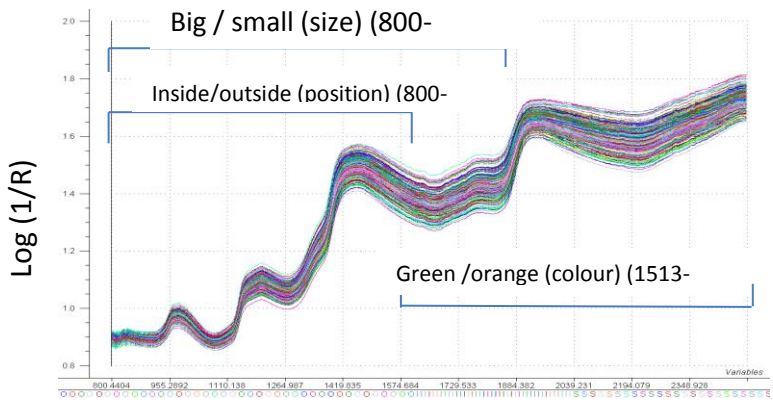

Figure 5.2.2.11. Absorbance spectra (log(1/R)) of 'Washington' navel orange fruit in the wavelength 800-2500 nm. (R= reflectance).

Principal component analysis (PCA)

Individual spectra from the six positions taken on fruit and the average spectra were tested to develop PCA and PLS models. Average spectra of the fruit showed better models than individual spectra at one position of the fruit. PCA was performed on the NIR spectra to compare the spectral characteristics of fruit from different canopy positions, sizes and colours. A PCA score plot, similar to a map of the samples, indicates how the different observations are explained by the principal components. Different observations which have the same characteristics would be grouped together in a score plot (Hanssens, 2011). The data distribution in the PCA score plot displayed two clusters in each PCA score plot for the green and orange fruit with an 87.5% level of accuracy (Fig. 5.2.2.12) for the inside and outside canopy fruit with an 86% level of accuracy (Fig. 5.2.2.13), for the large and small fruit with an 86% level of accuracy (Fig. 5.2.2.14). These clusters provided good discrimination between the different treatments.

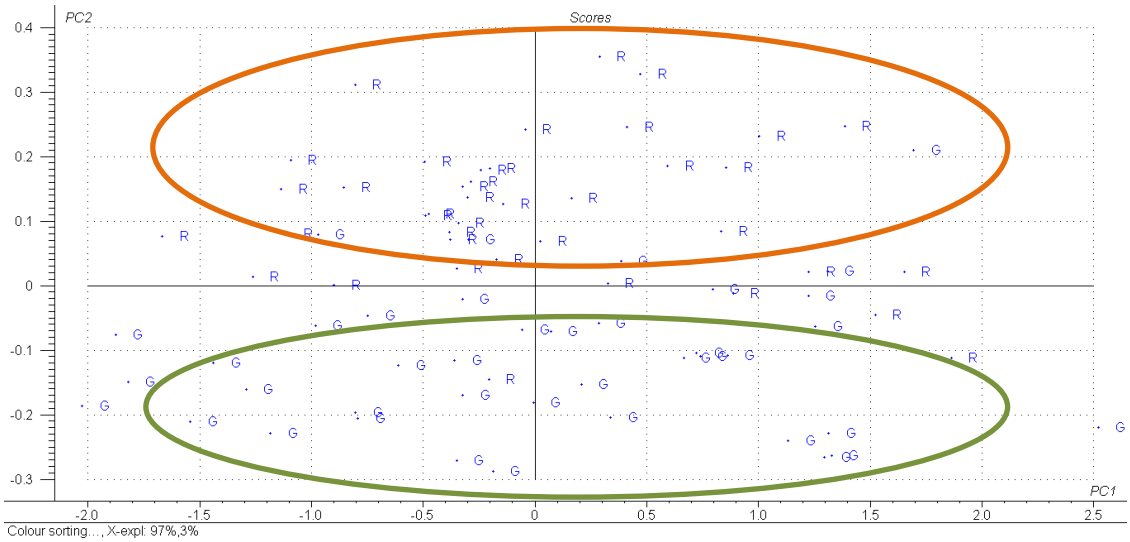

Figure 5.2.2.12. PCA plot for the two PC factors showing spectral ability to sort based on colour. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6°C. G represents green fruit and R represents orange fruit.

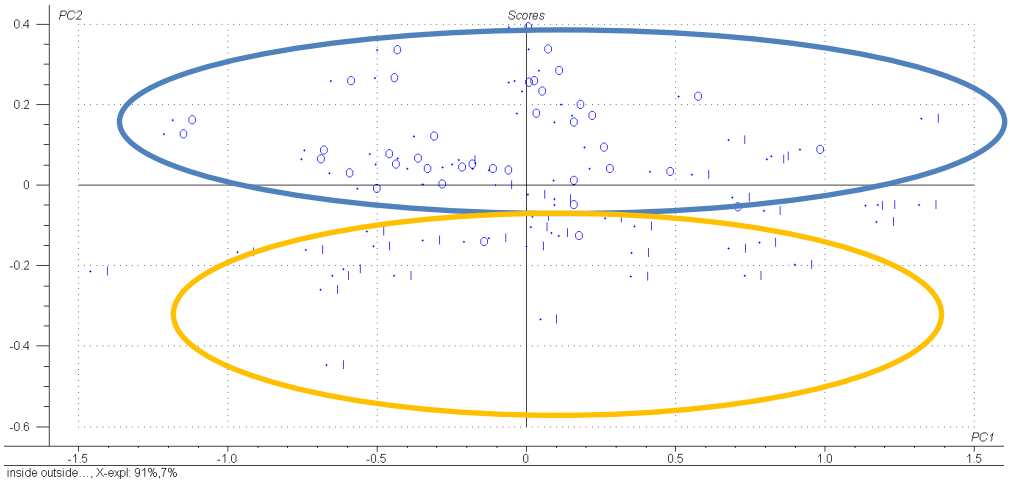

Figure 5.2.2.13. PCA plot for the two PC factors showing spectral ability to sort based on their origin within the tree canopy. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6°C. I represents fruit from inside and O represents fruit from outside.

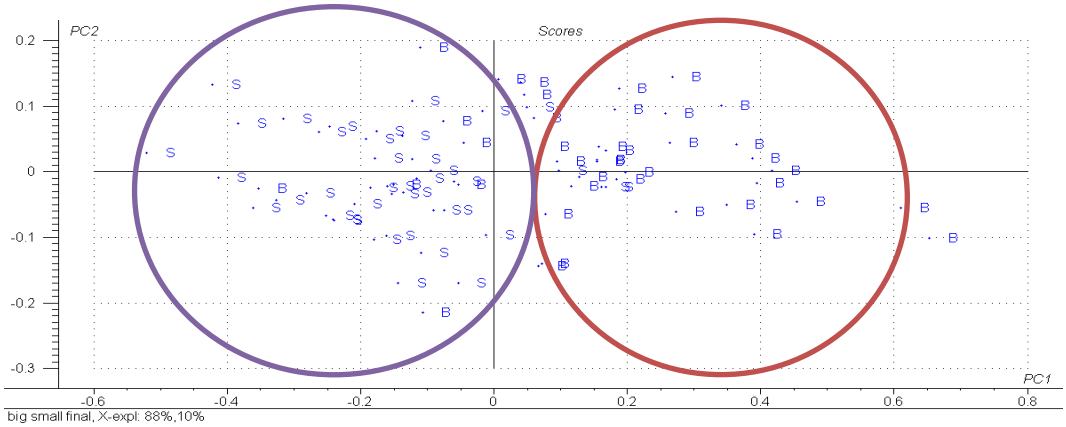

Figure 5.2.2.14. PCA plot for the two PC factors showing spectral ability to sort based on their size. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6°C. B represents large fruit (±80 mm) from inside and S represents small fruit (±60 mm).

The first two principal components for the orange and green fruit accounted for 100% of which 97% of the variance is explained by the principal component 1 and 3% by Principal component 2. There was a clear distinction between the green and orange fruit with principal component 2 using a spectral range from 1513-2500 nm (Fig. 5.2.2.12). This spectral range was used because it results in better discrimination between the green and orange fruit. The first two principal components for the inside and outside fruit accounted for 100% of the X (NIR spectra) variance, 91% of the variance is explained by the principal component 1 and 7% by Principal component 2. There was a clear distinction between the inside and outside fruit with principal component 2 using a spectral range from 800-1600 nm (Fig. 5.2.2.13). The first two principal components for the large and small fruit accounted for 98% of the X characteristic's variance, which 88% of the variance is explained by the principal component 1 and 10% by principal component 2 (Fig. 5.2.2.14). There is clear distinction between the big and small fruit using principal component 1 with a spectral range from 800-1800nm.

PLS prediction models

The NIR data were subjected to several pre-processing methods but the spectral pre-processing did not increase the accuracy of the prediction models. Prediction models were obtained by using the outside and inside fruit for prediction, the large and small fruit for second prediction and the green and orange for the third prediction for the different physical properties. Table 2 shows a summary of statistics for calibration and validation results for the different physical properties with PLS models. The prediction models for firmness and hue angle, chrome and lightness parameters were not reliable with a very low validation predictive R^2-values smaller than 30 % and an RPD level below or just above 1.5 indicating that the model cannot discriminate between values (< 1.5) or can only discriminate between very high and low values (Nicolaï, 2007). Mass prediction had higher R^2 value indicating that it could be possible to determine the mass of the fruit with the NIR but due to only the 6 different positions by which the NIR beam enters the fruit it was not possible to determine precise mass of the fruit.

Discussion

Citrus fruit which were harvested during 2011 from outside the canopy had a significantly higher CI index compared to fruit from inside the canopy which concurs with (Nordby and McDonald, 1995; Purvis, 1984). It is possible that the high light intensity in Citrusdal on the exposed fruit in the outside canopy, could result in fruit being more prone to sunburn thereby increasing fruit susceptibility to CI. High sunlight leads to an excess in photosystem 1 reduction and the rate of fixation of CO_2 cannot be maintained and $NADP^+$ is reduced, this will cause oxygen to compete for the electrons of photosystem 1 and will cause the formation of ROS and eventually cause lipid peroxidation and alteration in the membrane (Sevillano, et al. 2009). Free radicals and ROS and lipid peroxidation will increase and the membrane doesn't have the ability to recover.

Smaller fruit had a higher incidence of CI compared to larger fruit. There is competition for the photosynthates among different organs i.e. fruit to shoot as well as among fruit resulting in larger or smaller

fruit (Goldsmith and Koch, 1996). In citrus, the competition between fruit is more evident, than in other fruit trees. The reduction in the fruit numbers on a specific tree is due to the carbohydrate status of the specific tree (Goldschmidt et al, 1992). Larger fruit are considered to be a stronger sink during fruit development and could have a higher nutrient and carbohydrate status. In comparison to smaller fruit with low sink capacity which consists of less carbohydrate and nutrients. Some studies showed that carbohydrate metabolism is involved in the protection of tissue against the chilling stress. Purvis et al., (1979) reported a strong correlation between the reducing sugars and the resistance to the incidence of CI by stabilising the membrane. In grapefruit, reducing sugars were correlated with decrease in CI sensitivity of the fruits during the season (Purvis and Grierson, 1982). Larger fruit is a better sink which has more reducing sugars and carbohydrate reserves and is thus probably less prone to CI.

Fruit with green/orange rind colour were less susceptible to chilling injury than orange coloured fruit. In contrast, Grierson (1974) as cited by Purvis et al. (1979) reported that yellow grapefruit were more resistant to CI compared to green grapefruit. It was thought that the orange fruit will be less susceptible to CI but the results show the opposite. An explanation therefore can be that chlorophyll can protect the fruit against CI index. Pigments are considered a protective function against various pre- and postharvest stresses, for example photo protection in apples (Merzlyak and Chivkunova, 2000). It is possible that better coloured fruit could have been over mature, which could lead to a higher susceptibility to CI.

PCA plots models were successfully used to identify the different preharvest treatments. NIR models were used to discriminate among large/small fruit, inside/outside fruit and green/orange fruit without the use of different physical measurements. PLS models for firmness and colour were unacceptable due to a very low explained variance. Similarly Hanssens (2011) reported that the model for the compression force or firmness wasn't able to predict, possibly due to the small variation in firmness or robust measurement not picking up small differences in range where it is required. The variation in the firmness/hardness has to be more representative before being possibly used as a prediction model for firmness. If fruit is stored at cold temperatures and warm temperatures, a larger variation in firmness could be evident and then it may be possible to have a better prediction model for firmness.

The colour parameter (hue angle, chroma, lightness) models were not acceptable, probably due to the NIR wavelength used (800-2500 nm) which is above the visible range (450-700 nm). As recently reported by Magwaza et al. (2012b) models for the colour parameters were developed using the visible range of the spectrum. Fruit mass gave a better prediction model compared to the firmness and the colour parameters. However, on a sorting line, cheaper and more robust techniques are already currently being used to detect the mass and dimensions (L×h) of the fruit.

To conclude, it was possible to apply NIR spectral range using PCA plots to distinguish between fruit from different canopy positions, different sizes and different colours. Reliable prediction of colour parameters and firmness were difficult to obtain using integrated sphere NIR measurements from a wavelength range of 800-2500 nm, but a better prediction model for colour parameters could possibly be obtained using a broader spectrum 450-2500 nm. With the different parameters firmness, colour parameters, mass and the NIR spectrum using an integrated sphere, a good prediction model for the CI index could not be obtained. For future prospects the changes in biochemical product of the rind, for example carotenoids and lipid peroxidation, could be assayed and perhaps used for a prediction model for CI index if it could be reliably identified via NIR.

Table 5.2.2.3. Correlation between CI and physical parameters [mass, firmness, colour attributes (L, C, H)] of 'Washington' navel orange fruit measured before and after storage for 35 d at -0.6°C.

Variable 1	Spearman	p-value
Mass before	-0.37	<0.01
Mass after	-0.39	<0.01
Firm before	0.03	0.62
Firm after	0.02	0.73
H before	-0.24	<0.01
L after	-0.14	0.04

H after	-0.33	<0.01
C after	-0.19	<0.01
H after	-0.21	<0.01
Diameter	-0.32	<0.01
Length	-0.34	<0.01
C_B diff	0.17	<0.01

Table 5.2.2.4. An overview of statistics from calibration (n = 48), validation (n = 32) and of models for individual quality parameters.

	Calibration					Validation		
Treatment	parameter	r^2	RMSEE	RPD	Rank	r^2	RMSEP	RPD
Outside and inside	Mass before	62.46	25.20	1.63	6	45.35	31.30	1.36
	Mass after	33.73	32.00	1.23	3	27.13	35.50	1.17
	Firm before	13.18	20.40	1.07	1	27.49	18.10	1.17
	Firmness after	43.45	7.80	1.33	3	31.34	9.24	1.21
Large and small	Mass before	88.91	27.10	3.00	4	80.22	35.20	2.31
	Mass after	77.39	44.70	2.10	4	74.85	38.80	2.06
	Firm before	0.44	30.30	1.00	1	-5.89	30.60	0.98
	Firmness after	27.85	13.30	1.18	3	35.95	10.70	1.25
	C after	21.43	10.20	1.13	2	4.46	4.61	1.02
	H after	15.02	9.93	1.08	3	10.45	3.20	1.09
Green and orange	Mass before	38.04	36.10	1.27	1	26.23	31.20	1.17
	Mass after	41.05	34.80	1.30	2	1.94	35.10	1.01

A.3. Postharvest application of thiabendazole reduces chilling injury of navel orange fruit. PJR Cronje (CRI), Jeanine Hordijk (SU), Mareli Kellerman (SU) and Paul Fourie (CRI)

Materials and methods

'Washington' navel orange fruit was harvested on 25 July 2011 at Brakfontein, Citrusdal. In this experiment 600 'Washington' navel orange fruit of equal size and colour were harvested and divided into 20 fruit for each treatment with three replicates. Different fungicides, benomyl (Benomyl/Benlate®; 20 g/10 L), Carbendazim (Benazid®; 40 mL/20 L) and thiabendazole (Tecto®; 40 mL/20 L and ICA-TBZ®; 80 mL/20 L) were applied. These different treatments were applied to the fruit for 2 min in either cold water (10°C) or warm water (45°C). The controls used were 'Washington' navel oranges dipped in warm (45°C) water and cold (10°C) water for 2 minutes. Thereafter the fruit was stored for 35 d at -0.6°C and CI was scored as discussed above.

After the results of the first experiment were available, 'Cambria' and 'Autumn Gold' navel orange fruit (both late maturing cultivars) were harvested from Tien Riviere (32°.81'S, 19.08E) in Citrusdal on 14 Augustus 2011. For this experiment different concentrations of the TBZ (Tecto®) were tested viz. the commercial

recommended rate (40 mL/20 L) as well as dilutions of a half and quarter of this concentration. These different treatments were applied to the fruit for two minutes in either cold water (10°C) or warm water (45°C). Thereafter, the fruit was stored at -0.6°C for 35 d, kept at shelf life (20°C for 7 d), prior to scoring of CI incidence and severity.

The 2012 experiment consisted of applying TBZ as a water dip (warm vs. cold) and in a drench as well as in the wax. The experiment was repeated with 'Washington' navel followed by 'Autumn Gold' navel orange fruit. 'Washington' navel (900) orange fruit was harvested from WST-Karingmelksvlei in Citrusdal on 20 July 2012, divided into eight treatments with three replicates of 30 fruit per replicate while the rest of the fruit was used in the packline trial between the different treatments as pusher fruit to separate between treatments. 'Autumn Gold' navel orange fruit (700) were harvested from Rosedale in Robertson on 20 August 2012. For both sets of fruit the rind colour and size were within a narrow range to reduce variability. The eight treatments consisted of dipping, drenching and waxing. In the dip treatments the fruit were dipped for 1 min in 1000 ppm TBZ solution and in water for the control, these treatments were applied either in cold water (10°C) or hot water (35°C) and the fruit were allowed to air dry. The drench consists of pipes with openings, a water flow pump and a container with solution. The water or solution was pumped through the pipes out of openings onto the crate with fruit (Fig. 5.2.2.15). Fruit were exposed to the drench for 45 s using water (control) and a 1000 ppm TBZ solution whereafter the fruit were air dried. For the wax and drench treatments four different treatments were tested, i.e. first the application of only water in the drench and a clean wax, secondly drench with TBZ and clean wax, thirdly water in the drench and application of a TBZ wax, and lastly drench with TBZ and wax with TBZ.

Figure 5.2.2.15. The drench application used for 45 seconds on navel orange fruit.

The application of the wax was done with a custom-built experimental packline (Dormas, Johannesburg, South Africa) which is similar to packlines at commercial packhouses. This packline consists of four different units, an elevator which feeds fruit into the line, spray–on recycling washing system over eight brushes, a commercial coating applicator (JBT Foodtech, Brackenfell, South Africa) and an air drying tunnel. The drying tunnel uses very high volume air at a slow speed to dry the fruit. The coating applicator was calibrated using one pulsating nozzle (0.5 s on, 2 s off) at 22 $mL.min^{-1}$ (3 bar). The fruit moved across the packline at a set speed since the packline is speed controlled and the coating units have brush sweep paddles which move the fruit (Njombolwana, 2011). The wax used was the natural-based type with 18% solids consisting of carnauba-shellac formulations (875 High Shine, John Bean Technologies, Brackenfell, South Africa). The fruit were treated with clean wax with no addition as well as wax with TBZ at a concentration of 4000 $\mu g.mL^{-1}$. TBZ and wax were stirred throughout the trial with a magnetic stirrer. The fruit were exposed to the coating applicator for 20 s which resulted in a coating load of 1.2 $L.ton^{-1}$ of fruit.

After the application of the different treatments for the 'Washington' navel orange fruit, six fruit of each replicate were used to determine whether residues were below the maximum residue level (MRL) and then six fruit were divided into the flavedo, albedo and pulp tissue to determine the TBZ content in each. The rest of the fruit and the 'Autumn Gold' navel fruit were stored at a temperature of -0.6°C for 40 d, kept at shelf life (20°C for 7 d), prior to scoring of CI incidence and severity. The fruit was prepared for residues only in the following manner; two of the three replicates of the various treatments were used for TBZ residues for the Washington navel orange fruit. From each treatment combination six fruit were frozen (-20°C) after being weighed until further preparation. Fruit was defrosted, and by using a fruit blender the fruit was ground to a fine pulp (Salton Elite, Amalgamated Appliance Holdings Limited, Reuven, South Africa). For the

determination of TBZ distribution in the fruit, part of the flavedo, albedo and pulp of three oranges of each treatment were separated. The samples were frozen in liquid nitrogen and stored at a temperature of -80°C. Samples were submitted for TBZ residue analyses by an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town, South Africa). Extraction of the samples was done by using acetonitrile and matrix solid phase dispersion extraction. The analysis of the extraction was done using a liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA) (Njombolwana, 2011).

Statistical analysis

Data on CI and residues were analysed by one-way Anova and two-way Anova-tests using Statistical analysis system (SAS v 6.12; SAS institute, Cary, UC, USA). Each treatment was compared with the other using Fisher least significance differences with one-way Anova, which is a test to determine the difference between the treatments. The p-values illustrate the significance differences in the Figures presented. Where the *p*-value was smaller than 0.05 there was a significant difference between the treatments.

Results

CI significantly differs between fruit applied with Tecto in warm water and Tecto in cold water (Fig. 5.2.2.16).

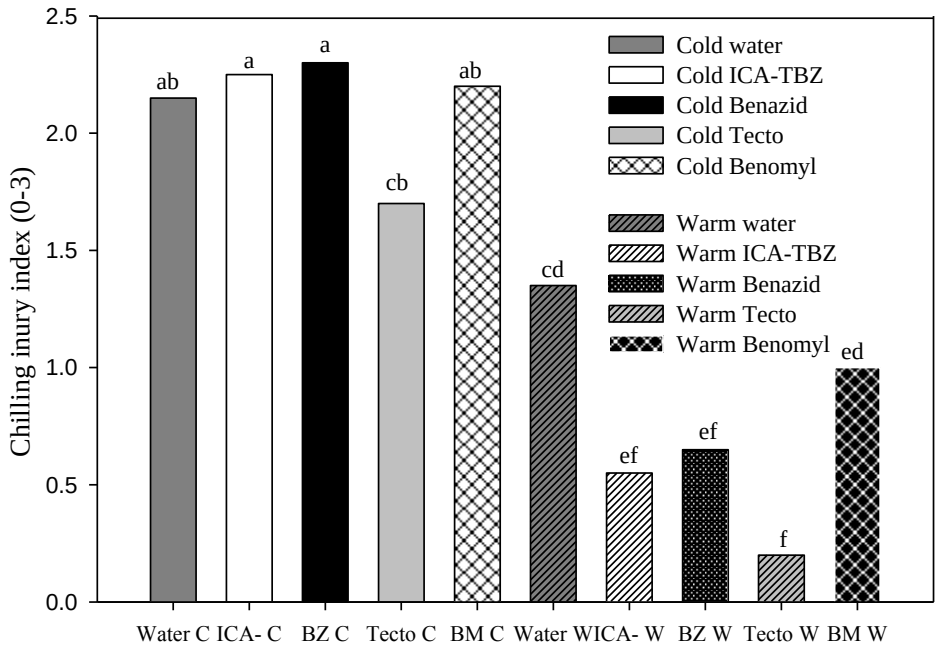

Figure 5.2.2.16. Difference in the chilling injury index (0-3) of 'Washington' navel oranges dipped with cold water 10°C and warm water 45°C, with different fungicides benomyl (Benomyl/Benlate®; 20 g/10 L), Carbendazim (Benazid®; 40mL/20L) and thiabendazole (Tecto®; 40 mL/20 L and ICA-TBZ®; 80 mL/20 L) for 2 minutes and stored for 35 d at -0.6°C and kept at shelf life (20°C for 7 d) done in 2011.

Using different fungicides in warm water was effective in reducing CI compared to applying the fungicide in cold water. In the dip experiment done in 2011 where four different chemicals of the benzimidazole family were each applied as dips to 'Washington' navel orange fruit, Tecto® significantly differed with the control and Benomyl; however, no significant differences were obtained from Benazid or ICA-TBZ (Fig. 5.2.2.17 and 18). Application of the four fungicides in cold water resulted in no significant differences in CI index, although Tecto® had the lowest incidence of CI compared to all the other fungicides used in cold water (Fig. 5.2.2.17).

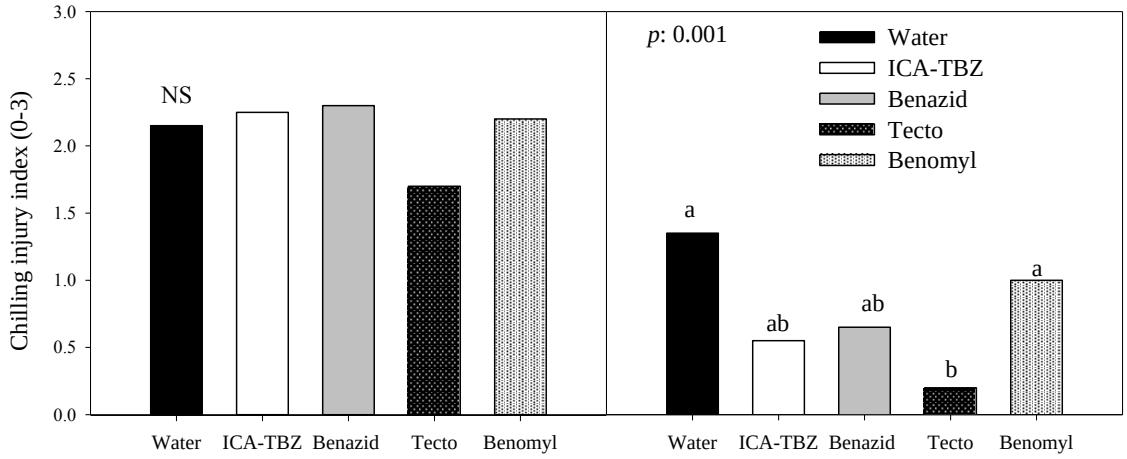

Figure 5.2.2.17. Difference in the chilling injury index (0-3) of 'Washington' navel orange fruit treated with different fungicides i.e. benomyl (Benomyl®; 20 g/10 L), Carbendazim (Benazid®; 40 mL/20 L) and thiabendazole (Tecto®; 40 mL/20 L and ICA-TBZ®; 80 mL/20 L) for 2 minutes in cold water 10°C (A) and warm water 45°C (B) prior to storage at -0.6 °C for 35 d plus shelf life (20°C for 7 d) ($p \leq 0.01$) done in 2011.

Figure 5.2.2.18. Example of the chilling injury incidence of 'Washington' navel dipped in Benomyl® and Tecto® for 2 min at 45°C and stored for 35 d at -0.6°C plus shelf life (20°C for 7 d) from 2011.

A dose response to Tecto® was evident in the CI of 'Autumn Gold' navel oranges applied with dilutions in warm water. At the lowest concentration, a quarter of the recommended rate Tecto® (40 mL.20 L^{-1}), there was a 52% and 36% higher incidence of CI in comparison to the ½ and full concentration respectively (Fig. 5.2.2.19). Less of a dose response was evident in the 'Cambria' navel orange fruit (Fig. 5.2.2.20).

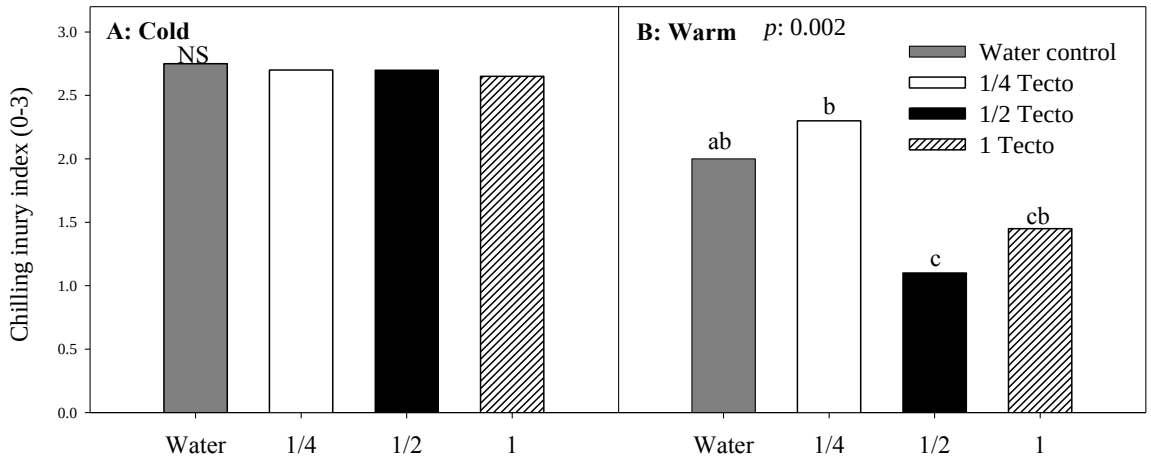

Figure 5.2.2.19. Difference in the chilling injury index of 'Autumn Gold' navel orange fruit treated with three concentrations of TBZ (Tecto®); full 40 mL/20 L, half 20 mL/L and quarter 10 mL/L, for 2 minutes in cold water (A) and warm water 45°C (B) and stored for 35 d at -0.6°C plus shelf life (20°C for 7 d) done in 2011 ($p \leq 0.01$).

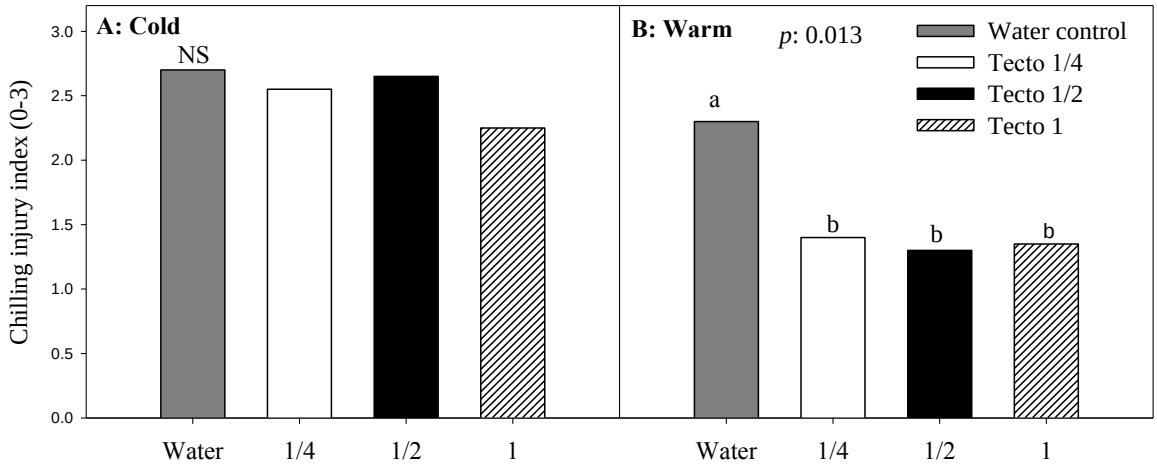

Figure 5.2.2.20. Difference in the chilling injury index of 'Cambria' navel orange fruit treated with three concentrations of TBZ (Tecto®); full 40 mL/20 L, half 20 mL/L and quarter 10 mL/L, for 2 minutes in cold water (A) and warm water 45°C (B) and stored for 35 d at -0.6°C plus shelf life (20°C for 7d) ($p \leq 0.01$) done in 2011.

During the second season of experiments (2012), application of TBZ in the drench, dip or wax resulted in reduction of CI of 'Washington' and 'Autumn Gold' navel orange fruit. Even though 'Autumn Gold' navel orange fruit dipped in warm water (35°C) for 1 min without TBZ reduced the incidence of CI significantly compared to the cold water (control), the dip application of warm water with TBZ was more effective in reducing the incidence of CI (Table 5.2.2.5). However, there wasn't a significant difference in the application of TBZ in warm or cold water, the application of fungicide in cold water numerically has the highest incidence of CI.

Table 5.2.2.5. Chilling injury index of 'Washington' and 'Autumn Gold' navel orange fruit as well as the TBZ residue loading application of TBZ (Tecto®) in the different treatments done in 2012. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax. The fruit were stored for 40 d at -0.6°C plus shelf life (20°C for 7 d). Residues were determined on a fresh fruit weight basis ($P \leq 0.05$).

	'Washington'		**'Autumn Gold'**
	CI index	**TBZ Residue (mg/kg FW)**	**CI index**
Cold water dip (15 °C)	1.09a	0.00c	1.28a

Warm water dip(35 °C)	0.81a	0.00c	1.03b
Cold water TBZ dip	0.80ab	2.63a	0.88bc
Warm water TBZ dip	0.42bc	2.82a	0.75c
Water drench + wax	0.25c	0.00c	0.17d
TBZ drench + wax	0.18c	0.97b	0.08d
Water drench + TBZ wax	0.13c	0.70b	0.05d
TBZ drench +T BZ wax	0.13c	2.95a	0.03d
***p*-value**	0.0002	0.0001	0.0001

*means with a different letter differ significantly at the 5% level (LSD)

All treatments which received wax (with or without TBZ) reduced CI in both cultivars and a 70% reduction was obtained compared to warm water dip treatments (Table 5.2.2.5). However when the drench and wax treatments (with or without wax) were analysed separately, for 'Autumn Gold' navel orange fruit the application of TBZ in the wax significantly decreases the incidence of CI further (Table 5.2.2.6).

Table 5.2.2.6. Chilling injury index of 'Washington' and 'Autumn Gold' navel orange fruit as well as the TBZ residue loading application of Tecto® in the different wax treatments done in 2012. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax. The fruit were stored for 40 d at -0.6°C plus shelf life (2°C for 7 d).Residue were determined on fresh fruit weight basis.

	'Washington'		**'Autumn Gold'**
	CI index	**TBZ Residue (mg/kg FW)**	**CI index**
Water drench + wax	0.25c	0.00c	0.17a
TBZ drench + wax	0.18c	0.97b	0.08ab
Water drench + TBZ wax	0.13c	0.70bc	0.05b
TBZ drench + TBZ wax	0.13c	2.95a	0.03b
***p*-value**	0.247	0.023	0.045

*Means in the same column with a different letter differ significantly at the 5% level (LSD)

Warm water dip of 'Washington' and 'Autumn Gold' navel orange fruit respectively, resulted in 84% and 95% higher CI index compared to fruit drenched in TBZ and applied with wax incorporated with TBZ (Table 1). The residue levels of TBZ measured from the 'Washington' navel orange fruit were high in all the treatments i.e. TBZ dip, drench or wax (Table 1). The residue of fruit dipped in warm or cold water TBZ, or where TBZ was applied in the drench and the wax was significantly higher compared to the treatments where TBZ was only applied in the drench or in the wax. The TBZ residues in the different tissue of the fruit i.e. flavedo, albedo, pulp, follow the same pattern at lower concentrations in the following order flavedo>albedo>pulp (Table 5.2.2.7). The residues were not significantly different for fruit dipped in warm water versus cold water TBZ.

Table 5.2.2.7. TBZ (Tecto®) residue loading in the flavedo, albedo and the pulp of 'Washington' navel orange fruit after various postharvest treatments. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax The fruit were stored for 40 d at -0.6°C plus shelf life (20°C for 7 d).

	TBZ residue (mg/kg FW)		
	Flavedo	Albedo	Pulp
Cold water dip (15 °C)	0.00c	0.00c	0.00c
Warm water dip(35 °C)	0.00c	0.00c	0.00c
Cold water TBZ dip	7.29a	0.18a	0.06ab
Warm water TBZ dip	7.59a	0.25a	0.07a
Water drench + wax	0.00c	0.00c	0.00c
TBZ drench + wax	2.84b	0.05b	0.03b
Water drench +TBZ wax	3.41b	0.05bc	0.04b
TBZ drench +TBZ wax	6.95a	0.14b	0.07a
p-value	0.0002	0.0002	0.0004

*Means in the same column with a different letter differ significantly at the 5% level (LSD)

Discussion

TBZ applied as a dip in 2011 and 2012 on the three different navel orange cultivars 'Washington', 'Autumn Gold' and 'Cambria', did reduce the incidence of CI significantly when applied in warm water. Similarly, Schirra et al. (1998) reported that the application of TBZ reduces the incidence of CI in 'Tarocco' oranges with concentrations of 200 ppm in warm water (50°C) and 1200 ppm in cold water. No significant differences were found between using ICA-TBZ®, Benazid® and Tecto®. The application of this fungicide in hot water was more effective in reducing CI, which could be due to the better uptake of the TBZ in the fruit rind. Increased efficacy of TBZ to reduce CI in combination with hot water was demonstrated previously in citrus fruit (McDonald et al., 1991; Schirra and Mulas, 1995). Schirra et al. (1996) also indicated that the enhanced activity of heated chemicals could be related to the higher deposition of the active ingredient on the fruit. Schirra and D'hallewin, (1997) explained that hot water dips induce a redistribution of the epicuticular wax and a melting of the waxy layer, which could aid in TBZ uptake into the rind.

Application of TBZ in a warm water dip (1 min for 35°C) reduced CI compared to the cold water dip. In contrast Lindhout, (2007) reported that dipping 'Lane Late' navel orange fruit in 50°C for 3 min reduced the incidence of CI, but adding TBZ (1000 ppm) did not reduce the incidence any further. The dip application with TBZ (Tecto®) at the higher temperature 45°C for 2 min in 2011 seemed to be 56% more effective in reducing the incidence of CI compared to the dip treatment for 1 min at 35°C for 'Washington' navel. This high temperature dipping is, however, commercially difficult because of the significant amount of heat transfer when fruit are processed in packing runs and the cost for electricity to heat the water to 45°C.

There was not a significant difference in the TBZ residue applied in a warm or cold dip, even though there was a significant difference in CI. This could indicate that the warm water, known to reduce CI (Schirra and D'hallewin, 1997) could to a large extent be responsible for the CI reduction. Some of the physiological effects of heat treatment are the accumulation of heat shock proteins or other molecular chaperones inducing other defence pathways (Lafuente et al., 1991). Heat shock proteins accumulate in response to sudden high temperatures (Lafuente et al., 1991). Therefore the reduction in CI by warm water TBZ could be the result of a synergistic effect of a warm water induced protective process as well as a currently unknown mechanism of TBZ; however, this hypothesis needs further testing. The maximum residue level which is measured for the whole fruit is 5-6 $mg.L^{-1}$ depending on the market (van Zyl, 2011). This was not exceeded with the highest value being 2.95 $mg.L^{-1}$ when applying TBZ in the wax and in the drench.

Even though the mode of action of TBZ is not known, the effect of TBZ may be associated with a decreased rate of rind senescence as shown with the reduction of rind colour development (Schiffman-Nadel et al., 1975). Benzimadazole treatments have also been associated with a decrease in senescence of wheat leaves (Mishra and Waygood, 1968), which is involved in the delay of protein degradation and net protein synthesis which is revealed through ratios of soluble and insoluble nitrogen fractions (Samborski et al., 1958, Lindhout, 2007).The use of different dilutions of TBZ (Tecto®) applied as postharvest dips in warm water indicated that a dosage threshold for effective uptake and protection exists i.e. a quarter (1/4) of the commercial recommended rate wasn`t effective in reducing the incidence of CI in 'Autumn Gold' but in 'Cambria' there was no concentration effect. The commercial recommended rate (40 mL/20 L) and the

application of half of the commercial recommended rate was effective in reducing the incidence of CI for 'Autumn Gold navel oranges which corresponds to the results by Schiffman-Nadel et al., (1972).

The application of wax was constantly effective in reducing the incidence of CI. Citrus fruit consists of a layer of epidermal wax and the cuticle, but this cuticle can form microscopic cracks (Cohen et al., 1994) and become damaged during the postharvest handling which allows moisture loss. Wax application is used to seal the fruit to water loss during export and cold storage. It is proposed that the waxing of fruit creates a physical barrier through preventing moisture loss (Lindhout, 2007). Lindhout, (2007) reported that the application of wax was most effective in reducing CI compared to other treatments and the additive effect of wax with other treatments i.e. heat treatments (3 min 50°C), the dip of fruit in TBZ and the application of methyl jasmonate. For 'Autumn Gold' navel orange fruit, fruit applied with only wax had a significantly higher incidence of CI compared to fruit with TBZ applied in the wax. The application of TBZ in the wax has the effect of both TBZ and wax which both reduce the incidence of chilling injury.

The average residue levels in the flavedo, albedo and the pulp of the fruit indicate that 96% of the TBZ residue is located in the flavedo of the orange fruit. The highest residue level in the albedo and pulp was with fruit applied with warm and cold dips followed by the application of TBZ in the drench and in the wax. In addition, when TBZ is only applied in the drench and not in the wax there is a lower residue in the flavedo compared to the application of TBZ in the wax. The application of TBZ in a dip had a higher residue level compared to the application of wax with TBZ even though the CI incidence of the fruit dip in TBZ was significantly higher compared to the fruit treated with wax. This could indicate that the wax application could to a large extent be responsible for the CI reduction.

To conclude, the application of TBZ was the most effective in reducing the incidence of CI compared to the other fungicides in the family of the benzimidazoles. In addition TBZ has a higher efficacy when applied in a warm water dip of 35°C or 45°C at 1 min or 2 min, respectively. The specific mechanism of TBZ is still unknown but the positive association of TBZ and hot water could be in the up regulation of stress defence mechanisms and enhancement of fruit resistance. The synergistic effect of the warm water protection process and the TBZ mechanism result in a reduction in CI. The application of postharvest wax was more effective in reducing the incidence of CI compared to the TBZ dip treatments. However, the application of TBZ with wax was overall the most effective in reducing the incidence of CI.

A.4. Various postharvest treatments to reduce CI of citrus fruit

The incidence of CI on various cultivars tested was not reduced by exposure to high CO_2 (10 and 20%) and ethylene (5 ppm) levels in a postharvest environment. In addition, it was show that variation in CI susceptibility exists between seasons as well as cultivars. In general, it is advisable that only fruit with a good colour development for example, an even red/pink rind for the 'Star Ruby' grapefruit and a deep orange for 'Nules Clementine' mandarin, be included in a cold sterilisation protocol.

Materials and methods

Fruit in all treatments and experiments in this project were stored for 32 days at -0.5°C and CI incidence was only determined subsequent to 7 days at ambient conditions.

Postharvest chemical treatments were applied in water by exposing fruit for 5 minutes to 1-MCP (ethylene blocker, 1000 ppb), Ethephon (ethylene in liquid form, 31.2 ml/10 L), Gibberellic acid (0.25 g/10 L) and thiabendazole (20 ml/10 L). Fruit was left to dry and was stored at -0.5°C for 32 d before being evaluated for CI. Waxes from JBT technology containing 14 and 18% solids were applied in a semi-commercial packline to Nadorcott mandarin, and two Navel orange cultivars, before storing at cold sterilisation temperature. Fruit weight loss and CI were determined. In order to decrease fruit sensitivity CO_2 and ethylene (5 ppm ethylene and 10 and 20% CO_2) were applied in a closed system continuous flow system for 24 h before storing at -0.6°C for 32 d. These gaseous treatments were applied to 'Nules Clementine' mandarin (Stellenbosch), 'Eureka' lemons (Stellenbosch) and 'Washington' navel (Paarl), thereafter fruit were cold stored. Chilling injury was scored and presented as discussed below. 'Marsh' grapefruit were harvested in Malelane according to position in the canopy in order to determine if it followed the same pattern of high susceptibility as outside fruit. Fruit were cold-stored for 32 d.

Scoring of CI incidence was done after storage at -0.6°C and after 1 week at shelf life (20°C) where the fruit was evaluated for any CI symptoms. The CI data were collected to illustrate incidence and expressed as percentage CI, as well as the severity of the injury expressed as CI index. The incidence data were collected as yes/no, whereas the severity fruit was scored from 0 to 3 and calculated with the following equation:

$$\text{Chilling injury index } (0\text{-}3) = \sum \frac{[\text{Chilling injury (scale 0-3)} \times \text{no. of fruit in each class}]}{\text{Total number of fruit in replicate}}$$

Chilling injury symptoms were scored as incidence (Yes vs. No) as well as severity according the rating 0 to 3 (0 = zero 1 = >10% CI; 2 = 10% - 50% CI; 3 = > 50% CI).

Figure 5.2.2.21. Example of visual rating scale of CI severity used in calculating the CI index.

Statistical analysis: Fruit in all treatments and experiments in this project were stored for 32 days at -0.5°C and CI incidence was only determined subsequent to 7 days at ambient conditions. Data of the CI index (ranked data) were analysed by using one-way Anova and two-way Anova (test interaction) using SAS (SAS v.6.12; SAS Institute, Cary, VC, USA). Each treatment was compared with the other using Fisher's least significance differences (LSD). The *p*-values illustrate the significance differences in the Figures presented. If the *p*-value is smaller than 0.05 there is a significant difference between treatments. With data that were not analysed by Anova the standard deviation was provided.

Results and discussion

Exposure of navel orange fruit to elevated CO_2 levels for 3 days prior to cold storage did not result in any significant reduction of chilling injury. There were a trend to suggest a positive effect on chilling incidence but in subsequent experiments on other citrus types (data not shown) no consistent effect could be seen (Fig. 5.2.2.22A). It was, however, also observed that the very high CO_2 (10 and 50%) exposure for 3 days did not result in any rind disorders or internal quality loss of Navels, lemons or grapefruit.

The application of various agrochemicals did result in some changes in CI of Navel orange fruit, but only TBZ significantly reduced the CI incidence. This result resulted in the expanded experiment reported above (Fig. 5.2.2.22B). However, the numerical increase after the 1-MCP application (an ethylene inhibitor) is interesting in light of the known positive effect of internal ethylene levels on protecting fruit against postharvest disorders such as chilling. This complex mechanism is poorly understood.

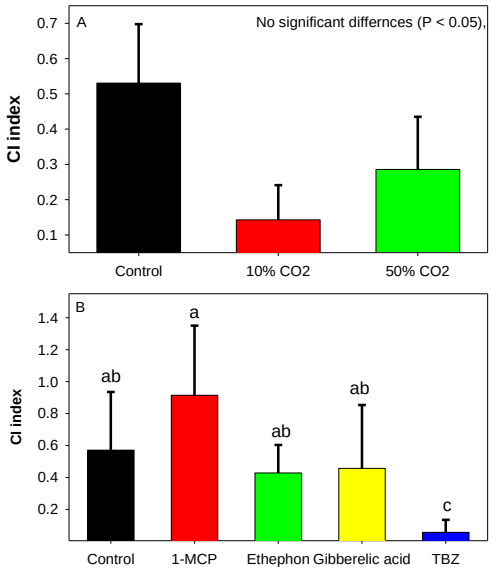

Figure 5.2.2.22 (A) The effect of postharvest CO_2 application for 24 hours on chilling injury incidence (index (0-3) of 'Navel' orange. **(B)** The efficacy of postharvest application of 1-MCP (1000 ppb), Ethephon (31.2 ml/10L), Gibberellic acid (0.25g/10L) and thiabendazole (20 ml/10 L). In both instances fruit were stored at -0.5°C for 32 days before being evaluated for CI. Different letters indicate significant differences at the 95% level.

During the experiment on 'Marsh' grapefruit and the relation to canopy position and CI the question was asked if the high tolerance to CI of inside (shaded) fruit is only true for the high-lycopene 'Star Ruby' cultivar. The opposite trend was seen after cold storage, which indicates that the confirmed positive relationship between inside/shading and chilling injury is true only for 'Star Ruby' grapefruit.

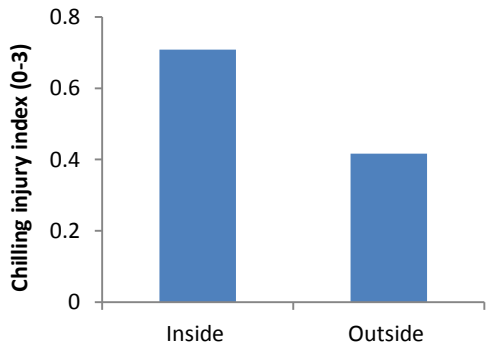

Figure. 5.2.2.23. Inside 'Marsh' grapefruit developed higher severity (although not significant at P<0.1) of chilling injury compared to the outside fruit. Fruit were harvested in Hectorspruit and cold stored at -0.5°C for 32 days + 7 days at ambient conditions prior to evaluation.

Postharvest wax on citrus fruit is applied to reduce weight loss (water vapour) as well as to serve as a carrier for fungicides. The positive impact on CI incidence is thought to be an additional benefit. In all four cultivars used the wax application reduced the incidence of CI in addition to reducing weight loss, but not always significantly (Figs. 5.2.2.24 and 5.2.2.25). This reduction of CI was without any TBZ in the wax. The difference in CI incidence between higher solids (14 vs. 14%) also indicates that in cold sterilisation markets waxes with higher solids could add CI protection.

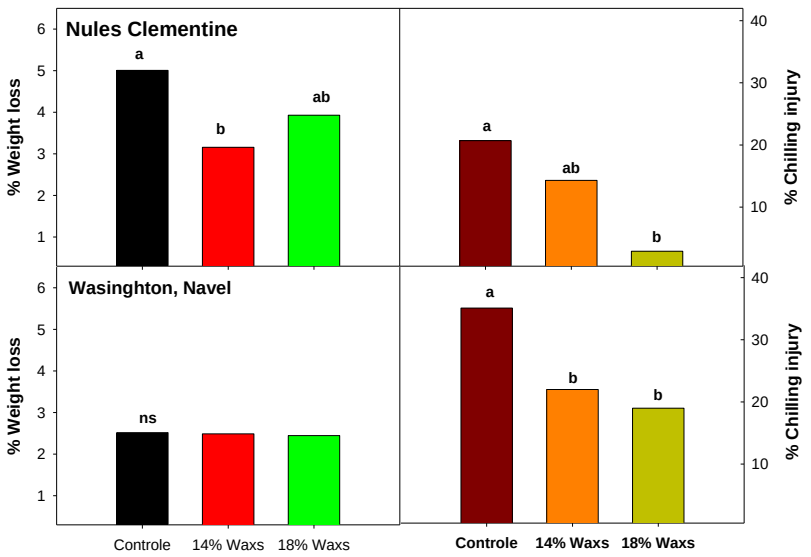

Figure 5.2.2.24. Influence of postharvest wax (14 and 18% solids) on weight loss and chilling injury of 'Nules Clementine' mandarin and 'Washington Navel' orange after 30 days at -0.6°C. Different letters indicate significant differences at the 95% level.

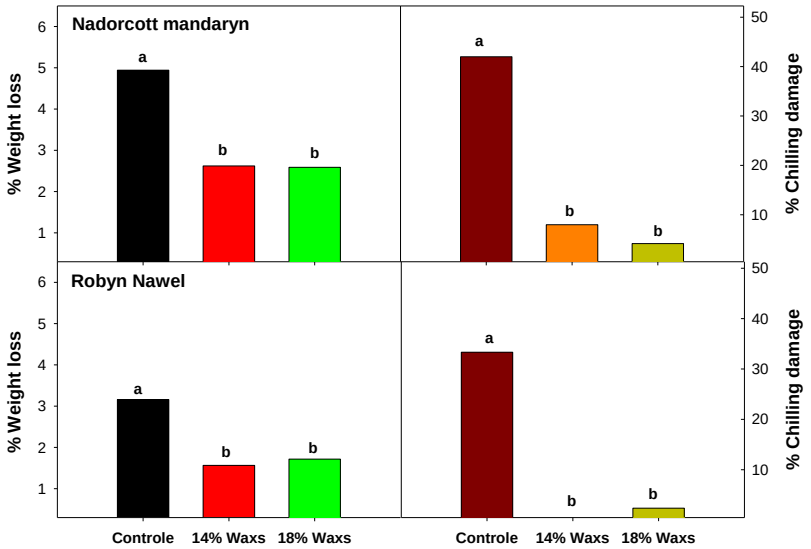

Figure 5.2.2.25. Influence of postharvest wax (14 and 18% solids) on weight loss and chilling injury of 'Nadorcott' mandarin and 'Robyn Navel' orange after 30 days at -0.6°C. Different letters indicate significant differences at the 95% level.

The direction of these experiments into CI of citrus fruit focused mostly on prevention of the disorder in a postharvest environment. The aim was to reduce the incidence of CI by applying to the rind a product that could either increase natural defences or impart a physical protective action. This direction had been decided on due to the extreme variation between fruit in susceptibility to CI, yet the fact that all fruit delivered to the packhouse could end up in a cold sterilisation market and must be able to handle the cold treatments.

The most promising results were the influence of TBZ on reduction of CI, and therefore an indication that the use of this fungicide could be a valuable step in managing the CI incidence in citrus fruit rind. This result was further studied in A4 and it is currently a recommendation to always include TBZ in the drench and wax in cold sterilisation programmes. The positive action of wax on chilling injury is thought to be due to a physical protective layer on the flavedo. It is thought that the wax does not stop the membrane damage caused by the 30 d exposure to -0.6°C, but rather inhibits the water loss from these damaged cells. The improved protection by the 18% compared to the 14% solids supports this argument as these products do not transport in the flavedo cells as TBZ does.

The non-significant effect of the CO_2 and Gibberellic acid treatments illustrates the difficulty of finding and developing an implementable technology for the purpose of increasing rind resistance against the severe cold disinfestation protocol South African citrus have to be subjected to in order to kill FCM larvae. The negative results of the high CO_2 exposure do not concur with previous research in which exposure to 10-40% CO_2 for a 1 to 14 day period reduced chilling injury of citrus fruit (Brooks & McColloch, 1937; Hatton & Cubbedge, 1974; 1975 and Palou *et al*., 2008). However these studies were done at much less severe temperature *viz.* 2°C compared to the -0.6°C in the experiment reported on. 'Marsh' grapefruit from the inside of the canopy developed a higher incidence of CI compared to the outside fruit. This was in contrast with the pattern now known to exist in 'Star Ruby' grapefruit and indicate that each cultivar does have particular aspects that influence chilling susceptibility and should be studied to understand in order to implement handling protocols.

Conclusion

The incidence of CI of citrus fruit in the market shows significant variation leading to the question why one fruit in a carton develops CI symptoms and the adjacent fruit not. It is concluded from this research that cultivar specific aspects, such as rind colour, are critical components determining fruit susceptibility to CI. In addition, no new treatments such as CO_2 exposure, has been identified that could reduce CI in all fruit. Indications are that cultivar and area could be influential in determining rind sensitivity to CI and was studied in A1. In addition, the application of TBZ in warm water is effective and further strategies should be developed in order to make full use of this technology in the packing process. The higher CI incidence of inside 'Marsh' grapefruit fit in with the known effect of lower carbohydrate in the flavedo of fruit from this position and illustrate that the pattern seen in 'Star Ruby' is an isolated phenomenon. It is therefore advised that producers exporting 'Marsh' grapefruit to cold sterilization markets could use fruit position as a factor to reduce CI incidence.

A.5. General Discussion and conclusion

Citrus fruit from South Africa are exported to countries such as the USA, Korea, Thailand and China with a mandatory cold sterilization treatment where fruit must be exposed to -0.6°C for 24 days to kill all insect larvae of false codling moth and fruit fly in the fruit. However, the cold temperatures can result in CI which negatively affects the external quality of the fruit and can result in dramatic financial losses. The first objective of the study was to investigate the effect of different pre- and postharvest factors affecting the CI incidence of navel orange fruit. In this study it was shown that cultivar, harvest date and site significantly influence the susceptibility to CI incidence of navel orange fruit. 'Washington' navel orange fruit was found to be more susceptible to CI compared to the 'Navelina', and therefore cultivar choice for specific steri-markets is an important part of a strategy to reduce the incidence of CI and to ensure postharvest quality of navel orange fruit.

Incidence in CI differs between the different areas in the Citrusdal valley and at the top of the valley (Tharakama), the coldest part with highest rainfall, both cultivars had higher incidence of CI compared to lower down in the valley i.e. Hexrivier and Ouwerf where it is warmer and dryer. It therefore could be construed that the lower preharvest temperature could negatively affect the postharvest incidence of CI. In addition, harvest date or maturity can affect the incidence of CI as immature and over mature fruit are generally more susceptible to CI compared to the fruit harvested at the optimal commercial harvest date.

It was hypothesised that there will be an increase in malondialdehyde as the fruit were stored longer; however, malondialdehyde (MDA) content (lipid peroxidation) did not show any specific trend in relation to CI. However, fruit that is harvested late vs early shows some difference with the fruit harvested late having a higher average MDA concentration. There is the possibility that there is no trend or that other compounds in citrus rind interfered with the measurements. For future research, the lipid peroxidation could be measured with high performance liquid chromatography (HPLC) to enhance accuracy. Measurement of ethylene production during storage showed a spike in the ethylene production for 'Washington' navel orange fruit stored for 21 or 35 days. However, 'Navelina', which was harvested later, did not develop CI or an increase in ethylene production.

Canopy position, fruit size and rind colour do affect the incidence of CI. Fruit from outside the canopy has a higher incidence of CI compared to fruit from the inside of the canopy. Due to the high light intensities in Citrusdal (the fruit growing area in this study), the fruit exposed to the sunlight, outside the canopy, could result in the fruit developing latent sunburn symptoms thereby increasing fruit susceptibility to CI, as seen in 'Star Ruby' grapefruit. Furthermore smaller fruit are more prone to CI compared to larger fruit and it is thought that larger fruit are generally a better sink, which would result in a higher carbohydrate accumulation in the flavedo and reduce CI susceptibility.

The NIR analysis was successful, after using PCA plots, to distinguish between fruit from different canopy positions and fruit sizes. However, the reliable prediction of colour parameters and firmness were difficult to obtain using integrated sphere NIR measurements from a wavelength between 800-2500 nm, but according to literature it is possible to predict the rind colour using wavelengths of 450-2500 nm which includes the visible spectrum. The biochemical changes of the rind such as carotenoid and lipid peroxidation could therefore be included in future studies to possibly be determined with this non-destructive tool in order to develop a more robust prediction model for CI.

Various postharvest treatments such as fungicide and wax application investigated in this study reduced the incidence of CI. The application of TBZ to 'Washington' 'Autumn Gold' and 'Cambria' in 2011in warm water (45°C) reduced the incidence of CI more than the application in cold water. The residues of the TBZ applied in cold or warm water did not differ significantly even though CI incidence was reduced. Therefore the reduction in CI by warm water TBZ could be the result of a synergistic effect of warm water induced protective process as well as a currently unknown mechanism of TBZ; however, this hypothesis needs further testing.

Approximately 96% of the TBZ residues were in the flavedo of the fruit rind and only a small portion in the albedo and pulp of the fruit. In a screening trial various commercial fungicides from the benzimidazoles family i.e. Benomyl®, Benazid®, Tecto®, ICA-TBZ® were tested for efficacy in reducing CI. However, the TBZ treatment of Tecto®, Benazid and ICA-TBZ® was overall the most effective in reducing the incidence of CI, Tecto® had numerically the highest efficacy. A dose response of TBZ fungicide as dip application was evident in 'Autumn Gold' navel fruit and a too low concentration, ¼ the commercial recommended concentration, was not effective in reducing the incidence of CI. It is therefore advisable that TBZ application must be higher than half of the commercial recommended concentration. The application of postharvest wax was more effective in reducing the incidence of CI compared to the TBZ dip treatments. The wax layer covers the fruit and protects the fruit against moisture loss, but the addition of TBZ in the wax was overall the most effective in reducing the incidence of CI.

For future prospects, it can be useful to determine a specific biochemical characteristic such as a change of the carotenoids, in the fruit rind which could be related to rind maturity or condition in order to determine the optimal harvest date to decrease CI. Further research that focuses on identifying non-destructive methods to assist in the sorting of the fruit according to rind quality is needed. Research on the use of NIR in postharvest technology in identifying fruit disorders offer the best opportunity to date and it is proposed that the NIR could be used to assay for example carotenoid content and possibly lipid peroxidation in the flavedo in order to develop a prediction model for CI susceptibility. To reduce the incidence of CI more research could be done around the mode of action of TBZ and how it affects the cellular structures and metabolism which is associated with chilling damage. In addition more research could be done to increase the effectiveness of wax application as well as identifying the components in the different waxes (polyethylene, shellac, bee wax and carnauba) which affect CI. In addition, the interaction of wax with different concentrations of TBZ, should also be optimised to increase the efficacy against the incidence of CI.

To conclude, cultivar and site differences have to be considered prior to orchard planting to avoid the high incidence of CI in exported fruit. Export of 'Navelina' to cold sterilization markets could be more suitable because the fruit were less susceptible to CI compared to 'Washington' navel orange. It is suggested that producers and exporters analyse previous season's data to ascertain which of these cultivars are more sensitive. In commercial handling particular attention should be given to 'Washington' navel orange fruit because of its high susceptibility to CI. Fruit have to be harvested at the commercial harvest date and not when immature or over mature, however, the ideal will be to determine the optimal rind maturity or condition to reduce the incidence of chilling injury. To reduce the incidence of CI on the packing line, fruit has to be sorted according to size and only bigger (±75-82 mm)fruit should be packed for export. Furthermore, fruit with sunburn or other lesions should not be packed for export to these cold sterilization markets. Wax application as well as TBZ in the wax (4000 ppm) is advisable to reduce the incidence of CI.

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B. Increased lycopene content in the fruit flavedo to reduce chilling injury of grapefruit during cold sterilization shipments

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Summary

Export of South African citrus fruit to certain markets must receive a cold treatment to sterilise insect larvae present in the fruit and involves a 24 day exposure to -0.6°C to kill the false codling moth as well as fruit fly larvae. This treatment, however, can result in chilling injury lesions in the fruit rind reducing the profit of exporting citrus fruit. Over several seasons it was ascertained that the amount of pink pigment (lycopene) accumulated in the flavedo of the 'Star Ruby' grapefruit has a protective action and if adequate concentrations are present in the flavedo they result in a significant reduction of chilling injury. Another objective in the study was to improve/enhance lycopene expression in the flavedo (rind). However, no postharvest treatments proved to enhance lycopene accumulation. It was only by increasing the amount of shade on the fruit from stage II (Oct-Dec) that higher levels were realised. It is concluded that 'Star Ruby' grapefruit growers should focus on developing a leafy tree canopy with adequate fruiting positions towards the main trunk. In addition, by sorting fruit during packaging on the distribution of visible pigment, the probability of economically devastating chilling incidence could be reduced. Preliminary commercial testing of this type of sorting has resulted in no reports of chilling injury from the market. The implementation of this low cost technology has helped to realise a consistent increase in cartons exported to a market that pays a 20-30% premium for high quality chilling-injury-free fruit.

Introduction

In the Southern hemisphere South Africa is the dominant force in grapefruit production and export due to the high quality and production of especially 'Star Ruby' grapefruit. Annually SA exports between 15-18 million 15 kg cartons (225 000 – 270 000 ton) grapefruit to various markets in the Northern Hemisphere. Saturation in traditional markets such as EU/UK led producers and importers to ask for management strategies to reduce the risk of chilling injury if exporting fruit under cold sterilisation protocols to new markets. Subsequently CRI undertook preliminary studies to determine the viability of exporting 'Star Ruby' grapefruit to the USA. From this study it was determined that the incidence of chilling injury was extremely variable but correlated well with rind colour. It was therefore expected that the accumulation of carotenoids in the flavedo could play a critical role.
Carotenoids are the principal pigments accumulated in citrus fruit, being responsible for the great diversity in coloration displayed in this genus. Colourful carotenoids are accumulated during citrus ripening and are influenced by different factors. 'Star Ruby' grapefruit is one of the most commonly produced pink/red grapefruit in the world. The pulp of 'Star Ruby' grapefruit is of a deep-bright coloration while the rind also develops pink/red pigmentation, but not as intense as the pulp (Alquézar et al., in publication Gmitter, 1995). Carotenoids are formed by condensation of isoprenoid molecules, forming a C_{40} basal colourless structure which suffers successive desaturation steps, leading to different molecules with diverse colour and properties. Carotenoid biosynthesis takes place in the plastids (chloro and chromoplasts), beginning with the fusion of two molecules of geranyl-geranyl pyrophosphate (GGPP), by the action of the phytoene synthase (PSY), that catalyses the first committed step of the pathway. After three successive desaturations, β-carotene is transformed into lycopene, the last linear carotenoid in the pathway. Further on, lycopene is cyclized by the action of a cyclase (βLCY) and transformed into β-carotene, which can be hydrolysed by the action of an enzyme known as β-carotene hydroxylase (βCHX) to start xanthophyll biosynthesis (Cazzonelli and Pogson, 2010).

Carotenoid accumulation in plants is affected by different environmental factors. It is well documented that light strongly affects colour development and consequently carotenoid content and composition in different plant tissues (Liu et al., 2004; Pizarro and Stange, 2009; Toledo-Ortiz et al., 2010). Evidence indicates that direct light promotes carotenoid accumulation but also influences the differentiation of chloro and chromoplasts (Pizarro and Stange, 2009). Moreover, it has been demonstrated that light directly regulates carotenoid biosynthesis, since it induced *PSY* transcription in *Arabidopsis* (Toledo-Ortiz et al., 2010) and affected the phytochrome-induced post-translational control of PSY in tomato fruit (Schofield and Paliyath, 2005).

In *Citrus* fruit, it is generally recognized that direct light exposure enhances fruit coloration in ordinary orange and mandarin fruit (Alquézar et al., 2008b). However, field observations indicate that pink/red grapefruit growing inside the canopy (shading fruit) developed a more intense red coloration in the rind than those

directly exposed to sunlight (Hwang et al., 2004; Alquézar et al., 2008b). Despite these apparent contrary effects of light on carotenoid accumulation in the rind of *Citrus* fruits, evidence of the influence of light on carotenoid biosynthetic genes is still lacking.

Lycopene content in the flavedo of 'Star Ruby' grapefruit is thought to determine or influence the susceptibility to chilling injury as lycopene is known to be an extremely potent anti-oxidant. This ability is thought to be part of the mechanism preventing cellular membrane damage during cold sterilization shipments. Currently no techniques are available to increase lycopene content as well as how to make use of this knowledge to reduce the chilling injury of rind in shipments to markets employing a cold sterilization (-0.6°C) protocol.

The project therefore had the following objectives: Elucidate the relationship between rind colour development and chilling injury and what contributes to the large variation between fruit. Are there differences in expression of rind colour between Kakamas (dry semi-desert) and Hectorspruit (subtropical) and do they influence chilling susceptibility? Can pre and postharvest strategies be put in place to improve rind colour development or reduce chilling injury? Can a postharvest management tool be developed to enable the sorting of fruit on colour, thereby reducing the risk of chilling injury incidence?

Results and discussion

Influence of canopy position on rind colour

The first experiment involved a study to determine the association between rind colour vs. canopy position in addition to the impact on chilling susceptibility. The data indicate (Fig. 5.2.2.26) that fruit in a sun-exposed outside position developed a significantly yellower fruit rind (high hue °) with low lycopene and β-carotene. These fruit had therefore a higher hue ° and a higher colour chart (CRI 35C) rating compared with the inside fruit that were shaded by leaves. In addition, the inside fruit showed a significantly lower incidence of chilling injury compared to the outside fruit which went a long way in explaining the preliminary results for this project. These findings were the same in both Kakamas and Hectorspruit and therefore only one set of data is shown (Fig. 5.2.2.26).

Rind colour and pigment content vs. chilling injury incidence

The objective of this experiment was to establish an association between rind colour development, pigment accumulation (lycopene and β-carotene) and incidence of chilling injury. In order to realise this aim fruit were visually classified from very pink (class 1) to very little pink/very yellow (class 6) (Fig. 3). After cold storage (-0.5°C for 35 d) the CI incidence was scored and the data indicated an increased CI as the rind colour decreased. In addition, this negative association was mirrored in the accumulation of pink pigments (lycopene and β-carotene) in the flavedo. This data set was a further strong indication of the close association between rind colour and susceptibility to chilling injury even if canopy position was not a factor due to fruit being randomly sampled from the canopy of a tree.

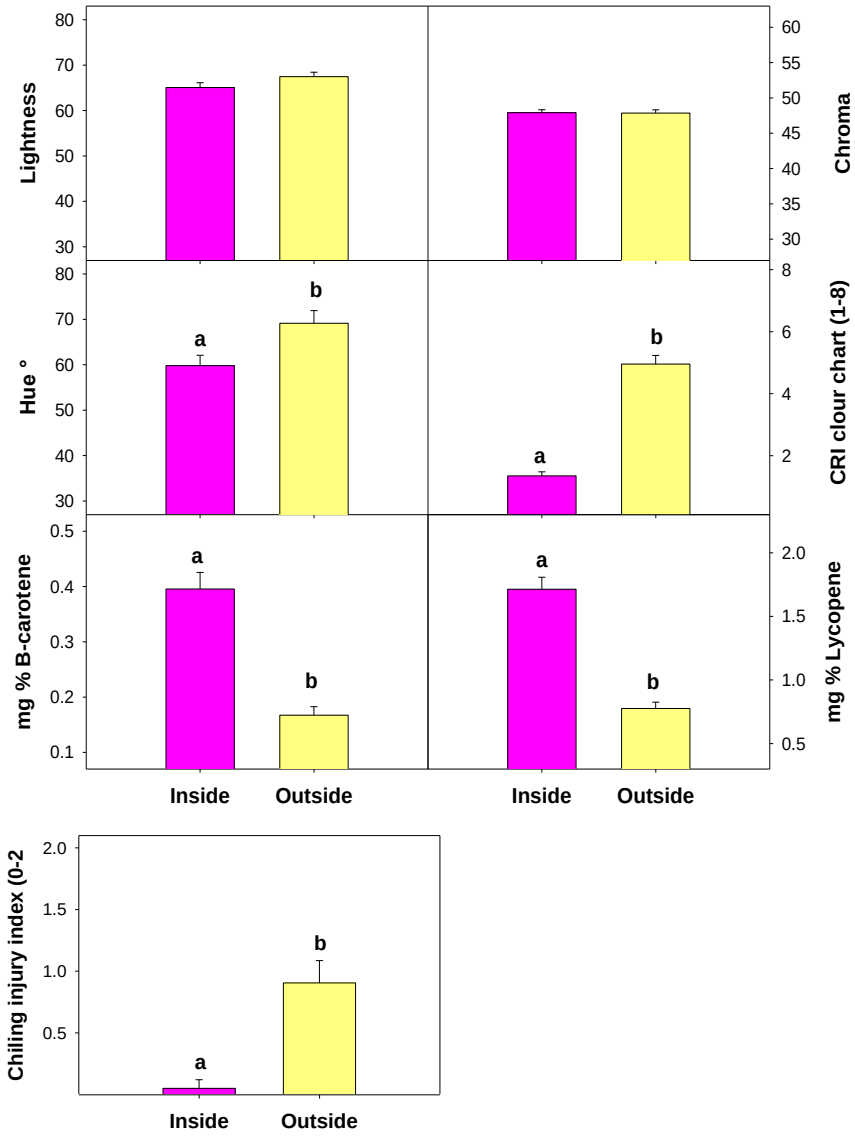


Figure. 5.2.2.26. The influence of canopy position on 'Star Ruby' grapefruit rind colour development (colorimeter measured values i.e. Hue°, Lightness, and Chroma) and CRI colour chart (1 most pink, 8 green), as well as lycopene and B-carotene content in the flavedo). The incidence of chilling injury as per position is indicated in the graph on the right.

Figure 5.2.2.27. The protective action of adequate pink rind colouration of 'Star Ruby' grapefruit against chilling injury is clearly illustrated with the yellow fruit on the right showing pitting due to chilling injury whereas the fruit on the left has no such symptoms even though both were stored at similar conditions.

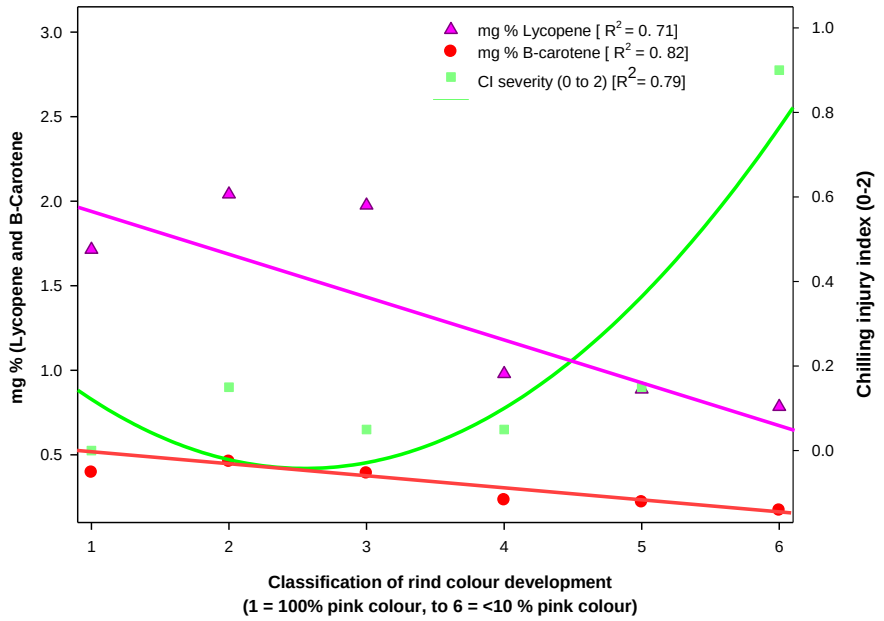


Figure 5.2.2.28. Measurement and classification of 110 'Star Ruby' grapefruit picked randomly from the canopy of a tree into six colour classes and storing the fruit for 35 d at -0.5°C prior to determining pigment content in the flavedo and chilling injury.

<u>Study of the mechanism of carotene accumulation in the flavedo in the shaded part of the canopy</u>

It was ascertained in previous experiments in this project that direct exposure of 'Star Ruby' grapefruit to light strongly influenced rind colour development. Mature fruit harvested from inside the canopy received a low photoactive radiation (PAR of 13 µmol/m^2/s) and registered a more pink/reddish colouration at harvest, reaching the *a/b* value of 0.41. On the other hand, fruit from the outside part of the canopy (light exposed) received a PAR more than 10x higher (175 µmol/m^2/s) than shaded fruit and developed a pale-yellow colouration with an *a/b* ratio as low as 0.04 (Fig. 5.2.2.29).

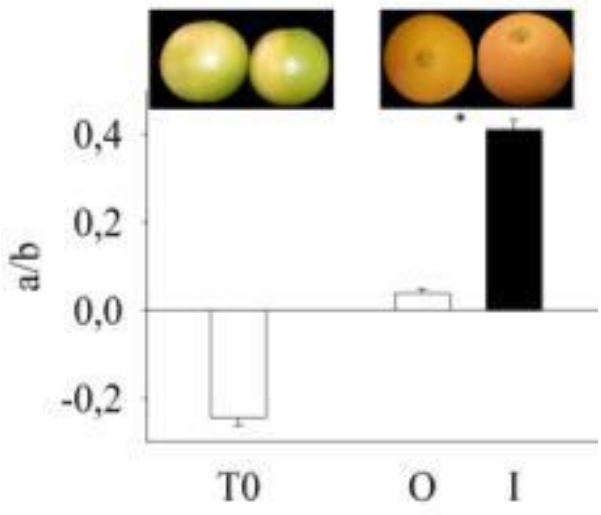


Figure 5.2.2.29. Colour (a/b Hunter ratio) of the rind of 'Star Ruby' grapefruit sampled after fruit set in Jul(NH)/Jan(SH) (Breaker, T0) and at maturity in Dec(NH)/Jun(SH) from outside (O) and inside (I) of the tree canopy. *asterisk indicates significant differences between fruit shaded and exposed fruit (Tukey p<0.05).

The promotion of rind colour by shading was paralleled by a substantial increase in total carotenoids. Thus, during the study period the flavedo of fruit exposed to light was around 20 µg/g FW, while that of shaded fruits was 69 µg/g FW. Moreover, lycopene (x14) and β-carotene (x4) experienced a dramatic increase in shaded fruit, while they remained almost unchanged in light-exposed fruit (Fig. 5.2.2.30). These results indicate that contrary to the general assumption that light increases carotenoid content, fruit shading enhanced carotenoid content in the rind of the red 'Star Ruby' grapefruit, suggesting an unusual regulation of carotenoid biosynthesis compared to other *Citrus* fruits.

Carotenoid biosynthetic gene expression increased in the rind of mature 'Star Ruby' fruit compared to breaker or colour break (T0) when the chlorophyll started to break down, but surprisingly this effect was of lower magnitude in shaded than in light-exposed fruit, contrary to carotenoid accumulation (Fig. 5.2.2.31). The expression of *PSY* was almost doubled in the rind of light-exposed fruit compared to shaded fruit (Fig. 5.2.2.31). Hence, the positive effect of light on PSY transcription described in *Arabidopsis* leaves (Toledo-Ortiz et al., 2010) was confirmed in the rind of 'Star Ruby' grapefruit. The same positive effect of light was registered on the transcription of other key genes of the carotenoid biosynthetic pathway, as *βLCY1* and *βLCY2*, enzymes that catalyse lycopene cyclization (Alquézar et al., 2009). Moreover, high PAR-levels also increased accumulation of the transcript corresponding to *βCHX,* which converts β-carotene into downstream xanthophylls. This positive effect of light on carotenogenic gene expression is well correlated with higher carotenoid levels, as has been described in tomato fruit, where lycopene and *β*-carotene accumulation increased concomitantly with the intensity of radiation (Alba et al., 2000; Schofield and Paliyath 2005; Dorais et al., 2008). In the rind of Satsuma mandarin, Ma et al. (2012) observed an increment in *β*-cryptoxanthin by red LED radiation concomitantly with the stimulation of most of the genes of the carotenoid biosynthetic pathway.

Contrarily, only blue LED radiation enhanced carotenoid accumulation and gene expression in cultured juice vesicles (Zhang et al., 2012). Other examples illustrated the diversity of light effects, as in *callus* of 'Red' Marsh where a lower carotenoid content was registered under light conditions or in the flesh of mature 'Cara-Cara' navel orange, where shading increased lycopene and *β*-carotene content (Wang et al., 2006). These observations suggest that the positive correlation between light and carotenoid accumulation appears not to be a general phenomenon in *Citrus* and that different tissue- or species-specific regulatory mechanisms may be operative. The response of the red 'Star Ruby' grapefruit to light appears to be a fixed at this stage, as increased the expression of biosynthetic genes (Fig. 6) but not carotenoid accumulation (Fig. 5.2.2.30). This unusual response may reflect alterations in the regulation of the biosynthetic pathway with respect to ordinary orange-pigmented fruits (Alquézar et al., 2009). A hypothesis to explain the higher accumulation of lycopene and β-carotene in the rind of 'Star Ruby' grapefruit inside the canopy would take into account a higher availability of early precursors for carotenoid biosynthesis combined with a lower expression of *βLCY* and *βCHX*.

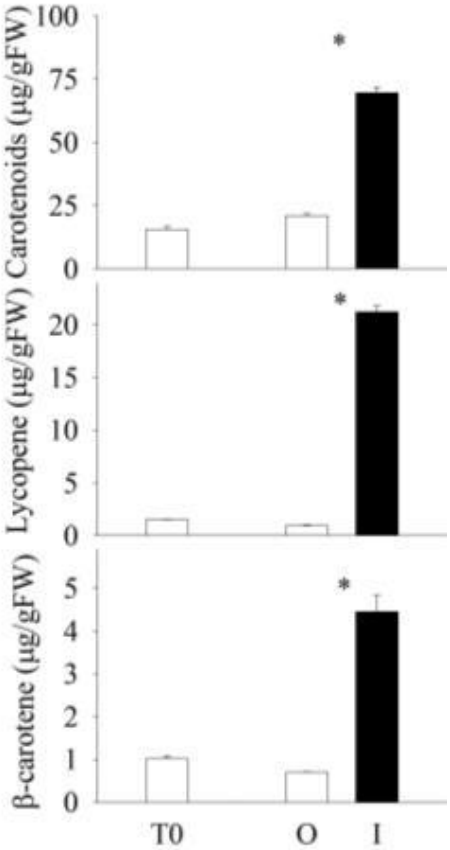


Figure 5.2.2.30. Total carotenoids, lycopene and β-carotene (µg/gFW) content in the rind of 'Star Ruby' grapefruit sampled after fruit set in Jul(NH)/Jan(SH) (Breaker, T0) and at maturity in Dec(NH)/Jun(SH) from

outside (O) and inside (I) of the tree canopy. * indicates significant differences between shaded fruit and exposed fruit (Tukey p<0.05).

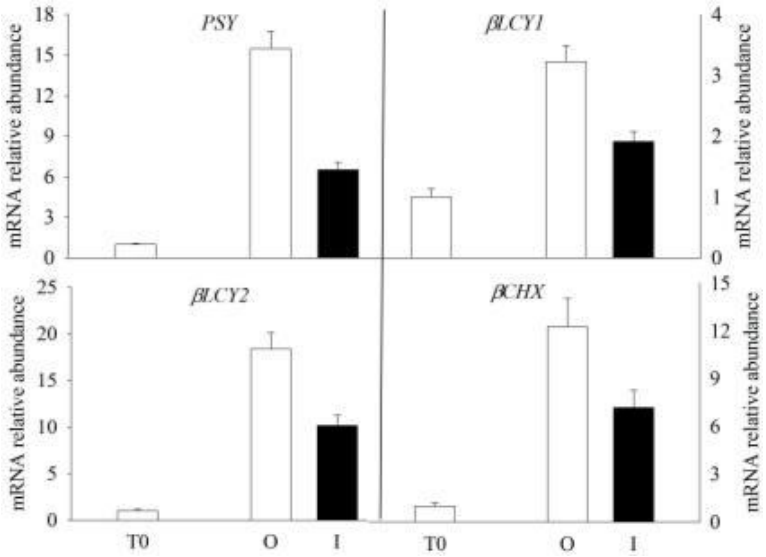


Figure 5.2.2.31. Relative expression of carotenoid biosynthetic genes phytoene synthase (*PSY*), β-carotene cyclase 1 and 2 (*βLCY1* and *βLCY2*) and *β*-carotene hydroxylase *(βCHX)* in the rind of 'Star Ruby' grapefruit sampled after fruit set in Jul(NH)/Jan(SH) (Breaker, T0) and at maturity in Dec(NH)/Jun(SH) from outside (O) and inside (I). The *asterisk indicates significant differences between shaded fruit and exposed fruit (Tukey p<0.05).

Changes in light irradiance have been also associated with differences in internal quality (Reitz and Sites, 1948; Sites and Reitz, 1949). However, only minor differences were detected in 'Star Ruby' grapefruit growing under the two light regimes studied. Soluble solids content was lower in shaded fruit (9.5) vs.10.2 in exposed fruit, hence determining a lower internal maturity ratio. By contrast acidity and juice content were not affected by light (Table 5.2.2.8). Similar results were obtained by Wang et al. (2006) in bagged Cara-Cara orange, where only a small decrease in glucose and fructose was described. A similar reduction in sugar content has been described in shaded 'Hongshigan' fruit (Tao et al., 2003).

Table 5.2.2.8. Internal quality of 'Star Ruby' grapefruit harvested in December from outside (O) and inside (I) the tree canopy.

	Outside	Inside
ºBrix*	10.2±0,12	9.50±0.20
Total acid (AT)	1.28±0.02	1.36±0.02
Ratio (ºBrix/AT)*	7.97±0.19	6.98±0.18
Juice (%)	43.3±0.72	43.8±0.93

* indicates significant differences (Tukey p<0.05).

Postharvest treatments to increase pigment synthesis and rind colour development

As a logical follow-up from the previous results, various techniques that are reputed in literature to influence carotenoid pathway and accumulation of pigments were tested in order to decrease CI susceptibility. For this part of the project outside fruit, of the same size, with a definite yellow side were used (10 replicates of 10 fruit each per treatment) due to them being more prone to CI. Three treatments were applied *viz.* high CO_2, Ethephon and high temperature for short duration ("wilting"). No data are shown for the wilting treatment as no significant results were recorded. For the CO_2 and Ethephon treatment the pigment content after cold storage was determined (Fig. 5.2.2.32). For all treatments the fruit were scored visually for chilling injury

incidence as well as colour improvement, however, no significant differences were seen between treatments and the data are therefore not shown.

The increase (non-significant) in pigments after the Ethephon application was expected as it is well documented that ethylene accelerates senescence processes in the rind of which carotenoid accumulation is one. However, this increase is too low and only of academic importance and could not be used commercially to improve rind colour. In the grapefruit industry 'Star Ruby' is sometimes exposed to ethylene gas, but more as a technique to reduce chlorophyll that to enhance lycopene. It is therefore more of an unmasking action.

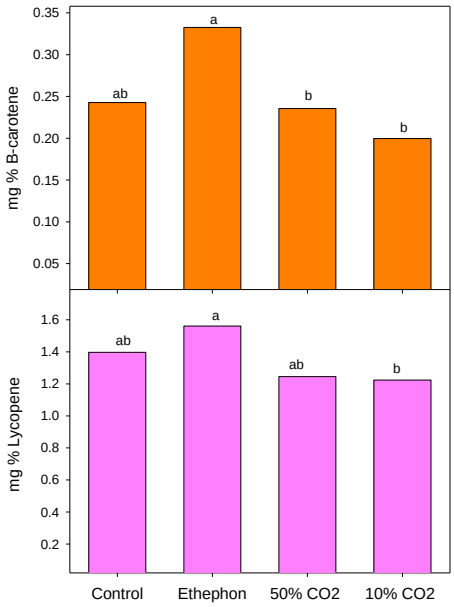


Figure 5.2.2.32. The influence of postharvest Ethephon (400 mg/L) application and exposure of 'Star Ruby' grapefruit to 10 and 50% CO_2 for 48h prior to 35 d cold storage at -0.5°C. Different letters indicate significant difference among treatments (Tukey $p<0.05$).

In an experimental test to determine if covering (shading) of individual fruit could increase pigment synthesis and therefore rind colouration, fruit were covered in December and January. At harvest the rind colour was scaled with the CRI colour chart (CRI 35C) as well as a percentage pink colouration value given prior to cold storage. This experiment was done to firstly ascertain when during a fruit developmental stage is it important that the fruit rind must be shaded/covered and secondly if an outside fruit has the potential to develop better rind colouration and thereby reducing chilling injury incidence. In Figures 5.2.2.33A and B, the improved colouration is shown (lower CRI colour chart value) as well the higher % pink rind colouration. In Figure 5.2.2.33C the difference of this treatment can be seen in the chilling injury incidence, with a lower CI in those fruit covered in December.

These results support the notion that all fruit on a 'Star Ruby' grapefruit tree have the potential to develop an adequate amount of pigment to impart CI protection. However, by being exposed to sun from December onwards the amount of pigment decreases to such an extent that the fruit becomes susceptible to CI. The technique used in the experiment is not practical but indicates that any horticultural technique available that makes economic sense and that will impart a reduction of direct sun on the fruit rind would possibly result in a higher percentage of fruit that could be exported to cold steri-markets.

A practical but expensive option is covering the orchard with shade netting to reduce the high PAR levels thereby reducing the bleaching effect of pigments from the rind. The second option will involve incorporating various management tools such as rootstock choice (more vigorous), irrigation and increased N-fertilising to ensure a large enough leaf canopy develops in which the fruit, after fruit set and initial fruit growth (Nov to Dec), move into the shade as the branches bend due to the weight accumulation. This movement into the shade should facilitate the development of pigments as well as inhibiting bleaching by the direct sunlight.

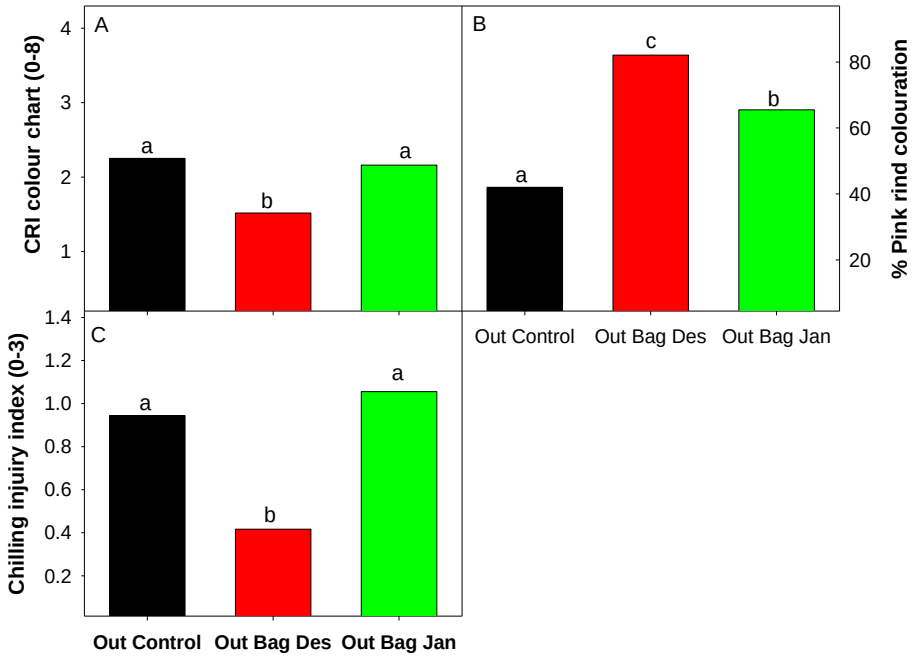


Figure 5.2.2.33. The impact of time of 'Star Ruby' fruit covered on chilling injury. Outside fruit were closed in December, January or left open (control). Fruit were harvested at commercial dates and cold stored. Different letters indicate significant difference among treatments (Tukey p<0.05).

Influence of TBZ on chilling injury

Certain postharvest fungicide treatments such as thiabendazole (TBZ) reduce the incidence of CI in 'Torocco' oranges (Schirra and Mulas, 1995) and grapefruit (Schirra et. al.2000). In addition, application in hot water (50°C for 3 minutes) was beneficial to reduce the incidence of CI. The aim of the experiment was to determine if different fungicides of the same chemical family i.e. Benzimadazole, Benazid®, Thiabendazole (Tecto® and ICA-TBZ®) influence CI susceptibility of 'Star Ruby' grapefruit as postharvest treatments. The different TBZ-family of products showed more variation in efficacy under cold water than hot water application (Fig. 5.2.2.34). Even though the differences were not significant it is of concern that Tecto (the most used product in citrus packhouses) showed the least amount of protection compared to the rest of the treatments. From the data it is clear that this family of chemical products do possibly change the physiology of the flavedo resulting in a protective action. Unfortunately this mode of action of TBZ in reducing CI susceptibility is not known but the significance of these treatments could lie in the association TBZ and hot water have with stress defence mechanisms.

The higher efficacy of warm water application could be due to the better uptake of the thiabendazole into the fruit rind. Increased efficacy of TBZ in combination with hot water was demonstrated previously in different fruit cultivars susceptible to CI, (McDonald et al., 1991; Schirra and Mulas, 1995). Schirra et al. (1996) also indicated that the enhanced activity of heated chemicals is due to the higher deposition of the active ingredient on the fruit. Schirra and D'hallewin, (1997) explained that hot water dips induce a redistribution of the epicuticular wax and a melting of the waxy layer. It can be speculated that the hot water fungicide treatment enhanced penetration of the fungicide through the epicuticular wax. Some of the physiological effects of heat treatment are the accumulation of heat shock proteins and other molecular chaperones and the induction of other defence pathways (Lafuente, 1991). It is recommended that the commercially recommended rate (40 ml/20 L) of TBZ should be applied in water at >40-50<°C if fruit is exported to a cold sterilisation market.

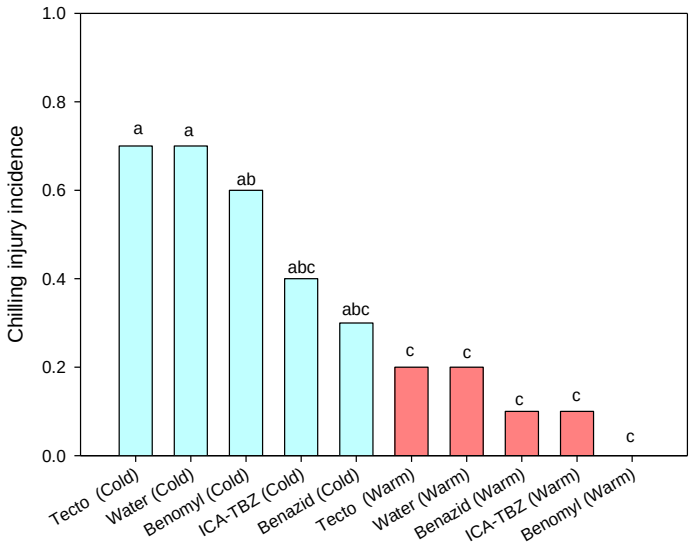


Figure 5.2.2.34. Various chemicals related to thiabendazole (TBZ) were applied to 'Star Ruby' grapefruit in cold (10-15°C) and warm (40-50°C) water for 3 min prior to cold store at -0.5°C for 35 d. Different letters indicate significant difference among treatments (Tukey $p<0.05$).

<u>Proposed new colour charts for 'Star Ruby' grapefruit exported to cold sterilisation markets</u>

The current colour chart develop by CRI and DAFF is focused on the total rind colouration (Fig. 5.2.2.35.) and does not take into account variation on the fruit surface as seen in especially the fruit from Kakamas were the direct sun exposure of a fruit result in a drastic variation from one to the other side of the fruit.

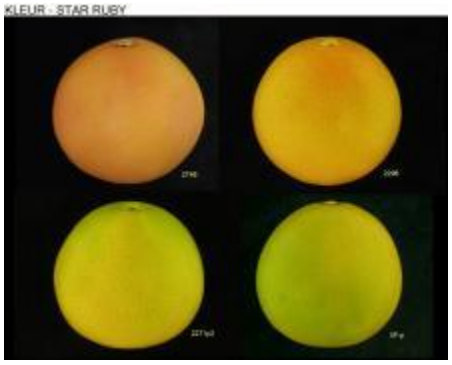


Figure 5.2.2.35. An example of the current CRI 'Star Ruby' grapefruit colour chart that focuses on the side coloration.

The colour charts included below were therefore an attempt to incorporate what was observed over the previous seasons in this study in relation to rind colour development and susceptibility to CI in a low cost packhouse sorting tool. The first observation that was incorporated in the charts was the fact that per fruit there could be significant variation from the top of the fruit compared to the sides. This is due to the effect of leaves at the calyx end supplying a deep shade in only some part of a fruit leaving the rest exposed to the direct sun. The second observation is that some fruit have a half that is pink and one that is yellow. This results in one area of the fruit having a deep pink colour with low susceptibility to CI and an area that is yellow and has a high susceptibility to CI. It was therefore felt that it is important that multiple aspects of the fruit should be used to describe and classify the rind colouration.

The Top View (from the calyx) chart is thought to give the best approximation of the whole fruit and should be the primary chart used to classify fruit. If need be further sorting can be done with either one of the side view charts.

Conclusions

The four questions related to the problem identification were answered in this study.

Firstly it was documented that canopy position and therefore direct sunlight exposure determine the amount of pigment in the rind at harvest and therefore are primarily responsible for the variation between fruit and on fruit, in rind colour. On a more fundamental scientific level it was found that the known lycopene synthesis pathway is not responsible for the higher accumulation in the shade and it is suggested that an unknown pathway could be responsible. Furthermore an association between adequate pink colouration and therefore lycopene content in the flavedo and a significant reduction in CI susceptibility was reported.

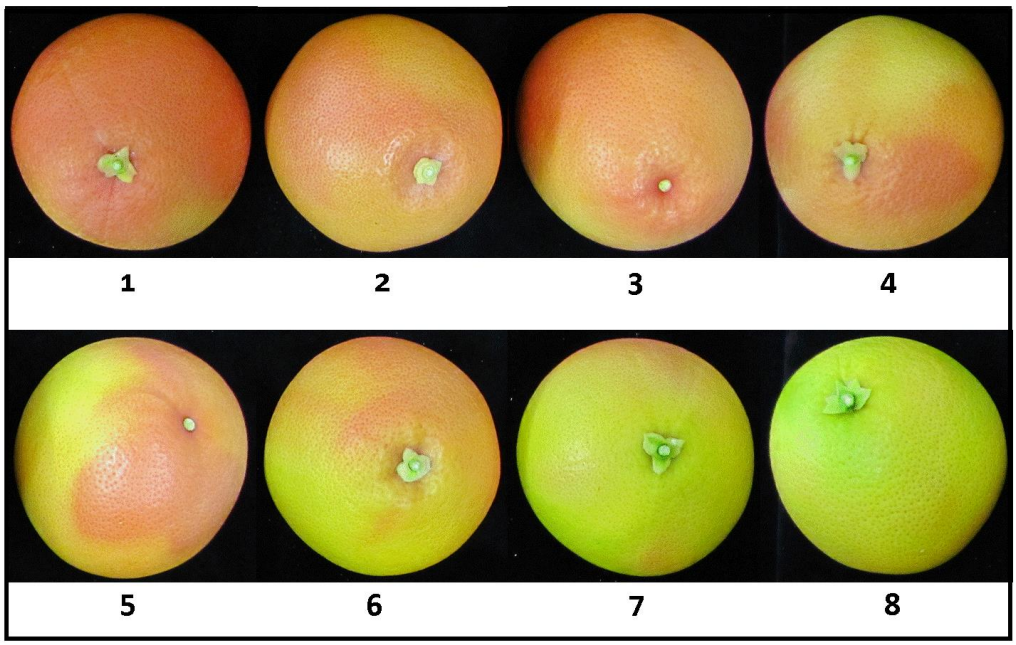
Secondly it was found that this aspect of high lycopene and low CI, was the same in fruit from the two different climates.

Thirdly no potential postharvest technology that was tested could enhance lycopene synthesis to such an extent that it resulted in a reduction in CI susceptibility. However, it was found that the application of TBZ in warm water (40°C) does impart CI protective action.

Lastly a colour chart was developed that could be used in the packhouse to sort fruit according to susceptibility to CI. The documentation of this relationship between high lycopene content in the rind in relation to low PAR and reduced CI susceptibility is a novel discovery and which has proved to be very valuable in safely increasing the export of 'Star Ruby' grapefruit to new cold sterilisation markets.

The following guideline is suggested and should be tested in semi-commercial trials in order to ascertain the relationship between rind colour and susceptibility of a producer's fruit to CI:

Colour chart	% Pink colouration	Risk of CI	Suggested postharvest treatment	Suggested market
1-2	100-80%	Very low	Normal practice or organic packing	All cold steri markets
3-4	60-80%	Low to moderate	Normal practice (Wax + TBZ)	All cold steri markets
5-6	<60%	High	All normal practice (High solid wax +TBZ)	Markets with shorter cold steri-protocol
7-8	<40%	Very high	All normal practice (High solid wax +TBZ)	Only non-cold steri markets



Top view chart

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C. The influence of postharvest storage on citrus fruit quality under high carbon dioxide and freeze conditions

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Summary

Postharvest exposure of citrus fruit to increased carbon dioxide concentration (CO_2) and low temperatures (<0°C) were examined. Negative, as well as positive results were obtained by the use of these technologies which enable the storage and shipping of fruit, but physiological disorders such as freezing and chilling injury can occur under these conditions. The effects and use of increased carbon dioxide and low temperatures are known for other horticultural commodities, but the information and application thereof on specifically citrus fruit, is lacking. Various internal changes occur when the fruit is placed under freeze conditions, but the most visible changes occur when the fruit are defrosted for a period. In the trial, Navel and Minneola fruit were stored at -0.5°C (control), under -2°C, or closed in a container with 10% or 0.039% CO_2 at 0.5°C for 4 weeks. Thereafter various factors were measured to determine the changes in the fruit, namely sugar (TSS) and acid (TA) levels, firmness, colour, as well as a photo record of the fruit before and after freezing. A record of the temperature during storage was also kept. Following the completion of the trial, clear internal and external change in the fruit became noticeable. Based on these results, it can be confirmed with certainty that there is a significant difference between freezing injury and high carbon dioxide exposure during shipment.

Introduction

South Africa is the 14th largest producer of citrus fruit (in terms of hectares), and is currently the second largest exporter of citrus worldwide. Citrus fruit, like other fruit, is susceptible to various postharvest diseases which can result in losses (Brown & Chambers, 1996). Taking the substantial distances from markets into account, the long shipping period can have a negative economic impact on the value of South African export citrus. The management of the cold chain and the correct handling of fruit are therefore vitally important to guarantee the delivery of quality fruit. Various physiological defects can however occur due to incorrect handling procedures, *viz*. rind pitting, chilling injury and internal decay.

The negative impact of postharvest losses as a result of low temperature damage is probably greater than is currently realised. The symptoms of low temperatures – chilling injury and freezing injury, is often still latent while the products are stored in cold storage and only become visible in the market when the fruit are stored under higher temperatures. In the market, deterioration of fruit due to chilling injury is also often confused with pathogen-induced causes or other physiological defects. Storage of fruit at low temperatures is the primary technology used to reduce the rate of fruit respiration, decay development, water loss as well as other associated physiological processes. Low temperatures will lead to a decrease in respiration, ethylene production, ripening, maturation, unwanted metabolic changes and spoilage (Hardenburg *et al.*, 1986). Hence the storage of plant products at low temperatures is considered the most effective strategy to maintain the quality of fruit and vegetables. Unfortunately, storage at low temperatures often poses more disadvantages than advantages for chilling injury-sensitive crops, like tropical fruit. Chilling injury-sensitive crops therefore do not always benefit from cooling, although these crops also start denaturation and exhibit a shorter shelf life if they are not chilled. This dilemma leads to substantial postharvest losses of horticultural commodities and therefore it becomes important to limit the negative effect of chilling injury through effective cold hedging management.

Citrus fruit are not stored in cold stores to the same degree as deciduous fruit. The latter is harvested from the tree before the fruit becomes overripe, while citrus fruit are almost stored on the tree until the point of marketing. There are, however, various exceptions to this statement. If citrus fruit are transported to the

market in quantities that are too large for immediate consumption, it must be stored in a cold store at 4-10°C (Kays & Paull, 2004).

Chilling injury is especially relevant for South African citrus shipped at -0.6°C for sterilisation of insect purposes. The South African citrus industry exports citrus to all countries in the northern hemisphere, but exports to the USA, Korea, Thailand and China must undergo a cold treatment for 24 days at -0.6°C beforehand, with pre-cooling at the same temperatures. Fruit to Japan are treated for 12 days at -0.6°C. This cold sterilisation protocol leads to the incidence of chilling injury in citrus (Hordjik, 2013). Chilling injury occurs due to exposure of fruit to a critical low temperature for a specific period (Ray & Patell, 2005), and can be observed as citrus rind spotting which deteriorates, forming necrotic lesions of 2-5 mm in the flavedo. A contributing factor to increased temperature damage is the rising use of containers instead of conventional bulk shipping of citrus. The increased management inputs to control the temperature of each container (20 pallets) in comparison to 200-300 pallets used in conventional shipping can lead not only to chilling injury, but also freezing injury of citrus fruit.

Freezing points of most vegetables and fruit lie between -0.5°C and -2.5°C and are dependent on the osmotic potential of the fruit cell content. Freezing injury is a physiological disorder that develops at temperatures below -2.5°C, and the forming of ice crystals is characteristic of this phenomenon (Wilkinson, 1970). Ice crystals that form within the fruit damage the membranes of the fruit juice cells and water loss through the rind increases as the fruit defrosts (Syvertsen, 1982). As a result of this water loss, the fruit will take on a dry texture over time and become unmarketable. Freezing injury can also cause loss of flavour through the initiation of the accumulation of bitter limonoid compounds within the fruit (Manners *et al*., 2003). A dramatic reduction in the marketability of citrus occurs when these fruit have been exposed to freeze conditions in the orchard or during cold storage.

Exposure of oranges to freeze conditions causes the formation of white spots or hesperidin crystals on the walls of the fruit segments. Slaugter *et al*. (2008) found that when Navel oranges were irradiated under a long wave ultraviolet light source, bright yellow fluorescent dots became visible on the rind surface of fruit exposed to freeze conditions. Freezing temperatures lead to drastic metabolic changes in the fruit which result in increased fermentation in fruit and the total loss of fruit quality. Freeze damage can occur in cold stores on fruit packed in the upper carton layer of pallets, due to the direct exposure to the cold air emitted by the fans. Fruit can also possibly be exposed to freeze damage during shipping. During the loading of ships, a temperature increase can occur in the containers. Air is then blown into the container at significantly lower temperatures (cold blasting) in order to decrease the fruit temperature to the required specification. This practice of cooling containers at temperatures lower than -2°C can result in freezing injury. In contrast to the freezing injury that can be induced in 1 hour, the damage that develops due to excessive carbon dioxide (CO_2) concentration is a more time-consuming process that changes respiration from aerobic to anaerobic and leads to the well-known negative impact on fruit quality (Kays & Paull, 2004). It is apparent from experience in the commercial shipping of citrus that the increased use of shipping in containers can lead to poorer control of CO_2 concentration.

On a metabolic level, more information exists regarding the effects of O_2 as opposed to CO_2 concentrations as a result of the in-depth research done on the conditions of anoxia and hypoxia in different plant material (Ratcliffe, 1995). In contrast, information regarding the effects of high CO_2 on fruit and vegetables after harvest is very limited, especially for citrus fruit. There are however, many similarities between the effects of low O_2 and high CO_2 on metabolism, where most of the effects result in the inhibition of various metabolic processes (Beadry, 1999; Kader, 1997a). Commodities differ significantly in their reaction to increased CO_2 and have a low tolerance for the gas limits that are employed to maintain quality in some instances (Watkins, 2000). Standard recommendations are made in order to lengthen the storage period for any crop as far as possible. The cultivar type and postharvest treatment of fruit before exposure to high CO_2 are factors that can influence the reaction of commodities to CO_2. It is worrying, however, that these factors are seldom taken into account during cultivar selection or in commercial applications (Watkins, 2000).

Carbon dioxide is added to the controlled atmospheric conditions under which apple fruit are stored in order to lower respiration and store fruit for long periods. The primary aim of CO_2 during storage is to enhance the holding ability of the fruit by reducing decay and preventing physiological disorders. CO_2 is particularly used in the storage of apples and pears and is not effective in citrus. Physiological disorders can occur by exposing citrus fruit to high CO_2 concentrations for relatively short periods before storage (Miller, 1946). High levels of carbon dioxide can also damage the fruit during cold storage and thus the duration of exposure to this gas must be limited (Ke & Kader, 1990). Exposure can lead to anaerobic respiration and a subsequent deterioration in internal quality. This phenomenon often occurs in containers during shipping when specification in terms of fresh air uptake is not conformed to. The recommendation is that citrus should be shipped at a ventilation rate of 15 m^3 air per hour and that the vents must not be closed.

It is currently debated in the citrus industry whether the exposure to increased carbon dioxide concentrations and reduced freezing temperatures have the same negative effect on the quality and symptoms of postharvest citrus fruit. The trial is designed to distinguish between the symptoms associated with both and to develop some practical guidelines to ensure the correct identification of a disorder.

Materials and methods

Two cultivars (harvested on 15 July in Citrusdal) were used, namely: Robyn, a late-maturing type of Navel, as well as Minneola mandarins. The fruit received all commercial treatments (wax and fungicide) in the Goede Hoop Citrus packhouse in Citrusdal, Western Cape, South Africa. There were 5 treatments, namely control (constant -0.6°C storage in open container), freeze for 24 h (before and after at -0.6°C in open container), 2 weeks freeze (before freezing -0.6°C and in open container), 10% CO_2 (in closed containers at -0.6°C) and 0.03% CO_2 (in closed containers at -0.6°C). Each treatment comprised 6 replicates each containing 10 fruit. Fruit were marked according to treatment, cultivar type and fruit number in order to perform measurements on the same fruit and position on fruit. Photos were taken before treatment, and the internal quality (taste, TA – titratable acidity, TSS – total soluble solids and percentage juice) was measured. The TSS, which is expressed in Brix, was measured using a refractometer. The acid content was measured using pipetting of NaOH in the fruit juice. Five drops of phenolphthalein were added to the respective juice samples. The firmness of each fruit was tested using the FTA (Fruit Texture Analyser). Colour measurements were performed using a Minoltachroma (Model CR200; Minolta Camera Osaka, Japan) and data was converted to Lightness, Chroma, and Hue^0 values. Lightness gives an indication of how dark or light the colour is, whereas Chroma indicates the colour intensity and Hue indicates which colour was measured. For both cultivars the fruit in control, the respective replicates of freeze 24 h and freeze 2 weeks, were packed on single layer plastic crates. Treatment 4 was placed in buckets, firmly sealed and rinsed with 10% CO_2 as an initial concentration. Treatment 5 was only placed in buckets and firmly sealed, i.e. exposed to 0.03% (or at least atmospheric concentration) CO_2. Temperature data loggers were installed in the fruit pulp for each treatment.

Two weeks after commencement of storage, the fruit used for treatment 24 freeze were taken out of the -0.5°C cold room and kept at -3°C for 24 h. From this point it was again placed at -0.5°C for the remainder of the storage period. On this same day the fruit used for the 2 week freeze treatment were also moved to -3°C. However, this treatment, in contrast with treatment 24 h freeze, was kept at -3°C until the end of the storage period (a further two weeks). All treatments were removed from the cold rooms and left for 3 days to defrost at room temperature. Gas was drawn from sealed buckets into a 10 ml syringe, and CO_2 and O_2 concentrations were determined with a gas chromatograph (Model N6980, Agilent Inc. Wilmington, USA). The same quality analyses (internal quality, colour and firmness tests) were performed as before storage. Following this, fruit were cut open and rind colour, as well as the incidence of hesperidin crystals were determined. Photos were taken again to point out characteristic symptom differences.

A sensory panel was used for the evaluation which comprised 3 judges (two men and one lady, between the ages of 22-25), who evaluated the taste of the fruit that received the respective treatments. A cryptic description, as well as a mark of 0-3 was awarded. 0 represents a value of a pleasant taste whereas 3 are totally unpalatable or disgusting.

The data were collected and converted in order to enable statistical analysis, analysis and conclusions. Al the statistics were performed using Fisher's test for LSD (Least significant difference) via an ANOVA with a confidence interval of 95%.

Results and discussion

The temperature in all 5 treatments over the course of the trial was indicated for hours in storage and showed the difference between the respective treatments (Fig. 5.2.2.36). For both cultivars, the temperature of the control and CO_2 treatments decreased as the fruit was taken from room temperature and placed in the cold room (only one line was indicated for both CO_2 treatments as no difference occurred). This temperature of -0.5°C was constant for the entire storage period. Two weeks after commencement of the trial, treatment 24 h freeze was frozen for 24 h at -3°C. With initial exposure to these cooler conditions, the fruit temperature initially increased (peaking at ± 0°C). Thereafter it decreased to -3°C. As the fruit was again placed in storage at -0.5°C, the fruit temperature fluctuated there for the remainder of the storage period. Treatment 3 was also stored in freeze conditions at -3°C after two weeks (in the middle of the trial period). The difference in this treatment, however, is that it was kept at -3°C until the end of the storage period (a further two weeks) and not stored at -0.5°C again. This treatment was therefore exposed to freeze conditions for a full two weeks, equal to half of the trial period. The fruit temperature also exhibited a peak increase in temperature when exposed to -3°C, but then decreased to -3°C and remained there until the end of storage.

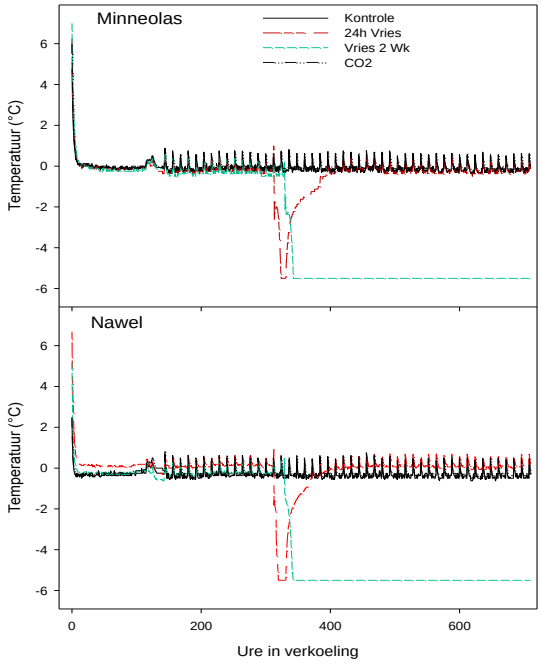


Figure 5.2.2.36. Temperature profiles for the various treatments across the entire trial period. Only one line is indicated for the two CO_2 treatments due to the same data for both.

The sharp increase in fruit temperature when exposed to temperatures under -0.5°C serves as evidence that freezing occurred. When a living cell freezes, it always coincides with a small temperature increase; when the conversion of a liquid to solid matter occurs, for example when water is converted to ice in the cell, energy is released. This energy is transferred as heat and released into the environment. The result is that an increase in temperature occurs and this can serve as a technique to determine at which temperature the fruit froze.

Significant differences occurred for both cultivars in terms of the firmness difference between measurements before and after storage (Fig. 5.2.2.37). The control treatment of the Robyn navel measured a firmness factor of approximately 1 before storage, compared to the Minneola factor of approximately 0.25. This is attributed to the cultivar properties, and the fact that mandarin is softer than a navel-type citrus. The carbon dioxide treatments (10% and 0.03% CO_2) of Minneolas' firmness after storage do not differ significantly to the control, although the CO_2 treatments of Robyn were significantly firmer than the control and can be linked to the storage in closed containers which could have resulted in lower moisture loss. After the freezing period, the difference in firmness increased significantly. This indicates that the fruit were softer and less firm. It appeared that navels lost more firmness after freezing in comparison to mandarins. In both cultivars, no significant difference in the firmness occurred between the 2 freezing treatments.

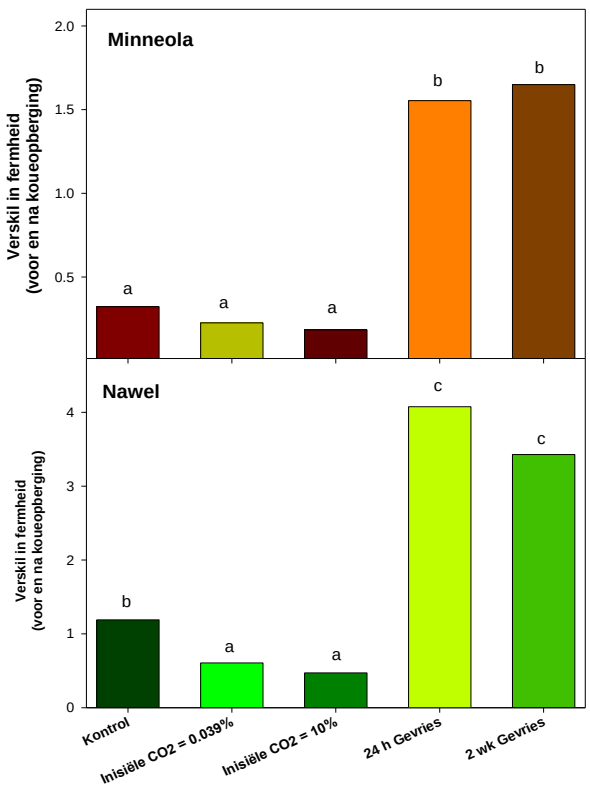


Figure 5.2.2.37. The difference in firmness (kg/cm^2) of Mineola and Robyn navel before and after cold storage after CO_2 and freeze treatments. Different letters between treatments indicate significant differences, according to the Fisher's least significant difference test ($P \leq 0.05$).

The firmness of the fruit decreased significantly after storage and during exposure to freezing conditions. Direct freezing injury occurs when ice crystals form inside the protoplasm of cells. In this case, it is not the cold temperatures, but rather the formation of these ice crystals that damages the fruit (Snyder & Paulo, 1995). It is believed that intracellular ice formation results in a mechanical disruption of the protoplasmic structure. Cellulose, together with other structural components, keeps the cell structure rigid. Cellulase is an enzyme that can potentially break down cellulose. When exposed to sub-zero temperatures the fruit cells can freeze, depending on the water potential of the fruit and this can result in the death of the cells. When exposed to warmer temperatures and as the fruit defrosts, it will increasingly start to respire. This occurs as a type of recovery reaction to damage. Enzymatic activity also increases. During freezing, the fruit also experiences water loss due to the formation of extracellular ice crystals (Figure 5.2.2.43). The cell dehydrates and can begin to disintegrate. The cellulose and microfibril structural components detach and the cell literally collapses. More cellulases are activated to destroy the damaged cells and attempt to form new cells. What transpires is a loss of structural support leading to fruit softening and firmness loss.

For both Navels and Minneolas, the treatment hours decreased after storage compared to the control measured before storage (Fig. 5.2.2.38). It is interesting that the sugar remained approximately the same for both cultivars before and after storage and there was no significant difference. A clear reduction in the juice percentage before and after storage can be seen in Navels and Minneolas. The freeze also indicates a lower juice percentage (compared to the control and other treatments) for both cultivars. The acid and Brix data do not appear to offer a good indication of freeze damage, or high CO_2 exposure. A more interesting aspect is the drop in juice percentage, and thus this technique has the potential to be used in commercial sorting to determine the incidence of postharvest freezing injury.

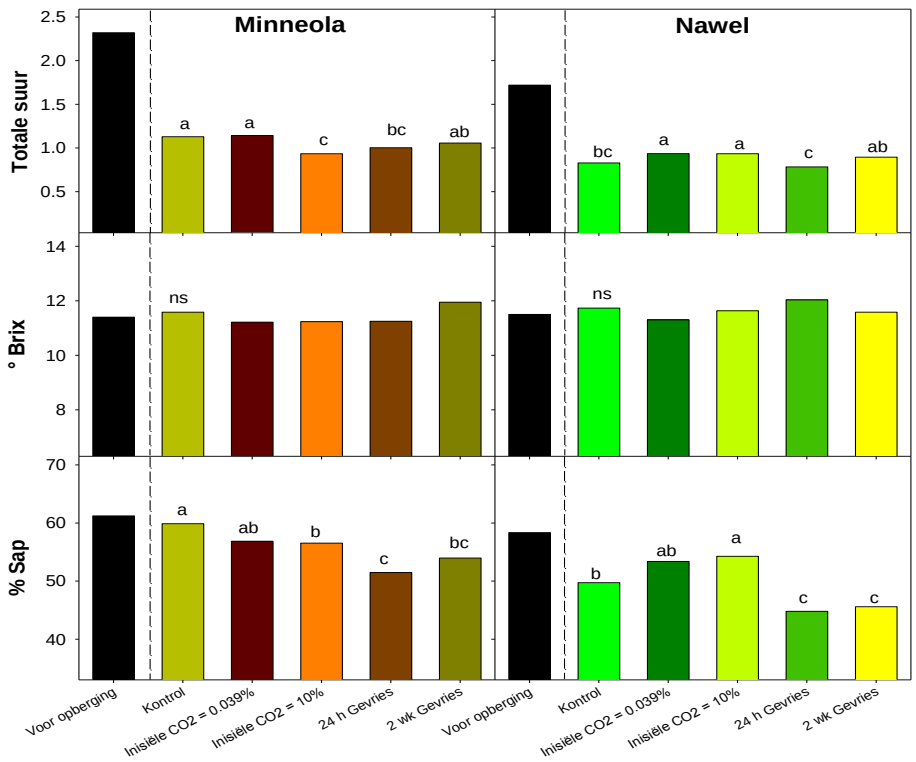


Figure 5.2.2.38. Details of change in internal quality of both cultivars before and after storage. Different letters between treatments indicate significant differences, according to Fisher's least significant difference test ($P \leq 0.05$).

Hesperidin crystals only formed during treatments 24 h freeze and 2 weeks freeze in the Navel and Mandarin fruit pulp, and not during the carbon dioxide (10% CO_2 and 0.03% CO_2) treatments. It is therefore directly linked to the exposure to freeze conditions.

The colour changes in Minneolas were generally more visible than in Navels (Fig. 5.2.2.39). Upon first impression the colour of both cultivars became darker, especially treatments 24 h freeze and 2 weeks freeze. Colour analyses were performed by evaluating three factors, namely lightness, Chroma and hue °. There was a significant difference in lightness° for both cultivars. The same trend was observed with regards to Chroma. Regarding hue°, there were also significant differences between the freeze treatments and the other treatments. The Minneola hue° and the CO_2 treatments did however also differ from the control, which was not the case for the Navel. The change in colour can be directly linked to damage caused to the flavedo and the possible breakdown of carotenes. Significant levels of chilling were not observed in any of the cultivars and the data are not shown. The symptom of chilling injury is sunken lesions spread across the entire fruit surface, and this should not be mistaken for freezing injury.

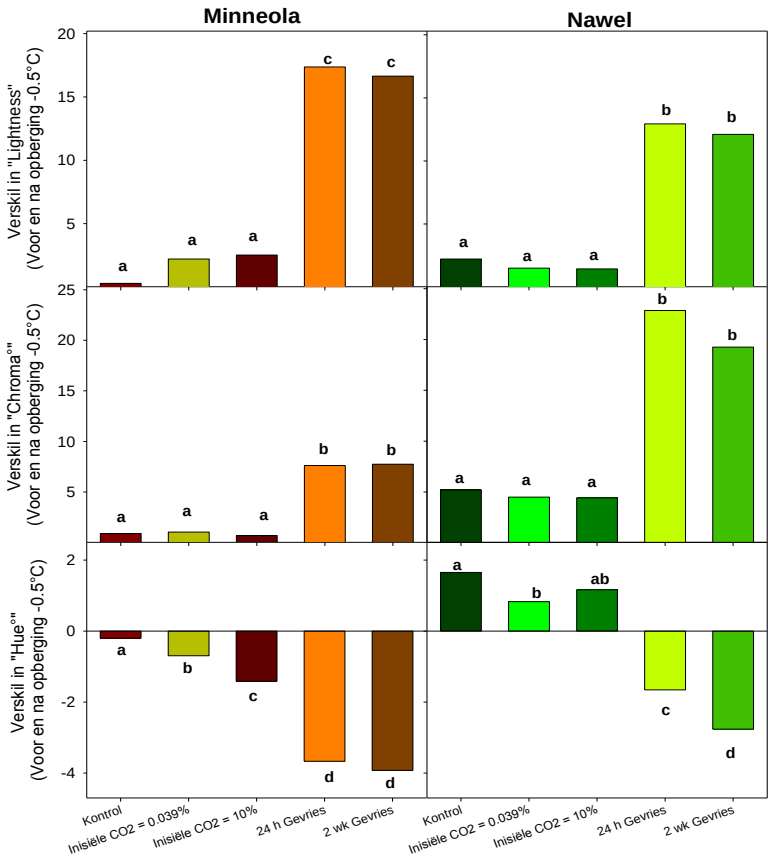


Figure 5.2.2.39. Change in rind colour before and after storage expressed in Hue°, Chroma°, and Lightness° for both cultivars. Different letters between treatments indicate significant differences, according to the Fisher's least significant difference test (*P*≤0.05).

The O_2 value of the Minneolas that received 10% CO_2 treatment was 5% lower compared to treatment 0.03% CO_2, and this confirms the difference between treatments and the effect that a high initial CO_2 concentration will cause (Fig. 5). There was a much larger difference in the percentage oxygen in Navels after treatments 10% CO_2 and 0.03% CO_2. 0.03% CO_2 was ± 18% and 10% CO_2 ± 3%. For Minneolas, the CO_2 measured in the container after storage was ± 5% higher during treatment 10% CO_2 compared to treatment 0.03% CO_2. It therefore correlates with the O_2 concentrations. There was a significantly larger difference in the CO_2 concentration of treatment 10% CO_2 compared to treatment 0.03% CO_2, but this too correlates well with other statistics. An interesting observation is that a higher carbon dioxide concentration was measured in Navels after storage during treatment 10% CO_2 compared to Minneolas, which could possibly be attributed to the difference between cultivars. The results indicate, however, that the fruit in the treatment were indeed exposed to high levels of CO_2.

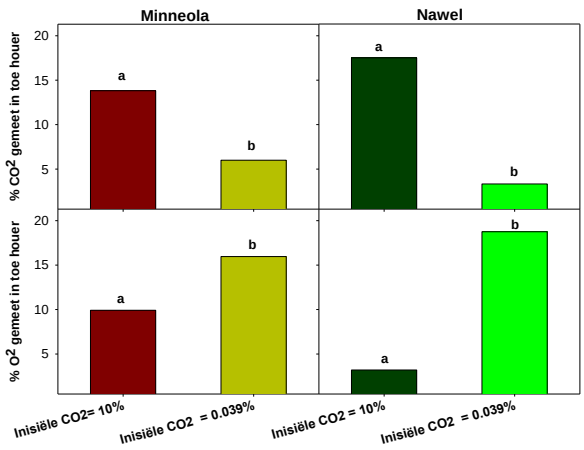


Figure 5.2.2.40. O_2 and CO_2 values measured for Minneola mandarin and Robyn navel after cold storage. Different letters between treatments indicate significant differences, according to the Fisher's least significant difference test (*P*≤0.05).

Figures 5.2.2.41 and 5.2.2.42 indicate the internal and external colour change in the Navel and Minneola fruit before and after the different treatments. Figure 5.2.2.43 shows the hesperidin crystals that formed in both Navels and Minneolas during treatments 24 h frozen and 2 weeks frozen.

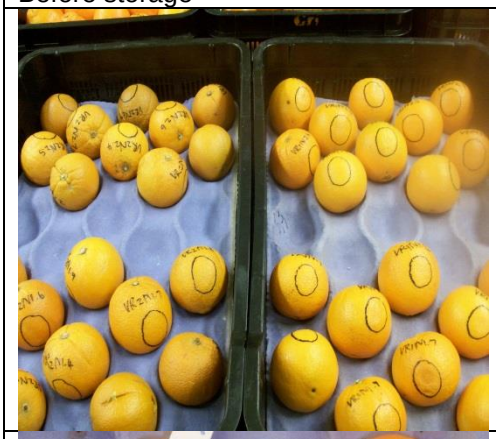
Before storage	After storage
	
	

Figure 5.2.2.41. Indicates the visual differences for navels before and after treatment.

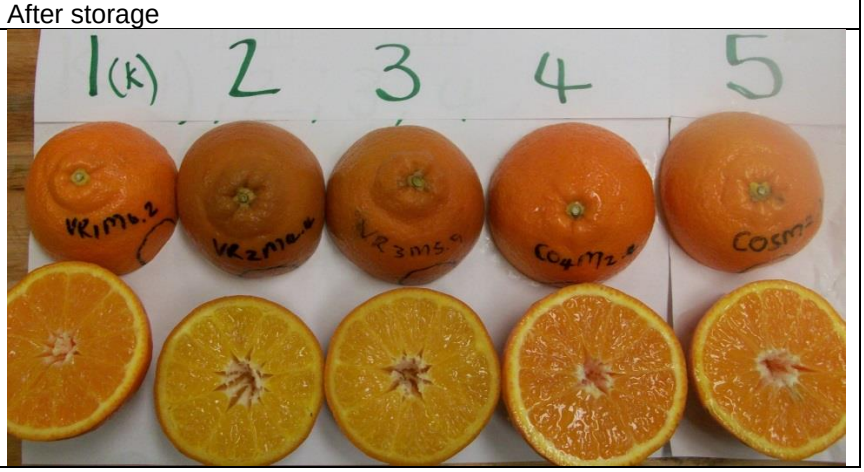
Before storage	After storage
	

Figure 5.2.2.42. Indicates the visual differences for Minneolas before and after treatment.

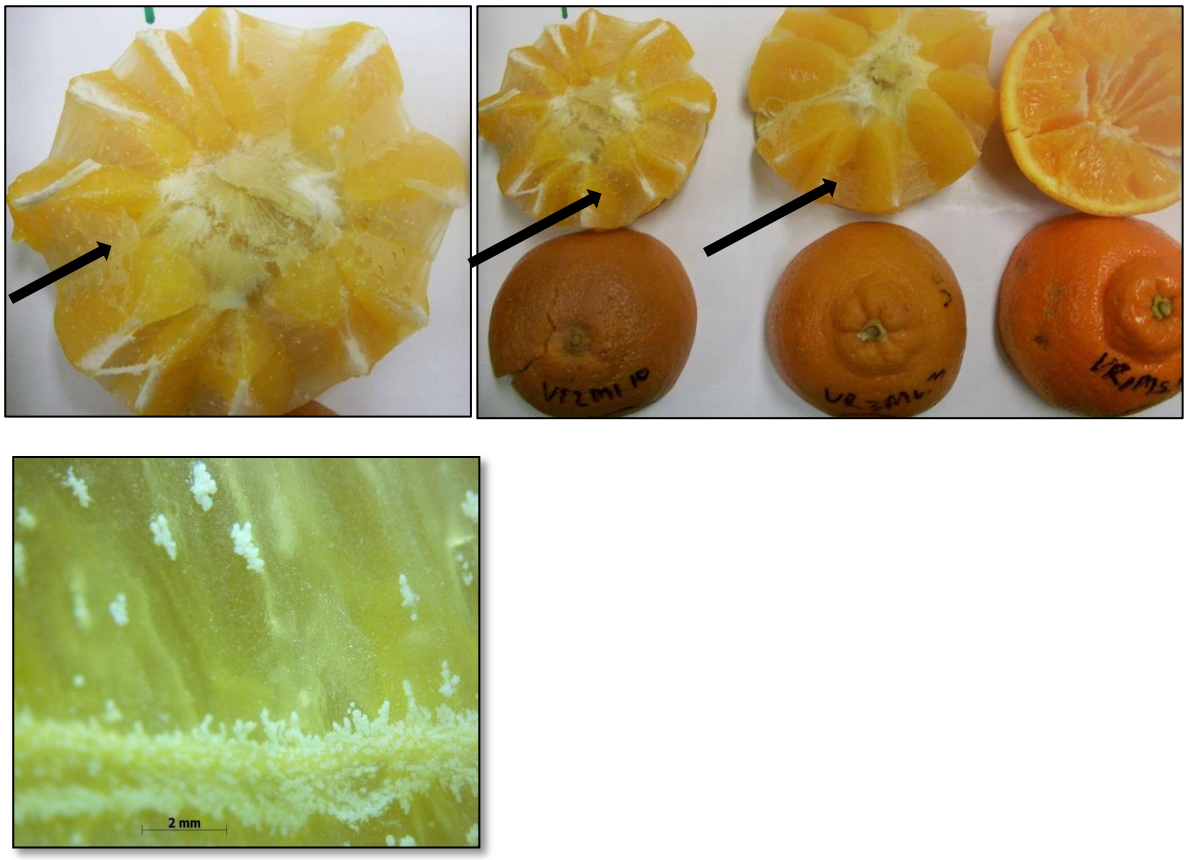


Figure 5.2.2.43. Clear hesperidin crystals visible on Minneola treatments 2 and 3 as well as the rind colour change from orange to grey/brown/orange.

Table 5.2.2.9. Navels before and after storage

	Hesperidin crystals	Taste (0-3)
Before storage	No	Pleasant – 1
After storage		
Control	No	No taste – 1
Freeze 24h	Yes	Rotten, alcoholic – 3
Freeze 2 weeks	Yes	Rotten, alcoholic – 3
10% CO2	No	Better acid, rotten – 2
0.03% CO2	No	Slightly rotten – 2

Table 5.2.2.10. Minneolas before and after storage

	Hesperidin crystals	Taste (0-3)
Before storage	No	Very sour, unpleasant – 2
After storage		
Control	No	Aroma deficiency – 1
Fr2	Yes	Disgusting – 3
Fr3	Yes	Disgusting – 3
$CO_2$4	No	Bitter – 1.5
$CO_2$5	No	Tart – 1

Conclusion

As globalisation and the production of agricultural products in new areas increases, fruit and other agricultural products are widely transported and shipped throughout the world. Management of the cold chain, the transport and handling of fresh products upon arrival at destination therefore have become critically important to ensure good quality. Fruit stored at high temperatures will exhibit deterioration and water loss, compared to fruit that can develop cold injury during storage at low temperatures. Thus significant management inputs are required in the postharvest handling of fruit in order to ensure optimal quality in the market place.
However, to date little research has been done on the effect of increased CO_2 and reduced temperatures (freezing) on specifically citrus fruit. Following the results of this trial, clear internal and external differences could be observed on fruit treated with CO_2, as well as fruit exposed to freezing conditions. More drastic changes were visible on fruit which underwent freezing injury. A visual inspection of the fruit colour and rind is sufficient to determine whether the fruit froze or not. A further important distinguishing factor is the forming of hesperidin crystals on the inside of the fruit on the vacuoles. These small crystals did not form during the carbon dioxide treatments, but did form during both of the freeze treatments. It is important to note that only citrus fruit containing hesperidin in the pulp will form the crystals. Cultivars such as grapefruit and lemons are therefore excluded.

The lower shipping temperature requirements for steri-markets than conventional markets is coupled with more challenges. It is also a much more expensive process and holds greater risks for freezing injury. The risks are substantial, but the potential income much greater. The use of shipping containers has increased significantly compared to conventional shipment. Very good management is required to maintain the cold chain during every phase of transport. Protocols must be compiled and issued regarding firmness, cold storage, sterilisation etc. of different mandarin cultivars. This would vastly assist in determining which cultivars should preferably be shipped to specific countries. Due to these factors, it is imperative that tangible knowledge is accumulated as well as guidelines for freezing injury. This knowledge must be conveyed to technical personnel or exporters and packhouses in the industry.

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Future research

Future research on chilling injury of citrus fruit should focus on three aspects.

- Improving rind condition preharvest and reducing variation between fruit.
- Identifying and testing new agrochemicals to impart an protective function on fruit rind during cold sterilisation
- Improve temperature control during pre-cooling and shipment to cold sterilisation markets especially in refrigerated containers.

Technology transfer

Publications

J Lado, P.J.R. Cronje. M-J Rodrigo, L Zacarìas. 2012. Influence of light on carotenoid accumulation in 'Star Ruby' grapefruit. Acta (In Press).

J. Lado, P. Cronje, M.J. Rodrigo y L. Zacarías. Tolerancia a las bajas temperaturas en frutos de pomelo 'Star Ruby': Implicación de los carotenoides y efecto en la síntesis de etileno. Avances en poscosecha de frutas y hortalizas. I. Recasens, J. Graell, G. Echeverría(eds). pp. 163-171. Edicions de la Universitat de Lleida

Snykant/Cutting edge no. 101. Handling guideline for the export of 'Star Ruby' grapefruit to the USA. Dr. Paul Cronje, Citrus Research International.

Presentations / papers delivered

J. Lado, P.J.R. Cronje. M-J Rodrigo, L Zacarìas. 2012. Influence of light on carotenoid accumulation in 'Star Ruby' grapefruit. 19-23 Nov. International Citrus Symposium, Valencia.

J. Hordijk. Influence of harvest period, cultivar and microclimate on incidence of chilling injury of citrus fruit. CRI symposium Drakensberg, August 2011.

CRI packhouse meetings

2012. ***Chilling and non-chilling rind disorder of citrus fruit.***
14-15 Feb Letsitele, Limpopo (105 persons),16-17 Feb Loskopdam, Mpumalanga (176 persons) , 21-22 Feb Nkwaleni valley Kwazulu Natal (47 persons),23-24 Feb Port Elizabeth Eastern Cape (151 persons) and 28-29 Feb Gordons baai, Western Cape (72 persons)

2013. ***Strategies to reduce chilling injury and improve rind condition of citrus fruit***.
29-30 Jan Polokwane, Limpopo (183 persons), 31-1 Feb Loskopdam, Mpumalanga (157 persons)

12-13 Feb Durban Kwazulu Natal (56 persons), 14-15 Feb Port Elizabeth Eastern Cape (176 persons) 19-20 Feb Stellenbosch, Western Cape (85 persons) and

2014. ***Presentation 1: Physiological rind disorders: Research update***
Presentation 2: Effective management of cold sterilisation.
29-30 Jan Polokwane, Limpopo; 31Jan-1 Feb Loskopdam, Mpumalanga; 12-13 Feb Durban KwaZulu-Natal; 14-15 Feb Port Elizabeth Eastern Cape and 19-20 Feb Stellenbosch, Western Cape

5.2.3 PROGRESS REPORT: Effect of different chemical applications on development of Peteca spot in lemons

Project 833 (2006/7-2014/5) by P.J.R. Cronje (CRI)

Summary

Peteca spot (PS) of lemon is a postharvest physiological disorder resulting in the collapse of the oil gland. Subsequently the oil leaks into the adjacent tissue and causes a darkened depression or sunken area. The occurrence can be severe, resulting in substantial economic losses without any specific pre- or postharvest practises that could be implemented to avoid or significantly reduce the incidence. PS occurs in all 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, the packing line and wax application. Preharvest Ethephon (2-Chloroethyl phosphoric acid) (200 mg/L and 400 mg/L) and AVG (aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L) applications to fruit resulted in no significant results in any treatment in 2013 due to too low PS incidence. It is hypothesised that if the internal ethylene synthesis is increased in sensitive fruit, i.e. immature fruit, a reduction in PS can occur due to a protective action and this will be further tested in 2014.

Opsomming

Peteka kol (PK) van suurlemoen is ŉ na-oes fisiologiese skildefek waar die olieklier in skeur en lek die olie uit in die omliggende weefsel en lei tot ŉ donker versonke letsel in die skil. Die voorkoms kan uiters hoog wees en lei tot ernstige finansiële verliese, en daar bestaan tans nie ŉ voorkomings of beheer maatreël nie. PK kom voor in alle suurlemoen areas in SA en daar word vermoed dat onvolwasse vrugteskille wat aan na-oes stres (soos hoë CO_2, verpakking en waks aanwending) blootgestel word, lei tot ŉ verhoogde voorkoms. In 2013 in voor-oes Ethephon (2-Chloroethyl phosphoric suur) (200 mg/L en 400 mg/L) en AVG (aminoethoxy-vinylglycine) (400 mg/L en 800 mg/L) toegedien op vrugte. Te lae voorkoms van peteka is in alle boorde wat behandel was gevind om betekenisvolle verskille op te merk. In geheel gesien kan uit vorige data afgelei word dat ŉ hoër vlak van interne geproduseerde etileen in die skil toe ŉ verlaagde sensitiwiteit tot PK in die skil lei a.g.v. ŉ onbekende beskermende funksie. Hierdie hipotese sal geduringe 2014 verder ondersoek word.

5.2.4 PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit

Project 958 (2009/10 – 2014/5) by P.J.R. Cronje (CRI)

Summary

Postharvest physiological rind disorders, such as staining and pitting, affect most citrus cultivars and have a significantly negative impact on return on investment for producers. Fluctuations in rind water balance, as influenced by ambient conditions during handling have been shown to play a part in inducing rind disorders. By altering the VPD (vapour pressure deficit) between harvest and wax application of Valencia and mandarin fruit a higher incidence of pitting was recorded. This supports the previous season's results which indicated the negative impact on rind condition of high moisture loss.

Opsomming

Naoes gepokte skil is ŉ fisiologiese skildefek wat bykans alle sitrus kultivars negatief kan affekteer en lei tot betekenisvolle finansiële verliese. Volgens verkeie bronne asook uit voorafgaande navorsing van CRI word vermoed dat die verandering in die hoeveelheid vog in die vrugskil, soos beïnvloed deur na-oes hantering, ŉ belangrike aandeel het in die indusering van skildefekte. Deur die dampdruk verskil in die periode na pluk en voor waks aanwending te verander, en sodoende die vogverlies uit die skil te verhoog, was daar getoon dat die voorkoms van gepokte skil verhoog word in Valencia lemoen en Nadorcott mandaryne. Hierdie bevinding strook met vorige seisoene se resultate wat daarop dui dat ŉ drastiese vogverlies uit die skil kan lei tot die ontwikkeling van fisiologiese skildefekte.

5.2.5 **FINAL REPORT: Non-destructive monitoring and prediction of postharvest rind quality of 'Nules Clementine' Mandarin and Valencia orange fruit**

Project 1031 (2010/11-2012/13) by P.J.R. Cronje (CRI), Lembe Magwasa & Linus Opara (SU), L. Terry (Cranfield University) & Bart Nicolai (KUL)

Summary

The aim of this study was to develop non-destructive methods to predict external and internal quality of citrus fruit. A critical review of the literature identified pre-symptomatic biochemical markers associated with non-chilling rind physiological disorders. The prospects for the use of visible to near infrared spectroscopy (Vis/NIRS) as non-destructive technology to sort affected fruit were also reviewed. Initial studies were conducted to determine the optimal condition for NIRS measurements and to evaluate the accuracy of this technique and associated chemometric analysis. It was found that the emission head spectroscopy in diffuse reflectance mode could predict fruit mass, colour index, total soluble solids, and vitamin C with high accuracy. Vis/NIRS was used to predict postharvest rind physico-chemical properties related to rind quality and susceptibility of 'Nules Clementine' to RBD. Partial least squares (PLS) statistics demonstrated that rind colour index, dry matter (DM) content, total carbohydrates, and water loss were predicted accurately. Chemometric analysis showed that optimal PLS model performances for DM, sucrose, glucose, and fructose were obtained using models based on multiple scatter correction (MSC) spectral pre-processing. The critical step in evaluating the feasibility of Vis/NIRS was to test the robustness of the calibration models across orchards from four growing regions in South Africa over two seasons. Studies on the effects of microclimatic conditions predisposing fruit to RBD showed that fruit inside the canopy, especially artificially bagged fruit, had lower DM, higher mass loss, and were more susceptible to RBD. The study suggested that variations in microclimatic conditions between seasons, as well as within the tree canopy, affect the biochemical profile of the rind, which in turn influences fruit response to postharvest stresses associated with senescence and susceptibility to RBD. Principal component analysis (PCA) and PLS discriminant analysis (PLS-DA) models were applied to distinguish between fruit from respectively, inside and outside tree canopy, using Vis/NIRS signal, suggesting the possibility of using this technology to discriminate between fruit based on their susceptibility to RBD. Results from the application of optical coherence tomography (OCT), a novel non-destructive technology for imaging histological changes in biological tissues, showed promise as a potential technique for immediate, real-time acquisition of images of rind anatomical features of citrus fruit. The study also demonstrated the potential of Vis/NIRS as a non-destructive tool for sorting citrus fruit based on external and internal quality.

Full dissertation available at URL: http://hdl.handle.net/10019.1/85578

Opsomming

Die studie het ten doel gestaan om nie-destruktiewe meeting metodes te toets en ontwikkel wat die interne en eksterne-kwaliteit van sitrusvrugte kan voorspel. In ŉ litratuuroorsig is biochemies verandering in die skil en wat geassosieer word met die ontwikkeling van fisiologies skildefekte geïdentifiseer, asook is die moontlikheid ondersoek om Naby Infrarooi spektroskopie (NIRS) as ŉ nie-destruktiewe tegnologie te gebruik om vrugte te sorteer. Eerstens was die optimale toestande waarby NIRS meetings van sitrusvrugte geneem moet word asook die akkuraatheid van die toerusting en chemometrika data-ontleding beproef. Daar is gevind dat die uitstralings-kop spektrofotometer in diffusie-weerkaatsings modus vrugmassa, skilkleur, totale opgeloste stowwe asook vitamien C akkuraat kan voorspel. Daarna van NIRS gebruik om na-oes fisies-chemiese eienskappe wat verband hou met skilkwaliteit en vatbaarheid vir skilafbraak van 'Nules Clementine' mandaryn. Deur gebruik te maak van "Partial least squares" (PLS) statistieke was gedemonstreer dat die skilkleur, droë massa (DM), totale koolhidrate en waterverlies akkuraat voorspel kon word. Chemometriese analises het ook getoon dat optimale PLS modelle vir DM, sukrose, glukose en fruktose verkry kan word deur modelle te skep wat gebaseer is op "Multiple scatter correction" (MSC) spektrale voor-verwerking. ŉ Belangrike stap in die ontwikkeling van NIRS gebaseerde indeling is om die robuustheid van die kalibrasiemodelle te toets en was gedoen deur vrugte te meet en sorteer van vier boorde en oor twee seisoene. ŉ Verder eksperiment om die impak van mikroklimaat op die skil se vatbaarheid vir fisiologiese defekte te ontwikkel het getoon dat vrugte wat binne in die blaardak ontwikkel (lae vlakke van sonlig) ŉ laer DM, hoër gewigsverlies het en was ook meer vatbaar vir skilafbraak. Die resultate dui daarop dat verskille in mikroklimaat oor die seisoen asook in die blaardak die skil se biochemiese profiel beïnvloed, wat lei tot ŉ negatiewe reaksie op na-oes stres en verhoogde voorkoms van fisiologiese skilafbraak. Die ontwikkelde "Principal component analysis" (PCA) en PLS-diskriminant analise modelle was daarna suksesvol toegepas om vrugte te skei na NIRS meetings, op die basis van vrugpossies in die blaardak. Nuwe, nie-destruktiewe tegniek, nl. "Optical coherence tomography" (OCT) was suksesvol getoets as manier om ŉ fotografiese beeld te skep van histologiese veranderinge in die skil. Die resultate dui op die potensiaal van die onontginde tegnologie om intak biologiese-materiaal te analiseer. Hierdie studie

het getoon dat daar wesenlike potensiaal is om NIRS verder te ontwikkel tot ŉ tegnologie wat gebruik kan word om vrugte te sorteer gebaseer op eksterne (skil) asook interne (pulp).

Introduction

South Africa exports approximately 120 000 tons of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) per year, making it the third largest producer and exporter of Clementine mandarins after Spain and Morocco (Citrus Growers' Association of Southern Africa, 2012). 'Nules Clementine' mandarin fruit are prone to develop a progressive non-chilling physiological rind disorder during postharvest storage, referred to as rind breakdown (RBD). Although RBD is a superficial rind disorder that does not compromise the edible internal portion of the fruit, it, dramatically decreases fruit market value. On some citrus sorting lines, cameras are already being used to detect the presence of rind lesions such as disorders and colour development. However, these are not suitable for detecting RBD, since the disorder characteristically does not show visual symptoms during packhouse grading and packing, but develops about 3 to 5 weeks after harvest (Cronje et al., 2011a). In the export-orientated South African citrus industry, this delayed symptom development is extremely problematic. A previous study by Cronje (2009) reported that fruit harvested from inside the canopy had lower sugars and were more susceptible to RBD. These findings suggested that rind carbohydrate concentrations could be used as potential biochemical indicators of fruit susceptibility to RBD. A major challenge facing the citrus industry and researchers alike is to develop non-destructive technology to determine quality on the packing line. As such, the use of visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS) to evaluate fruit physiological defects and predict postharvest quality attributes of fruit are topical in postharvest research (Cozzolino et al., 2010).

Existing analytical methods for the quantification of carbohydrates in fruit rind and biomaterials are expensive, time consuming, and require specialised sample preparation. Increasing consumer demand for internal quality attributes such as sweetness and nutritional content, coupled with industry demand for innovative tools for rapid and cost-effective detection and monitoring of physiological disorders, have spurred considerable interest among researchers on the application of non-destructive methods of citrus fruit quality monitoring and evaluation. These non-destructive instrument-based methods for assessing fruit quality will assist citrus packers to segregate fruit based on their desired quality attributes and also susceptibility to rind physiological disorders. Internationally, there are considerable research activities on the investigation of Vis/NIR-based sensors for determining various quality factors in a range of fruit and vegetables, including citrus (Nicolaï et al., 2007). As such, Vis/NIRS has become one of the most used technologies for the non-destructive evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Miyamoto and Kitano, 1995; Nicolaï et al., 2007; Cozzolino et al., 2010; Wedding et al., 2013). Vis/NIRS has been applied to packhouse fruit sorting lines for TSS quantification of citrus and other fruit since the mid-1990s (Kawano et al., 1993). In contrast, very limited research has been conducted to evaluate Vis/NIRS to predict and monitor physiological disorders and rind physiological disorders of citrus fruit in particular (Zheng et al., 2010). Vis/NIRS offers a potential for the non-invasive assessment of the chemical composition and changes thereof, such as carbohydrate content in the rind of intact fruit that might predispose fruit to the development of rind disorders. Based on current literature information, the overall aim of this study was to develop non-destructive methods based on Vis/NIR spectroscopy to predict external and quality of citrus fruit during postharvest storage.

Non-destructive evaluation of citrus fruit quality using Vis/NIR spectroscopy

A preliminary study was conducted in Chapter 4 to determine optimal conditions of near infrared (NIR) spectroscopy (NIRS) measurements and evaluate the accuracy of this technique to detect physico-chemical properties of citrus fruit. Most of the previous NIRS investigations on citrus fruit have focused on assessing specific internal quality attributes and did not integrate quantitative assessment of external and internal quality attributes in one system (Gómez et al. 2006; Lu et al., 2006; Liu et al., 2010; Jamshidi et al., 2012). This study evaluated the feasibility of three Fourier transform near infrared (FT-NIR)-based spectrophotometers namely, a fibre-optic probe for solid samples (SP), an integrating sphere (IS) and an emission head (EH), for integrated non-destructive measurements of external and internal quality attributes of 'Valencia' oranges [*Citrus sinensis* L. (Osb.)]. The results showed that calibration models developed using the EH predicted mass, colour index, and vitamin C better than other spectrometers (or acquisition modes), while the IS gave the best prediction for TSS. However, the prediction statistics for the TSS obtained using EH were also comparable (RMSEP = 0.65°Brix) to those of IS (RMSEP = 0.58°Brix). Considering that consumers can detect a difference in taste of about 1°Brix, an RMSEP of 0.65°Brix for EH showed that the model possesses the ability to assign the oranges to specific taste requirements of consumers.

Good correlation between spectral information obtained by the EH spectrophotometer and quality attributes measured by conventional methods (mass, colour index, TSS and vitamin C) demonstrated the potential of

this FT-NIR instrument as a non-destructive tool to holistically evaluate fruit external and internal quality parameters. Although mass was predicted with high accuracy, the future use of NIRS in predicting mass is questionable considering that there are cheaper and more user-friendly methods. However, if FT-NIR could be successfully developed to automatically and simultaneously measure a combination of internal and external quality attributes, as shown to be possible using EH, investing on the technology and model development could be justified. Considering that before the current study, very limited studies have sought to assess the suitability of NIRS for integrated assessment of internal and external quality parameters in intact oranges (Sánchez et al., 2013), this research made a significant contribution towards on-line non-destructive evaluation of citrus fruit.

Considering that the chances of successful commercial implementation of non-destructive technology depends on model robustness, external validation of calibration models using samples from different orchards, growing regions and production season has become a critical issue in Vis/NIRS and an active area of research (McGlone et al., 2002; Guthrie et al., 2005b; Zude et al., 2008). In this study, sufficient variation of quality attributes was added to calibration and validation data sets. This was shown on calibration models developed using all fruit which performed better in predicting all quality parameters compared to models developed based on small sample population. The better model performance developed across a population of fruit with larger range in the quality attribute in question demonstrated the importance of having enough variability in the reference samples tested.

High predictive performance of calibration models when validated using fruit from another location demonstrated a high level of model robustness. However, before EH FT-NIR spectrophotometers can be successfully implemented on a commercial sorting line, validation across different seasons would be advisable. Guthrie et al. (2005a, b) recommended that optical and reference sampling for all citrus fruit should be at any position around the equatorial position of the fruit in order to best represent the entire fruit. In the current study, the spectra taken from the equatorial position of orange fruit was used for the PLS calibration and validation. The problem with this approach is that in commercial packing lines, it is currently not possible to align fruit in a manner that allows the NIR device to always scan the equatorial cross-sectional portion of the fruit. It is recommended that semi-commercial studies be conducted where spectra are acquired at random from fruit rolling on a conveyor belt to test the predictive ability of the NIR device and model, to overcome the effect of spatial differences in quality of fruit, which may result in significant increase in the prediction error. Williams and Sobering (1993), Cléments et al. (2008) and Davey et al. (2009), reported that pigments and colour results obtained using an NIR instrument, which includes a visible range of the electromagnetic spectrum were better than those without visible range. Therefore, the modification of the lamps and detectors of the NIR instruments to incude the visible range of the spectrum (350 to 750 nm) would enable colour to be measured from principle, rather indirectly through PLS models against chromameter measured colour space. Based on this recommendation, subsequent experiments conducted to develop calibration models to predict susceptibility of 'Nules Clementine' mandarin fruit to RBD used Vis/NIRS. The use of chemometrics to analyse Vis/NIR spectra collected from intact 'Nules Clementine' mandarin fruit at harvest, was explored to predict the rind biochemical profile after eight weeks of storage.

A study to evaluate the ability of Vis/NIRS to non-destructively predict rind physico-chemical profile of 'Nules Clementine' mandarins and susceptibility to RBD was conducted. The studies further explored different chemometric analysis of spectra acquired from intact fruit at harvest to predict postharvest rind physico-chemical properties related to rind quality and susceptibility to RBD. Due to the discrete nature of RBD scores, correlating it with complex Vis/NIRS data was difficult and partial least squares (PLS) model statistics were poor, suggesting that the technique is not able to accurately predict the disorder. The complexity of biological factors involved in the development of RBD (Cronje et al., 2011a, b) may also account for the difficulty of developing a reliable prediction model for RBD. Nevertheless, rind physico-chemical properties such as rind sugars and dry matter content, which could be used for potential on-line application of Vis/NIRS, were predicted with accuracy for up to eight weeks of fruit storage. Previous studies have shown that RBD-susceptible fruit located inside the canopy had lower rind sugar concentration and dry matter content (Cronje et al., 2011a). Taking this into account, the ability of Vis/NIRS to predict rind sugars and dry matter content as possible biochemical indicators of fruit susceptibility to RBD was explored. The good prediction statistics for these rind properties which predispose fruit to RBD revealed the ability of Vis/NIRS and chemometrics to predict postharvest behaviour of mandarins and hence susceptibility to the disorder. The chemical composition of citrus fruit varies from stem to blossom end and from sun to shade sides of the same fruit (Peiris et al., 1999). This would limit the implementation of the Vis/NIRS for a real-time on-line sorting system, unless spectra acquisition is repeated at several positions around the fruit in order to minimise the effect of variation within fruit. However, this might not be practically compatible with a typical speed of commercial sorting lines, which may be as high as 10 fruit per second (Nicolaï et al., 2007). Therefore, parallel sorting lines with turning fruit and a number of spectrophotometers might overcome this problem, although costs of implementation would be high.

Vis/NIRS models obtained in the current study also demonstrated the importance of model robustness in predicting new populations of fruit. Adding orchard and seasonal variability to the calibration models reduced prediction errors of target analytes. These observations are similar to those by Wedding et al. (2013), who reported improved model performance when more seasonal variability was included in the calibration set. Although the models developed in this study showed high levels of robustness, it is important to stress that special attention should be paid when new populations of fruit from different orchards or growing regions and production or marketing seasons are evaluated. Considering the observed variation between growing locations and seasons, it is advisable to upgrade calibration models, using fruit from successive seasons and orchard locations. Spiking existing calibration models with a few samples from the target prediction orchard will potentially improve model performance and reduce calibration time and costs.

Although this study has demonstrated the considerable potential of Vis/NIRS to predict biochemical properties associated with RBD, certain aspects could be improved. The performance of calibration models for sucrose was not satisfactory and had very high prediction errors, which could be attributed to high skewness in data distribution. The skewness has recently been shown to affect interpretation of PLS model statistical parameters such as RPD, which was developed for biological data sets showing normal distribution (Bellon-Maurel et al., 2010). The use of the standard deviation (the numerator in the RPD ratio) of highly skewed data with many low values samples, such as the distribution of RBD, may not be statistically correct. In addition, there is still no definite upper or lower limit of carbohydrate concentration in which the disorder occurs or does not occur. Hence, further research still needs to be conducted to explain whether Vis/NIRS-predicted carbohydrate concentration is useful for determining fruit susceptibility to postharvest RBD.

Principal component analysis (PCA) and PLS discriminant analysis (PLS-DA) models based on spectra acquired before harvest were able to discriminate fruit based on their position within the canopy. The accuracy of the two regression methods was very high indicating that both methods could be used, individually or in combination, for screening purposes. The results of this study indicated that fruit located inside the canopy were more susceptible to RBD than outside fruit. Therefore, the ability of Vis/NIRS to discriminate between inside and outside fruit suggests the potential of this technology to discriminate fruit based on their susceptibility to RBD. In practical terms, the capability of Vis/NIRS to non-destructively segregate fruit based on their origin within the canopy, suggested the use of this technology as an on-line deciding tool to classify individual fruit destined for either local (inside fruit) or export market and long term storage (outside fruit).

Biochemical profile of fruit located in different positions of the canopy

Recent results from Cronje (2009) demonstrated that outside fruit with higher sugar content developed less RBD than inside fruit. The hypothesis was that limited photosynthetically active radiation (PAR) on shaded portions of the canopy reduced fruit photosynthesis rate and rind carbohydrates, which in turn increase susceptibility to RBD. However, these studies were conducted in one citrus growing region of South Africa (Stellenbosch), therefore, the proposed hypothesis for the mechanism underlying the development of RBD may not be generalised for all growing regions.

To test the hypothesis describing the effect high or low light on the sensitivity of ‘Nules Clementine’ mandarin fruit to rind sugar concentration and RBD incidence, the study in Paper 6 was extended to include more orchards from other citrus growing locations. The main finding of this study was that fruit position within the canopy and exclusion of sunlight by bagging fruit during growth had a significant effect on rind physiological and biochemical properties as well as susceptibility to RBD. In all four orchard locations, ‘Nules Clementine’ mandarin fruit borne inside the canopy were more susceptible to RBD compared to other preharvest treatments, while bagging of inside fruit resulted in the highest incidence of the disorder. These results indicated that regardless of orchard location, reduced exposure of fruit to sunlight during growth was the major preharvest factor contributing to the disorder. The results of the current study were similar to those reported by Cronje et al. (2011a, b), who observed higher RBD on shaded fruit compared to sun-exposed outside fruit. In contrast to disorders such as rind staining in ‘Fortune’ mandarins (Almela et al., 1992; Duarte and Guardiola, 1995) and rind breakdown in ‘Navel’ oranges (Agustí et al., 2001) where peel disorders were associated with sun/heat exposure, RBD in ‘Nules Clementine’ occurred on inside fruit because the disorder is associated with low mineral and carbohydrate allocation and therefore premature senescence (Cronje et al., 2011a). These findings of the current study provided further evidence of the pre-harvest conditions affecting postharvest behaviour of citrus rinds in relation to RBD. In one orchard (Citrusdal), the concentrations of sucrose, glucose and fructose in the rind of bagged fruit from inside and outside canopy positions were lower than those of unbagged fruit. Reduced radiation by bagging has previously been reported to negatively affect non-structural carbohydrates concentration of citrus fruit (Hiratsuka et al., 2012).

An opposite pattern was observed in rinds of fruit harvested from two other orchards (Porterville and Paarl), which had higher concentrations of non-structural carbohydrates (fructose, sucrose and total carbohydrates) than sun-exposed fruit. For fruit grown in Stellenbosch area, sucrose of bagged inside fruit was also higher compared to outside fruit. In these three orchards, shaded fruit containing higher non-structural carbohydrates were more susceptible to RBD during storage, suggesting that the carbohydrate content was not a limiting factor in the susceptibility of 'Nules Clementine' mandarins to RBD in these regions. Different carbohydrates responses to reduced sunlight indicated that water soluble sugars might not be a universal biochemical marker or the primary cause for RBD, since it is highly dependent on orchard and location.

Although non-structural carbohydrates of fruit from different locations responded inconsistently to preharvest canopy position and light reduction treatments, rinds of sun-exposed outside fruit had significantly higher dry matter content (30 to 35 g/100g FM) than inside fruit (27 to 30 g/100 g FM), indicating a close relationship between sunlight level within the tree canopy and the levels of accumulated structural carbohydrates. The strong negative correlation between RBD and rind dry matter shown in the PCA plots in Chapters 7 and 9 confirmed that rind dry matter content could be used as a potential biochemical indicator of fruit susceptibility to the disorder. The results obtained using Vis/NIRS to predict rind dry matter was another positive breakthrough towards on-line predicting RBD. Therefore, as a possible benchmark for fruit harvested in the studied orchards, it is recommended that for fruit destined for export market or long term storage, rind dry matter should not be below 30 g/100 g FW at harvest. However, detailed studies to quantify change in dry matter content as fruit develops would still be required to determine the optimal range of dry matter to minimise or eliminate incidence of RBD.
The shortcomings of the study were that physico-chemical properties of fruit were only determined at the beginning of storage and in the final evaluation date (after 8 weeks) and not throughout the storage period along with the recording of RBD incidence. Taking this limitation and inconsistent response of rind sugars of fruit from different orchards to preharvest canopy and light reduction treatments into account, a more detailed investigation was conducted. The assessment of such biochemical markers and correlation constituted the principal framework of this thesis which was projected towards understanding the mechanism of RBD, which in turn could lead to a pre-symptomatic detection and/or prediction of the disorder. In this part of the study, the focus was on several rind quality parameters and composition of sugars, organic acids, and phenolic compounds in rinds of harvested inside and outside canopy, and also in bagged fruits. Another objective of this comprehensive study was to investigate physiological changes associated with senescence during postharvest storage.

Analysis of sugar concentration patterns in the rind of mandarin fruit during postharvest storage showed that sucrose levels declined which may be due to its hydrolysis to monosaccharides, glucose and fructose. Rind fructose concentration gradually increased during storage, whereas glucose declined, indicating the possibility of its use as a substrate during senescence-associated respiration. Osmotic adjustment resulting from a decline in sucrose (a solute with the lowest contribution to osmotic potential) and accumulation of fructose may have contributed to RBD development. The proposed hypothesis is that during postharvest storage, higher osmotic potential due to increased fructose resulted in cellular water loss, leading to progressive shrinkage of the vacuole, which in turn induced cell and oil gland collapse, allowing RBD to develop. Similar to previous results shaded inside fruit had higher mass loss during storage and this corresponded with higher levels of RBD. In accordance with literature (Alférez et al., 2003, 2005, 2010; Alférez and Burns, 2004; Alquezar et al., 2010), changes in the water status of the rind was one of the primary postharvest factors contributing to the susceptibility of 'Nules Clementine' mandarin fruit to RBD. The low correlation coefficient (0.22) between the RBD and mass loss observed in this study, suggested that development of the disorder might be attributed to other factors, in addition to weight loss during postharvest storage. Due to the complexity of biological factors involved in the development of RBD, we have not been able to establish the upper limit or lower limit in which the disorder occurs.

The findings discussed above, support the hypothesis that reducing PAR levels around an individual fruit reduced fruit photosynthesis rate, which in turn reduced accumulation of solutes, osmotic potential, decreased rind condition and ultimately, increased fruit susceptibility to RBD. From a horticultural perspective, the incidence of this disorder can therefore be controlled by maintaining sufficient light penetration within the canopy by pruning, maintaining constant and high humidity condition in postharvest environment, and therefore reducing the rate of fruit senescence and water loss.

Identification of potential pre-symptomatic biochemical markers for RBD

Detailed review of the literature showed that existing knowledge of chemical changes in the rind of citrus fruit that could be used to predict fruit susceptibility to RBD is limited, with most research focusing on content and concentration of carbohydrates. It was therefore crucial to explore other potential pre-symptomatic biochemical indicators or markers descriptive of rind condition and fruit susceptibility to RBD, which could be

detected by Vis/NIR spectroscopy. In the quest to identify further biochemical indicators correlated to RBD, non-volatile organic acids and flavanone glycosides were selected as possible candidates because of their high concentration in rinds of citrus fruit. In a study like this one, it is customary to first address major compounds known to have a marked response to stress. From these experiments, a total of seven phenolic acids, including three hydroxybenzoic acids (ρ-hydroxybenzoic and vanillic), and five hydroxycinnamic acids (chlorogenic, caffeic, ρ-coumaric, ferrulic, and sinapic) as well as three flavanones (narirutin, hesperidin and didymin) were identified and quantified. The method separated 10 phenolic compounds faster (52 minutes) than 120 minutes as reported by Li et al. (2006), Kelebek (2010) and Kelebek and Selli (2011).

At harvest, the rind of fruit harvested from outside the canopy had significantly lower hesperidin concentration compared to shaded fruit. Narirutin concentration in the rind of inside bagged fruit was 1.7-fold higher than outside fruit indicating the positive effect of reduced light availability on the concentration of this polyphenolic compound. Reasons for the observed responses of hesperidin and narirutin are unclear, since preharvest canopy position effects on postharvest flavanone glycosides' profile of stored citrus fruits, remain largely unknown. Manthey (2004) and Xu et al. (2008a, b) reported that phenolic compounds contribute a major proportion to the total antioxidant capacity of the citrus rind. Therefore, results in the current study strengthen the hypothesis that the elevation of FGs could possibly be involved in the defence mechanism of the fruit due to stressful postharvest storage conditions. It can therefore be speculated that elevation of these phenolic compounds (flavanone glycosides) could possibly be involved in the defence mechanism of the fruit, due to stressful postharvest storage conditions.

Sun-exposed outside fruit had higher concentrations of ascorbic acid compared to shaded fruit and this observation was consistent with previous results. Ascorbic acid accounts for a great proportion of the antioxidant capacity of citrus fruit (Sdiri et al., 2012). The high concentration of ascorbic acid observed in sun-exposed fruit from the outside position of the canopy, could also explain the increased tolerance of these fruit to RBD. These observations were consistent, reporting results of fruit from Stellenbosch and Citrusdal on experiments conducted during 2011 and 2012, respectively. These findings suggest the possibility that ascorbic acid concentration in the rind could be used as a potential biochemical marker of fruit position within the canopy and susceptibility to RDB.

Application of optical coherence tomography (OCT) to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins

In order to gain a better insight of the mechanism of RBD, the feasibility of OCT, as a potential non-destructive technique to visualise microstructural changes associated with the development of the disorder, was investigated. Immediate and non-destructive acquisition of images showing histological and microstructural features of intact rind tissues was demonstrated. The study showed that OCT is capable of acquiring immediate, non-destructive and contactless cross-sectional images rind structures in real-time mode by measuring their optical reflections. Comparing OCT images with conventional microscopy images would have allowed better assessment of the applicability and effectiveness of the new OCT technique. Therefore, OCT images obtained in this study were compared with light and electron microscopy images from the literature.

OCT images have lower resolution than conventional microscopy, but they have very high contrast for an image taken *in situ*. The fundamental limitation of the OCT in the current study was imposed by the source bandwidth, which was about 7 µm. The dense tissues of intact flavedo and albedo are strongly scattering, resulting in the attenuation of OCT signal, which resulted in low image resolution at greater depths than 1.1 mm. Therefore, although we successfully demonstrated the potential of OCT in imaging rind microstructural features associated with RBD, the sensitivity of the technique still needs to be enhanced to increase signal-to-noise ratio, hence improving the image resolution. In future studies, this could be achieved by using a higher (stronger) wavelength to reduce scattering, and/or a broader spectrum. Essentially, the use of different optical parameters and improved image processing techniques may be crucial to improve the performance of this system.

The OCT images showed that the oil glands stayed intact in unaffected fruit and gradually collapsed on RBD affected fruit. At advanced stages of the disorder, the collapsed oil glands became increasingly deformed and flattened. Other previous studies reported that oil glands are not involved in the development of non-chilling rind disorders on certain mandarin cultivars such as 'Fortune' (Almela et al., 1992), 'Encore' (Medeira et al., 1999; Vitor et al., 2000) and 'Clementine' (Assimakopoulou et al., 2009). Therefore, the disruption of the oil gland during the development of RBD in 'Nules Clementine' mandarins offers some new biological information about the mechanism of the disorder. It was therefore hypothesised that, in 'Nules Clementine' mandarins, oil glands are the primary sites of damage in RBD-affected fruit and that rupture of these oil

bodies, in turn, release phytotoxic oil into the surrounding cells, causing the collapse of the oil gland and damage to adjacent cellular structures.

Until the present study, there has been lack of quantitative information on oil gland volume of fruit with different degrees of non-chilling physiological disorders. The study therefore successfully demonstrated the feasibility of OCT to visualise dimensional changes of rind microstructures and to quantify the degree of collapse of the oil glands with increased level of the disorder. The merits of this work are that it opens the future possibility for monitoring by spotting the oil gland that is in the process of collapsing (they usually become darker) and then follow it over a period until the final symptoms of RBD are clear. This information could point researchers in this field a step further towards understanding the actual mechanism of RBD and not just the symptoms. Understanding oil gland development in citrus fruit might assist in identifying some features of the oil gland and perhaps rind tissue that could provide information about the growing conditions to which the fruit was exposed. The next logical step in future studies would be targeting specific spots on fruit with the potential of developing the disorder and monitor these over time to gain information regarding the time when cellular changes take place. Although OCT has been used extensively in the medical field for visualisation of internal structures of humans and animals (Kamensky et al. 1999; Gladkova et al. 2000), the application as an imaging system in plant science or plant materials is currently in its infancy (Meginsky et al., 2010; Verboven et al., 2013) and therefore only available to researchers for experimental purposes.

Conclusions

This study makes a significant contribution to the potential prediction of citrus external and internal quality. It showed that FT-NIR fitted with an emission head can be used as a tool to rapidly assess rind colour, fruit mass, TSS, and vitamin C levels in 'Valencia' oranges. Results from this study also improved understanding of the mechanism of RBD. The study further demonstrated the importance of dry matter content, ascorbic acid and flavanone glycosides content on 'Nules Clementine' mandarin rind and how these compounds vary across canopy position and bagging treatments. These findings showed how these quality parameters and compounds could be potentially employed as biomarkers in predicting rind quality and fruit susceptibility to RBD. Lastly, this study has successfully demonstrated the feasibility of OCT as a novel non-invasive tool to visualise dimensional changes of rind microstructures and to quantify the degree of collapse of the oil glands with increased level of RBD.

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5.3 PROGRAMME: FRUIT PRODUCTION AND QUALITY

Programme coordinator (Acting): Tim G Grout (CRI)

5.3.1 Programme summary

Demands for water in South Africa are increasing, from informal shack dwellers to farmers in remote rural areas. It is therefore of huge significance that we secured funding from the Water Research Commission to conduct research on quantifying citrus water use and water stress before quotas for growing citrus are changed or enforced. So far this research has focused on the best modern techniques to use in measuring sap flow (5.3.2). Several years ago, requests were made for research on humic acid and it is now clear that humates and fulvate, when applied with N, P or K, can increase the microbial activity in some soils and reduce leaching of N. A long-term trial is being conducted to determine whether humates and fulvate can also reduce fertiliser applications in citrus (5.3.3). Research to determine the best formulations for the uptake of micronutrients applied as foliar sprays has been completed and sulphates were found to be the best for copper, manganese and iron. It was also found that leaf cuticle thickness varied with different citrus types (5.3.4). Research on possible benefits of silicon was also completed and frequent applications to glasshouse trees were shown to reduce infection by *Alternaria alternata.* However, an attempt to confirm a similar suppression of citrus black spot in the field after monthly applications of silicon failed due to the low levels of citrus black spot present in the orchard (5.3.5).

Programopsomming

Die behoefte aan water in Suid-Afrika neem toe, van informele gemeenskappe tot produsente in die afgeleë, landelike gebiede. Daarom is dit van groot belang dat ons fondse van die Waternavorsingskommissie verseker het om navorsing te doen oor kwantifisering van die watergebruik van sitrus en waterstres, alvorens kwotas vir groeiende sitrus verander of afgedwing word. Tot dusver het die navorsing daarop gefokus om die beste moderne tegnieke te gebruik om sapvloei te meet (5.3.2). ʼn Paar jaar gelede is versoeke gerig om navorsing oor humussuur te doen en dit is nou duidelik dat “humates” en “fulvate”, wanneer toegedien word saam met N, P of K, die mikrobiese aktiwiteit in sommige gronde kan verhoog en die loging van N kan verminder. ʼn Langtermyn eksperiment word gedoen om te bepaal of “humates” en “fulvate” ook die toedienings van bemesting in sitrus kan verminder (5.3.3). Navorsing om die beste formulasies vir die opname van mikro-voedingstowwe wat as blaarbespuitings toegedien word te bepaal is afgehandel, en sulfate is gevind om die beste vir koper, mangaan en yster te wees. Dit is ook gevind dat die dikte van die blaar se kutikula tussen verskillende sitrustipes varieër (5.3.4). Navorsing oor die moontlike voordele van silikon is ook afgehandel en gereelde toedienings aan bome in glashuise het getoon om die infeksie deur *Alternaria alternata* te verminder. ʼn Poging om soortgelyke onderdrukking van sitrus swartvlek in die veld te bevestig, na maandelikse toedienings van silikon was egter nie suksesvol nie omdat die vlakke van sitrus swartvlek in die boord te laag was (5.3.5).

5.3.2 PROGRESS REPORT: A novel approach to water and nutrient management in citrus

Project 986 (August 2010–March 2017) by J.T. Vahrmeijer (CRI), N.J. Taylor, M. Sam & B. Teklemicael (UP)

Summary

External funding for research on citrus water was secured from the Water Research Commission through a project (K5/2275//4) entitled “Quantifying Citrus Water Use and Water Stress at Tree and Orchard Scale”, which started in April 2013. The aim of this study is to: analyse the water use, yield, fruit size and quality of selected Valencia, navel orange, grapefruit and/or soft citrus cultivars for different canopy architectures in summer and winter rainfall regions; including a detailed analysis of water stress in relation to yield and quality for a selected cultivar at a single location. Three main objectives were identified and are: i) to validate citrus water use by comparing different sap flow techniques using an appropriate technique such as lysimetry, cut stem and/or eddy covariance, ii) to measure and model citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size and iii) to determine the influence of water stress on fruit set, fruit yield, and pre- and post-harvest fruit quality for a selected cultivar and single location.

Water loss (transpiration) from potted citrus trees, measured with different sap flow techniques, was compared with water loss measured with a gravimetric method (lysimeter). Initial results indicate that the compensation heat pulse method (CHPM) correlates the best with results from the lysimeter. However, this research is still in progress. Water use and water use efficiency measurements in different aged orchards began near Citrusdal in the winter rainfall region. The climate in this area is influenced by the local steppe and is a BSh according to Köppen and Geiger classification. Installation of sap flow equipment took place

from 29 July until 2 August 2013 in four orchards on the Patrysberg farm (32° 27' 15'' S 18° 58' 03'' E), which consisted of five and 13-year old 'Midknight' Valencia (Troyer/Carizzo rootstocks) and 12 and 23-year old 'Bahianinha' Navel (Troyer/Carizzo rootstocks) orchards. Data collection is still in progress that include stomatal conductance, leaf and stem water potential, evaporation, soil water content and Eddy covariance measurements.

Opsomming

Buite fondse vir navorsing in sitrus watergebruik, is van die Waternavorsingskommissie verkry. Die projek (K5/2275//4) getiteld "Quantifying Citrus Water Use and Water Stress at Tree and Orchard Scale", het in April 2013 begin. Die algemene doelwit van die studie is om water gebruik, opbrengs, vruggrootte en kwaliteit van geselekteerde kultivars van Valencia, nawel lemoene, pomelos en/of sagte sitrus, vir verskillende blaardakgroottes, in somer en winter reënvalgebiede te ontleed. Ingesluit by die studie is 'n detail ontleding van die verhouding tussen waterstress, opbrengs en kwaliteit van 'n geselekteerde kultivar in 'n bepaalde gebied. Drie doelwitte is geïdentifiseer: i) om sitrus watergebruik te valideer deur verskillende sapvloeitegnieke met 'n tegniek soos gravimetrie (lysiemeter), afgesnyde stam en/of eddy kovariansie te vergelyk, ii) om sitruswatergebruik en doeltreffendheid gedurende verskillende seisoenale groeistadiums te meet en te modelleer en iii) om die invloed van waterstress op vrugset, vrugopbrengs en voor- en na-oes vrugkwaliteit vir 'n geselekteerde kultivar in 'n bepaalde gebied, te bepaal.

Verlies in water (transpirasie) van sitrusbome in houers, soos gemeet met verskillende sapvloeitegnieke, is met die gravimetriese verlies in water (lysiemeter) vergelyk. Aanvanklike resultate dui daarop dat die kompensasie hitte pulse metode (CHPM) die beste korrelasies met die gravimetriese metode gee. Hierdie navorsing is egter nog nie voltooi nie. Die studie in die watergebruik en -doeltreffendheid van verskillende ouderdom sitrusboorde in die winterreënvalstreek, naby Citrusdal, het begin. Die klimaat van hierdie area word beïnvloed deur die omliggende berge en is 'n BSh-klimaatstreek volgens die Köppen and Geiger klassifikasie. Sapvloeisisteme is vanaf 29 Julie tot 2 Augustus 2013 in vier boorde op die plaas Patrysberg (32° 27' 15'' S 18° 58' 03'' E) geïnstalleer. Die boorde bestaan uit vyf en 13 jaar oue 'Midknight' Valencia (Troyer/Carizzo onderstamme) en 12 en 23 jaar oue 'Bahianinha' nawels (Troyer/Carizzo onderstamme). Die insameling van data vind nog plaas wat metings van huidmondjiegeleiding, blaar- en stamwaterpotensiaal, evaporasie grondwaterinhoud en eddy kovariansie, insluit.

5.3.3 PROGRESS REPORT: Study on the effect of humic and fulvic acids on fertiliser application in citrus

Project 1028 (April 2011 – March 2016) by J.T.Vahrmeijer (CRI) & A. Gatabazi (UP)

Summary

Results from an experiment to determine the influence of humates and fulvate on the microbial activity of soils, showed that humates and fulvate increase the dehydrogenase (microbial activity) in a sandy clay and sandy clay loam soil. Total bacterial counts in both soils, after two weeks of incubation, increased when humates and a fulvate combined with N, P and K were mixed with the soils. After four weeks the bacterial and fungal counts were the highest in the soils treated with humates and fulvate combined with N, P and K fertilisers compared to the soils containing no humates and fulvate. In an experiment with leaching columns it was found that humates and fulvate mixed with N, P and K fertilisers reduced the leaching of N in both soil types, while inconsistent results were found for K and P. The results from pot trials clearly show that humates and fulvates combined with N, P and K fertilisers increased the pH and EC of the leachate and significantly reduced N and P leaching in both soils, but did not reduce K leaching. In general the N, P and K content of the leaf and bark increased when humates and fulvate combined with N, P and K were added to the soil. However, humate and fulvate combined with N, P and K did not increase the N, P and K content of the roots in the sandy clay soil.

Initial results from a long term field trial, to determine if humates and fulvate can be used to reduce fertiliser application in citrus orchards, showed that irrigation scheduling plays an important role in reducing N leaching at orchard level. When humates and fulvate were mixed with liquid fertilisers, the initial N leaching directly after fertilisation, was not reduced. But results from subsequent irrigation events suggest a decrease in N leaching. This study is available as an M.Sc. thesis entitled "Nitrogen, phosphorus and potassium availability as influenced by humate and fulvate soil amendment" from the library of the University of Pretoria.

Opsomming

Resultate van ŉ eksperiment om die invloed van humate en fulvate op die mikrobiologiese aktiwiteit van gronde te bepaal, het aangetoon dat dehidrogenase (mikrobiologiese aktiwiteit) in ŉ sandklei- en sandkleileemgrond verhoog. Na twee weke van inkubasie het die totale bakteriese telling in beide gronde, wanneer humate en fulvaat tesame met N-, P- en K-kunsmis met die grond gemeng word, verhoog. Na vier weke was die bakteriese- en swamtellings die hoogste in die gronde wat met humate en fulvaat en N-, P-, K-kunsmis behandel is. In ŉ ander eksperiment met loogbuise, is dit gevind dat N loging in beide gronde verminder indien N-, P- en K-kunsmis met humate en fulvate gemeng word, terwyl wisselende resultate vir K en P gevind is. Die resultate van potproewe het duidelik aangetoon dat humate en fulvaat, tesame met N-, P- en K-kunsmis, die pH en EG van loogwater verhoog asook N en P loging verminder, terwyl K loging nie afgeneem het nie. In die algemeen het die N-, P- en K konsentrasies van die blare en bas toegeneem vir die humate en fulvaat, tesame met N-, P- en K-kunsmis, behandeling. Humate en fulvaat, tesame met N-, P- en K-kunsmis, het egter nie die N, P en K konsentrasie van die plantwortels verhoog nie.

Aanvanklike resultate van ŉ langtermyn veldproef, om te bepaal of humate en fulvate gebruik kan word om kunsmis toediening in sitrusboorde te verminder, dui daarop dat besproeiing ŉ belangrike rol speel in die vermindering van N loging op boordvlak. Humate en fulvate wat met vloeibare kunsmis gemeng is, het geen invloed gehad op die aanvanklike loging van N nie, maar uit die resultate van opvolgende besproeiings wil dit voorkom of die N loging verminder. Hierdie studie is beskikbaar as ŉ M.Sc. tesis getiteld “Nitrogen, phosphorus and potassium availability as influenced by humate and fulvate soil amendment” in die biblioteek van die Universiteit van Pretoria

5.3.4 FINAL REPORT: The evaluation of different formulations of micronutrients on foliar uptake

Project 1037 (April 2012–March 2014) by J.T.Vahrmeijer (CRI) & S.M. Scholly (UP)

Summary

Potted Valencia ‘Midknight’ trees were used to evaluate the absorption of foliar applied micronutrients. All experiments were done in a greenhouse at the University of Pretoria. Two techniques of leaf analysis were evaluated by spraying selected leaves with different formulations of manganese (amino acid-chelate, EDTA-chelate, and $MnSO_4$) and boron (H_3BO_3). Treated leaves, as well as the leaf directly above and below the treated leaf were sampled and prepared for analysis by pressing the leaf-sap from half of the samples. The other half of the leaves were prepared by microwave assisted digestion. The concentration of Mn and B were determined with ICP-OES and the results from the two methods were compared. Microwave assisted digestion proved to be the more efficient and sensitive method. For the main experiment different types of formulations, concentrations and sampling times for foliar applied Mn, Zn, Fe, B and Mo were evaluated. The most effective formulation and concentration, as a multiple of the FSSA (Fertiliser Society of South Africa) recommended concentration, in parenthesis are as follows: Cu: $CuSO_4$ (8x); B: H_3BO_3 (2x); Mo: $Na_2MoO_4.2H_2O$ (8x); Mn: $MnSO_4$ (8x); Zn: Zn-Amino acid (8x); Fe: $FeSO_4$ (4x). It was also found that transport of the applied elements occurred to the leaf directly above and below the treated leaf. The optimal sampling times after application for the different elements were: Cu – 24 h, Mo & B – 48 h, Mn & Zn – 96 h, Fe – 192 h. An electron microscopic study (SEM) was done of the leaf surfaces of different citrus types. ‘Washington’ navels had the thinnest cuticles in young leaves and ‘Midknight’ Valencia had the thickest abaxial cuticle in young and mature leaves. The thickest adaxial cuticle was that of ‘Satsuma’ mandarins in young and mature leaves. This study is available as an MSc thesis entitled “Evaluating the effectiveness of different formulations of micronutrients on foliar uptake by *Citrus sinensis* (L.) Osbeck cv. Valencia” from the library of the University of Pretoria.

Opsomming

Valencia ‘Midknight’ bome in 10 L potte is gebruik om die opname van mikro-elemente te evalueer. Alle eksperimente is in ŉ kweekhuis by die Universiteit van Pretoria uitgevoer. Twee blaarontledingstegnieke is geëvalueer deur sitrusblare met verskillende formulasies van mangaan (aminosuur-chelaat, EDTA-chelaat, en $MnSO_4$) en boor (H_3BO_3) te spuit. Die behandelde blare, sowel as die blare direk bo en onder die behandelde blaar, is geneem vir ontledings. Blaarsap is uit helfte van die blaarmonsters gepers en die ander helfte van die blaarmonsters is met behulp van mikrogolf geassisteerde vertering voorberei. Mn en B konsentrasies is met behulp van ICP-OES bepaal en die resultaat van die twee tegnieke is met mekaar vergelyk. Dit is bevind dat die mikrogolf geassisteerde vertering die meer effektiewe en sensitiewe metode is. Vir die hoofeksperiment is verskillende formulasies en konsentrasies van Mn, Zn, Fe, B en Mo, asook die optimum tydperk na toediening vir monsterneming geëvalueer. Die mees effektiewe formulasie en konsentrasie, as ŉ veelvoud van die aanbevole konsentrasie van die MVSA (Mistofvereneging van Suid Afrika) vir elke element in hakies, is soos volg: Cu: $CuSO_4$ (8x); B: H_3BO_3 (2x); Mo: $Na_2MoO_4.2H_2O$ (8x); Mn:

$MnSO_4$ (8x); Zn: Zn-Amino acid (8x); Fe: $FeSO_4$ (4x). Die optimale tye vir monsterneming na toediening vir die verskillende elemente is: Cu – 24 hr, Mo & B – 48 hr, Mn & Zn – 96 hr, Fe – 192 hr. ŉ Elekronmikroskopie studie (SEM) van die blaaroppervlakke van verskillende sitrustipes is gedoen. Die dunste kutikula van jong blare is waargeneem by 'Washington' nawels. 'Midknight' Valencia het die dikste abaksiale in jong en ouer blare gehad en 'Satsuma' mandaryne die dikste adaksiale kutikula. Hierdie studie is beskikbaar as ŉ MSc tesis getiteld "Evaluating the effectiveness of different formulations of micronutrients on foliar uptake by *Citrus sinensis* (L.) Osbeck cv. Valencia" by die biblioteek van die Universiteit van Pretoria.

Introduction

Research on foliar fertilisation started in the latter part of the 1940s and was mainly focused on micronutrients in high value horticultural crops. Even though macronutrients are needed in higher amounts than micronutrients, both are equally important. The development of high concentration soluble fertilisers and equipment to apply overhead irrigation, herbicides, fungicides and insecticides as sprays boosted this practice immensely (Fageria *et al*. 2009).

Perennial fruit crops such as citrus can benefit greatly from foliar nutrition since the deep roots can deplete the soil in time, and soil amendments are not easily applied without damaging these roots (Abd-Allah, 2006). Foliar nutrition can be very advantageous during certain stages of fruit development where the nutritional status and the quality of the fruit crops are enhanced (Mengel, 2002). According to Rashid (2006), the deficiency of certain micronutrients in soil can be of economic significance in some crops, which can then be alleviated by the foliar application of these elements. In some instances foliar applications can be more cost effective and efficient than soil application, since foliar applications can be made together with herbicides, fungicides and insecticides (Fageria *et al*. 2009; Rashid, 2006). Foliar sprays of micronutrients can also be used to supply micronutrients to different plant parts where elements (Mn and Cu) that are less mobile in the phloem cannot be transported readily in the plant tissue. Once the nutrients are in the cell, they can move between cells via the plasmodesmata which ensure adequate dispersal of the nutrients in that specific tissue. The foliar application of nutrients can also be used as an alternative nutrient supply method to plants growing in soils with low nutrient availability and for plants in peak nutrient demand (Fernandez & Eichert, 2009).

When manganese (Mn) is applied to soils, it can be adsorbed very quickly (seconds to minutes) to negatively charged surfaces in the soil and become unavailable to plants (Silber *et al*. 2009). Ozaki (1955), as cited by Mengel (2002), reported that a sprayed solution of manganese sulphate ($MnSO_4$) is more effective than Mn-chelates to alleviate a Mn-deficiency in beans and peas. Foliar application of zinc (Zn) is generally worth the effort on perennial fruit trees (Rashid, 2006). In a trial with coffee and citrus, zinc sulphate ($ZnSO_4$) was more effective than the chelated Zn in a quarter of the field trials to increase the Zn concentration to adequate levels (Rosolem & Sacramento, 2001, as cited by Mengel, 2002). Foliar application of copper sulphate ($CuSO_4$) has led to leaf scorching on many occasions, and this may be because copper ions (Cu^{2+}) are bounded strongly to the apoplast. This phenomenon can cause Cu^{2+} to be translocated to the fruit of the plant by transpiration (Fox & Guerinot, 1998). Time of application plays an important role in the effectiveness of foliar applied iron (Fe), usually as chelates. Since Fe is not very mobile in the phloem, it is not readily absorbed by older leaves or transported to developing leaves (Kosegarten *et al*. 1999). Fe is especially available when applied as iron sulphate ($FeSO_4$), since the divalent Fe ion (Fe^{2+}) can pass through the plasmalemma readily to be used in other physiological processes. Chelated iron (Fe-EDTA, Fe-EDDHA and Fe-EDDS) applied as a foliar spray may be more available to the plant since chelation keeps it in a soluble form. By using chelates, the translocation of Fe to other plant parts is increased significantly (Ylivainio *et al*. 2004; Cerdán *et al*. 2009). Fe is trivalent when applied as a chelate, and must be reduced to Fe^{2+} before it will enter the cytosol (Kosegarten *et al*. 1998).

When a nutrient (N, P, K, Mg, Mo, Zn and occasionally B) is deficient, it is transported from older to younger leaves (Brown & Shelp, 1997), since the older leaves will absorb nutrients more effectively due to a larger leaf area and because the cuticles of older leaves are generally more damaged and have cracks through which the solutes can move (Mengel, 2002). Some micronutrients are not transported from older foliage, (Bouma, 1997) and are not as mobile in the phloem of the plant, e.g. Mn and Cu (Mengel, 2002).

Objectives

1 Evaluation of different formulations of micronutrients on uptake

Many factors affect the penetration of the foliar applied solution and since the existing knowledge on this is limited, the response from foliar sprays is very inconsistent, and many experiments cannot be successfully reproduced (Fernandez & Eichert, 2009). To evaluate the efficiency of leaf uptake of micronutrients, different formulations of zinc (formulations: $ZnSO_4$, chelate, amino acid), copper (formulations: $CuSO_4$, chelate, amino acid), iron (formulations: $FeSO_4$, chelate, amino acid) and manganese (formulations: $MnSO_4$, chelate, amino

acid) were applied as foliar sprays to potted citrus trees at different concentrations under controlled conditions.

2 Comparative study of leaf anatomy of main citrus groups

A comparison among leaf surfaces of lemons (*Citrus limon*), grapefruit (*Citrus paradisi*), Valencias (*Citrus sinensis*), navel oranges (*Citrus sinensis*) and mandarins (*Citrus reticulata*) and different ages of their leaves is needed to detect possible differences in foliar spray uptake. Scanning electron microscopy was used to determine whether different citrus groups (lemons, grapefruit, Valencias, navel oranges and mandarins) and different ages of those leaves have considerable changes in their leaf surfaces that may influence the uptake of foliar sprays.

Materials and methods

The establishment of the citrus trees for foliar spray experiments

Young Valencia "Midknight" grafted on Carizzo citrange was used in this study. The trees were transplanted into 10 L aerated plastic containers with an appropriate growth medium. These trees were kept in a climatically controlled greenhouse on the Experimental Farm of the University of Pretoria to acclimatise for a few weeks before the start of the experiments.

Evaluation of contact time and the type of formulation (SO_4, chelate and amino acid) on the absorption of Mn, Zn, Cu and Fe

The influence of contact time between the micronutrient solution and citrus leaves on absorption was determined by applying different micronutrient formulations to the leaves and sampling at different time intervals. The micronutrients were dissolved in deionised water and all solutions applied to the leaves contained a 1 ml L^{-1} pH buffer (Allbuff, Tsunami Plant Protection (PTY) Ltd, Heidelberg, RSA) and 0.05 ml L^{-1} surfactant (Breakthru S240, Evonik Goldschmidt, GmbH, Essen, Germany). Copper solutions ($CuSO_4.5H_20$, Cu-EDTA and Cu-amino acid chelate) contained 0.281 g L^{-1} elemental Cu; Fe solutions ($FeSO_4$, Fe-EDTA and Fe-amino acid chelate) contained 0.408 g L^{-1} elemental Fe; Mn solutions ($MnSO_4$ + Na_2MoO_4, Mn-EDTA and Mn-amino acid chelate) contained 0.549 g L^{-1} elemental Mn, and the $MnSO_4$ solution also included 0.05 g L^{-1} Na_2MoO_4 (0.017 g elemental Mo). The Zn solutions ($ZnSO_4$, ZnO, ZN-EDTA and Zn-amino acid chelate) contained 0.001 g L^{-1} elemental Zn, which was much lower than recommended by the FSSA (2003), because the low water solubility (0.0016 g L^{-1}) of ZnO (International Zinc Association, 2011) was brought into consideration, so that all the Zn treatments contained the same amount of Zn in solution. A control treatment was included where distilled water, surfactant and buffer at the aforementioned rates were used.

Treatments were applied in a split-split plot design in a completely randomized block design, and each treatment was replicated four times. Four branches on each tree were marked and a single leaf from each branch was dipped into the solution so that the entire leaf surface was covered with the solution. Measures were taken to ensure that no dripping occurred on the rest of the tree. Treated leaves, the leaf directly above and directly below the treated leaf (from here on referred to as 'above' and 'below') were sampled at 12, 24, 48, 96 and 192 hours after application. Leaves were washed with 10% acetone and rinsed with distilled water, then dried at 65°C for 24 hours. Washing of samples is recommended to ensure decontamination of the applied solutes (Labanauskas, 1968, as cited by Jones, 1991). Samples were then digested by microwave assisted digestion with a 1400 W Multiwave 3000 (Anton Paar GmbH, Graz, Austria). Digested samples were analysed by ICP-OES (inductively coupled plasma) to determine micronutrient concentrations.

An optimal foliar spray concentration for each element (Mn, Cu, Zn, Mo, Fe, and B) and each formulation for that element (salt, amino acid and chelate) was determined by applying five different concentrations: 0.5, 1, 2, 4 and 8 times the recommended concentration from the Fertiliser Society of South Africa (FSSA, 2003). However, for B and Mo no chelate or amino acid formulation could be found and therefore B was only applied as H_3BO_3 and Mo as $Na_2MoO_4.2H_2O$. In Table 5.3.4.1 a summary of the concentrations of each element for the different formulations applied is presented. Treated leaves and the leaves directly above and below the treated leaf were sampled at 24 h for the Cu treatments, at 48 h for Mo and B, at 96 h, for the Zn and Mn treatments and at 192 h for the Fe treatments.

Table 5.3.4.1. Type of formulation and the concentration of each element applied.

Element	Type of formulations	Element concentration g L^{-1}				
		*0.5x	*1x	*2x	*4x	*8x
Zn	$ZnSO_4$ / ZnO / chelate / amino acid	0.449	0.897	1.794	3.588	7.176
Cu	$CuSO_4$ / chelate / amino acid	0.141	0.281	0.563	1.126	2.251
Fe	$FeSO_4$ / chelate / amino acid	0.204	0.408	0.816	1.632	3.264
Mn	$MnSO_4$ / chelate / amino acid	0.275	0.549	1.098	2.196	4.392
B	H_3BO_3	0.175	0.350	0.699	1.399	2.798
Mo	$Na_2MoO_4.2H_2O$	0.025	0.050	0.100	0.200	0.400

*Multiple of the recommended concentration from the Fertiliser Society of South Africa (FSSA, 2003)

Method validation

Manganese and B concentrations from leaf-sap were compared with results from microwave-assisted digested leaves. Boron (H_3BO_3) at 0.350 g L^{-1} and different formulations of Mn (amino acid-chelate, EDTA-chelate, and $MnSO_4$) at a rate of 0.549 g L^{-1}, irrespective of formulation, were sprayed on leaves of young potted Valencia 'Midknight' trees. A buffer solution and wetting agent were included in the solutions. Treated leaves, as well as the leaf directly above and below the treated leaf were sampled after 48 h, washed with 10% acetone and then rinsed with deionized water. The leaf-sap from half of the samples was immediately extracted by press and membrane filtered. The other half were oven dried at 65°C for 18 hours and prepared with microwave assisted digestion. The concentrations of Mn and B for the two different methods were determined with ICP-OES and compared with each other.

Comparison of the leaf surfaces of different age and types of citrus

The leaf surfaces of lemons (*Citrus limon*), grapefruit (*Citrus paradisi*), Valencias (*Citrus sinensis*), navel oranges (*Citrus sinensis*) and mandarins (*Citrus reticulata*) and different leaf ages were compared with each other to determine if differences exist that may influence the uptake of foliar sprays.

Samples from lemons, grapefruit, Valencias, navel oranges and mandarins were collected from commercial orchards in the Groblersdal area. Young and mature leaves were sampled and three to four leaf pieces (1 mm x 4 mm) were dissected from the middle region of the lamina and fixed for at least four hours at 5°C in a 100 mM phosphate buffer, containing 3% (v/v) glutaraldehyde, at pH 7. Samples were then post-fixed for four hours in 2% (w/v) osmium tetroxide in a phosphate buffer before dehydration in an ethanol series and held in 95% ethanol for two hours. Tissue segments were then critical-point dried, gold-coated and photographed with a scanning electron microscope (SEM) on Polaroid film using an accelerating voltage of 15 kV (Bondada *et al*., 2006). Images were taken of flat leaf surfaces and broken leaf edges.

Results and discussion

Method validation

Microwave assisted digestion proved to be the more efficient and sensitive method for micronutrient determination with an ICP-OES.

Micronutrient applications to citrus trees

In Table 5.3.4.2 results from the foliar study are shown, depicting the most effective formulation and the optimal sampling time for each element.

Table 5.3.4.2. Optimal sampling time after application for each element, and formulations with the best elemental foliar uptake by the leaf in descending order

	Cu	B	Mo	Mn	Zn	Fe
Optimal Sampling Time	24h	48 h	48 h	96 h	96 h	192 h
Highest absorption	$CuSO_4$	H_3BO_3	Control	$MnSO_4$	Zn Amino acid	$FeSO_4$
↓	Cu Amino acid	Control	$Na_2MoO_4.2H_2O$	Mn-EDTA	$ZnSO_4$	Fe Amino acid
	Cu-EDTA			Control	Zn-EDTA	Fe-EDTA
	Control			Mn Amino acid	ZnO	Control
Lowest absorption					Control	

Mn

The $MnSO_4$ treatment (Table 5.3.4.2) had the highest Mn concentration, in the treated leaves, for all Mn treatments. This is in accordance with findings from Ozaki (1955), as cited by Mengel (2002), that $MnSO_4$ will reduce deficiencies more effectively than chelated Mn. The highest concentrations for $MnSO_4$ were detected at 8x the recommended concentration of the Fertiliser Society of South Africa (FSSA, 2003) and the optimal sampling time was found to be 24 h after treatment application.

Zn

Zn concentrations were highest for the Zn amino acid treatments (Table 5.3.4.2) at all sampling times. This is in accordance with Srivastava & Singh (2005) who concluded that chelated Zn penetrated more efficiently through the leaves than inorganic salts, but in contrast with Ceatano (1982) and Boaretto *et al.* (2002) who concluded that absorption and translocation of a commercial Zn chelated product were the same as for $ZnSO_4$. Highest Zn concentrations were detected at 8x the recommended concentration of the FSSA for ZnO which was in excess (>200 mg kg^{-1}) according to FSSA (2003). Optimal sampling time was at 96 h after treatments were applied.

Cu

The highest Cu concentrations were measured in the leaves treated with $CuSO_4$ (Table 5.3.4.2). Fox & Guerinot (1998) reported that $CuSO_4$ caused leaf scorching on many occasions, but no leaf scorching was observed in this trial. Treated leaves had the highest Cu concentrations at 8X the recommended application rate of $CuSO_4$ (FSSA, 2003). The highest Cu concentrations were detected after 12 h. In a study done by Chamel (1988), uptake of Cu by the plant leaves increased as the Cu concentration increased. He found that localization of Cu was more than 90% at the application site after 24 h of application.

B

The highest B concentrations (Table 5.3.4.2) were measured in the treated leaves 48 h after the application of H_3BO_3 with the lowest B concentration at 0.5x and the highest at 2x. Chamel (1988) reported that 78-93% of B was retained in the treated leaf 24 h after the foliar application of boric acid. He also found that B was homogenously spread throughout the treated leaf and did in fact enter the plant tissues.

Fe

Fe treatments showed a definite increase in Fe concentration over time. On average $FeSO_4$ (Table 5.3.4.2) showed the best overall Fe concentrations. Leaves treated with different Fe formulations showed increased uptake with increase in concentration. Highest Fe concentrations were detected at 4x for the $FeSO_4$.

Mo

In the experiment to determine optimum sampling time the Mo concentrations (Table 5.3.4.2) for treated leaves was the highest after 96 h. However, the leaves of the control had higher concentrations of Mo, which indicated that the plants may have adequate Mo (0.05-3.0 mg.kg^{-1} Mo) (FSSA, 2003) and therefore the Mo concentration of the treated leaves did not increased. For this experiment the Na_2MoO_4 was applied with $MnSO_4$ that also may have influenced Mo uptake due to the antagonistic interaction between Mo and SO_4^{2-} (Kannan and Ramani, 1978, as cited by Kaiser *et al*., 2005).

In the second experiment where the Mo concentration of the applied solution was increased, the Mo content of the treated leaves was the highest for the 8x treatment.

Citrus leaf surface comparison

The measured thickness in leaf cuticle differed among the abaxial and adaxial surfaces, as well as between new and old leaves for the different citrus types. In young leaves the abaxial cuticle thickness ranged from 430 - 2290 nm and the adaxial cuticle thickness ranged from 460 - 2170 nm. In mature leaves the abaxial cuticle thickness ranged from 1300 - 2250 nm and the adaxial cuticle thickness ranged from 1630 - 2490 nm. Differences were observed among citrus types so that Washington navels had the thinnest cuticle in the young leaves. Midknight Valencia had the thickest abaxial cuticle in young and mature leaves and the Satsuma mandarins had the thickest adaxial cuticle in young and mature leaves.

Conclusion

In the preliminary study the microwave-assisted digestion method proved to be more sensitive and efficient and therefore sample preparation for the rest of the study was done through microwave- assisted digestion. Leaf concentrations indicate that there are differences in the absorption of the different formulations of the elements. Optimal sampling times after application for each element were: Cu – 24 h, Mo & B – 48 h, Mn & Zn – 96 h, Fe – 192 h and the most effective formulation and concentration, as a multiple of the FSSA recommended concentration, are: Cu: $CuSO_4$(8x); B: H_3BO_3(2x); Mo: $Na_2MoO_4.2H_2O$(8x); Mn: $MnSO_4$(8x); Zn: Zn-Amino acid(8x); Fe: $FeSO_4$(4x).

Results from the electron microscopic study showed that the thinnest cuticles in young leaves were those from Washington navels. Midknight Valencia had the thickest abaxial cuticle in the young and mature leaves and the thickest adaxial cuticle was found for Satsuma mandarins in young and mature leaves.

Future research

Further studies may include different crops with different types of cuticles as well as other nutrient formulations. Radioactive labels may also be used to track nutrients in the plant. Leaf age can be included in parameters for future foliar application studies.

Technology transfer

1. Foliar uptake of Mn and Zn formulations at various time intervals by Valencia trees
   SM Scholly, PJ Robbertse, JT Vahrmeijer
   Combined Congress 20-23 January 2014, Grahamstown, South Africa
2. Comparison of leaf cuticle thickness among some citrus cultivars
   SM Scholly, PJ Robbertse, JT Vahrmeijer
   Post Graduate Symposium, University of Pretoria, South Africa
3. Comparison of leaf cuticle thickness among some citrus cultivars
   SM Scholly, PJ Robbertse, CF van der Merwe and JT Vahrmeijer
   Combined Congress 21-24 January 2013, Durban, South Africa
4. The evaluation of different formulations of micronutrients on foliar uptake – Progress presentation 1
   SM Scholly, PJ Robbertse, JT Vahrmeijer
   Zinchem, Benoni, South Africa
5. The evaluation of different formulations of micronutrients on foliar uptake – Progress presentation 2
   SM Scholly, PJ Robbertse, JT Vahrmeijer
   Zinchem, Benoni, South Africa
6. The evaluation of different formulations of micronutrients on foliar uptake – Progress presentation 3
   SM Scholly, PJ Robbertse, JT Vahrmeijer
   Zinchem, Benoni, South Africa
7. Evaluating the effectiveness of different formulations of micronutrients on foliar uptake by Citrus sinensis (L.) Osbeck cv. Valencia – M.Sc. thesis
   Available from the library of the University of Pretoria

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5.3.5 FINAL REPORT: The role of silicon in the control of brown spot (*Alternaria alternata*) under laboratory conditions and black spot (*Phyllosticta citricarpa*) in an orchard

Project 1057 (2013-2014) by N.M. Asanzi (UP) & J.T. Vahrmeijer (CRI)

Summary

The use of silicon (Si) in several economic crops to reduce fungal infection motivated the investigation of Si application in citrus and its role in the alleviation of brown spot (*Alternaria alternata*), under controlled conditions, in mandarins (*Citrus reticulata*) and black spot (*Phyllosticta citricarpa*) under field conditions in lemon trees (*Citrus limon*). The *Alternaria alternata* trial was conducted in a greenhouse at the University of Pretoria and consisted of two mandarin cultivars ('Fairchild' and 'Nova') and three Si (0, 500 and 1000 $mg.L^{-1}$) treatments with five replicates. Leaves from the two mandarin cultivars and different treatments were selected and inoculated with isolates of the brown spot pathogen. After inoculation, the leaves were placed on a moist filter paper in a Petri dish to provide a highly humid environment. Lesions from *Alternaria alternata* occurred three days after inoculation with the highest disease incidence registered five days after inoculation. Disease severity in both varieties was significantly reduced for the 1000 $mg.L^{-1}$ Si treatment. The field trial was conducted in a commercial orchard of 12 year-old 'Eureka' lemon trees (*Citrus limon*) grafted on rough lemon rootstocks in the Brits region. Potassium silicate (K_2SiO_3) had been applied as a drench (2000 $mg.L^{-1}$ Si at 5 $L.tree^{-1}$) once a month since February 2013 to a section of the orchard to ensure

a sufficient increase in the Si-content of the trees. As a result of the low incidence of black spot in the orchard, the effect of Si on black spot in lemons could not be assessed.

Opsomming

Die gebruik van silikon (Si) in verskeie ekonomiese gewasse om swaminfeksies te verminder het die ondersoek na die toediening van Si in sitrus gemotiveer. Die rol van Si om bruinvlek (*Alternaria alternata*) onder gekontroleerde toestande in mandarynbome (*Citrus reticulata*) en swartvlek (*Phyllosticta citricarpa*) onder veldtoestande in suurlemoenbome (*Citrus limon*) te onderdruk, is geëvalueer. Die *Alternaria alternata* studie is in ʼn glashuis by die Universiteit van Pretoria uitgevoer en het bestaan uit twee mandaryn kultivars ‘(Fairchild’ en ʼnova’) en drie Si (0, 500 and 1000 mg L^{-1}) behandelings met vyf herhalings. Blare van die twee mandaryn kultivars en van die verskillende behandelings is geneem en geïnokuleer met *Alternaria alternata* spore. Die geïnokuleerde blare is op nat filtreerpapier in ʼn Petribakkie geplaas. Letsels van *Alternaria alternata* is na drie dae van inokulasie waargeneem en na vyf dae het die meeste letsels voorgekom. Die voorkoms van *Alternaria alternata* letsels het betekenisvol vir die 1000 mg L^{-1} Si behandeling, verminder. Die veldproewe is in ʼn kommersiële boord van 12 jaar oue ‘Eureka’ suurlemoenbome (*Citrus limon*), wat ingeënt is op growwe skil, in die Britsomgewing uitgevoer. Kaliumsilikaat (K_2SiO_3) is as ʼn wortelbehandeling (2000 mg L^{-1} Si teen 5 L $boom^{-1}$) een keer per maand, vanaf Februarie 2013, in ʼn gedeelte van die boord toegedien om die Si-inhoud van die bome te verhoog. Weens die lae voorkoms van swartvlek in die boord kon die effek van Si op die afname van swartvlek nie geëvalueer word nie.

Introduction

Plant surfaces, below and above the ground, serve as a protective barrier between the plant’s interior and the environment. The cell wall, with its cuticle, constitutes the first line of defence against attacks from insects, fungi and bacteria. Bacteria and fungi break through the defence by chemical means, whereas pests, including phytophagous insects, mainly utilize mechanical means to penetrate plants (Epstein, 1999).

In an experiment on rice (*Magnaporthe grisea)* disease expression was reduced by the increase of silicon (Si) in the plants, with a substantial decrease in lesion length, rate of lesion expansion, and infection area (Seebold et al., 2001). Several studies in monocots (rice and wheat) and dicots (cucumber) supplied with Si, have shown that in response to fungal infection antifungal compounds such as phenolics and phytoalexins, are produced by the plants to reduce fungal infection (Seebold, 1998; Fawe et al., 1998). Silicon is also able to activate defence mechanisms. For example, in the roots of cucumber plants that were infected and colonized by *Pythium spp*, the fungi growth was inhibited by Si that enhanced the activity of chitinases, peroxidases and polyphenoloxydases (Chérif et al., 1994). These biochemical responses are induced by soluble Si, suggesting that soluble Si may play an active role in improving host resistance to diseases by stimulating defence reaction mechanisms (Fawe et al., 1999). Fawe et al., (1999) reported that the application of Si in cucumber stimulates auto defence mechanisms as a result of a gene expression that enhances the protein responsible for the transformation of soluble silica (SiO_2) to insoluble silica at the site of the attempted penetration of fungi into epidermal cells. Silicon is known to be involved in cell-wall reinforcement and also enhances plant resistance to insect pests such as stem borer and plant hopper by providing a mechanical barrier against probing and chewing insects (Ma & Takahashi, 2002).

Alternaria alternata is the causal agent of brown spot, a fungal disease common in most humid and semi-arid growing citrus regions (Timmer, 1998). The disease may affect tree growth, causes considerable crop loss and renders fruit unacceptable to consumers due to blemishes. The main control of the disease is the spraying of high volumes of fungicide to ensure good leaf coverage. However, high volume sprays can result in excessive runoff from foliage and fruits on the outer canopy, which is a waste of money and may pollute the environment (Timmer et al., 2000). Another challenge faced with *Alternaria alternata* fungicide spray programmes is the difficulty to predict when the disease will occur, due to the short incubation period that varies from 24 h to 120 h (Fourie et al., 2009). This limits the effectiveness of spraying a fungicide for prevention and periodic application can only reduce the infection rate for a short period of time (Timmer et al., 2000). The evidence of the role of Si in reducing fungal infection rate suggests that it could be a possible avenue to mitigate *Alternaria alternata* (Fawe et al., 1999; Ma &Takahashi, 2002).

Objectives

- To determine the influence of Si application on *Alternaria alternata*
- To determine under laboratory conditions if silicon (Si) reduces *Alternaria alternata* infection of young leaves in two mandarin (*Citrus reticulata*) cultivars (‘Fairchild’ and ‘Nova’).
- To determine the influence of Si application on *Phyllosticta citricarpa*

- To assess under field conditions the impact of root and foliar applied Si to 'Eureka' lemon (*Citrus limon*) trees on *Phyllosticta citricarpa* infection.

Material and methods

Increase of Si content of two mandarin cultivars

The experiment was conducted in a glasshouse at the experimental farm of the University of Pretoria (S25°44' E28°15') from August 2013 – November 2013. The Si content of small potted mandarin (*Citrus reticulata*) cv. 'Fairchild' and 'Nova' trees was increased by applying a Si-solution (K_2SiO_3) to the roots once a month for the duration of the study. The experiment consisted of four Si treatments of 0, 500, 1000 and 2000 $mg.L^{-1}$. The small trees were subjected to a complete randomized design with five replications. At the end of the trial root and shoot tissues were oven dried at 60°C for 48 h and dry mass determined followed by leaf analysis with ICP-OES to determine Si concentration. Daily climatic variables (temperature and relative humidity) were measured with data loggers.

Alternaria alternata pathogenicity test

Pathogenicity was tested by inoculating the leaves of two mandarin cultivars 'Fairchild' and 'Nova' with *Alternaria alternata* according to the method described by Dhingra and Sinclair (1995). Spores of *Alternaria alternata* were cultured on agar in a Petri dish. After seven days of incubation the fungi were carefully removed from the agar surface and filtered through cheesecloth to separate the mycelium and spores. The concentration of spores was determined using a haemocytometer. Uniform quantities of the fungi (5×10^5 $spores.mL^{-1}$) were placed on the adaxial leaf surface of the two mandarin cultivars according to the method of Kohmoto *et al*. (1991). The experiment was conducted at room temperature and fungal growth (disease severity) was scored at 48 h, 72 h, 96 h, 120 h, 144 h and 168 h after inoculation based on the leaf area covered with black necrotic spots (Rezende *et a*l., 2009).

Field trial

The experiment on the role of Si on *Phyllosticta citricarpa* was conducted at a commercial farm in Brits on 12 year-old trees of cultivar 'Eureka' (*Citrus limon*) grafted onto a rough lemon rootstock with plant spacing of 2.4 x 5 m. The trial consisted of two parts. Potassium silicate (K_2SiO_3) was applied as a drench (1000 mg L^{-1} Si at 5 L $tree^{-1}$) once a month from February 2013 to a section of the orchard (10 trees) to ensure a sufficient increase in the Si content of the trees. The foliar application of Si was conducted by spraying the Si solution to the leaves, until run-off, once a month. The Si concentration of the leaves was monitored regularly by sampling young and old leaves from the sunny and shaded part of three trees per treatment. Silicon concentration was determined with induced couple plasma optical emission spectrometry (ICP-OES) using a microwave-assisted acid-base digestion method adapted from Haysom & Ostatek-Boczynski (2006). According to the farm manager, *Phyllosticta citricarpa* infection normally occurs between October and December in this orchard, hence an assessment of disease infestation was done during this time.

Results and discussion

Si uptake in mandarins

The Si concentration of the leaves of the two mandarin cultivars increased significantly ($P \leq 0.01$) with increase in Si application rate and with time (Figure 5.3.5.1 and 5.3.5.2). The highest Si concentration in the leaves was recorded for the 1000 $mg.L^{-1}$ root application. These results are in accordance with previous reports that Si concentration in the leaves increased with Si application rates (Epstein, 1999; Mitani & Ma, 2005) and that Si uptake decreases at very high concentrations (2000 $mg.L^{-1}$) (personal communication with Prof M.D. Laing, UKZN).

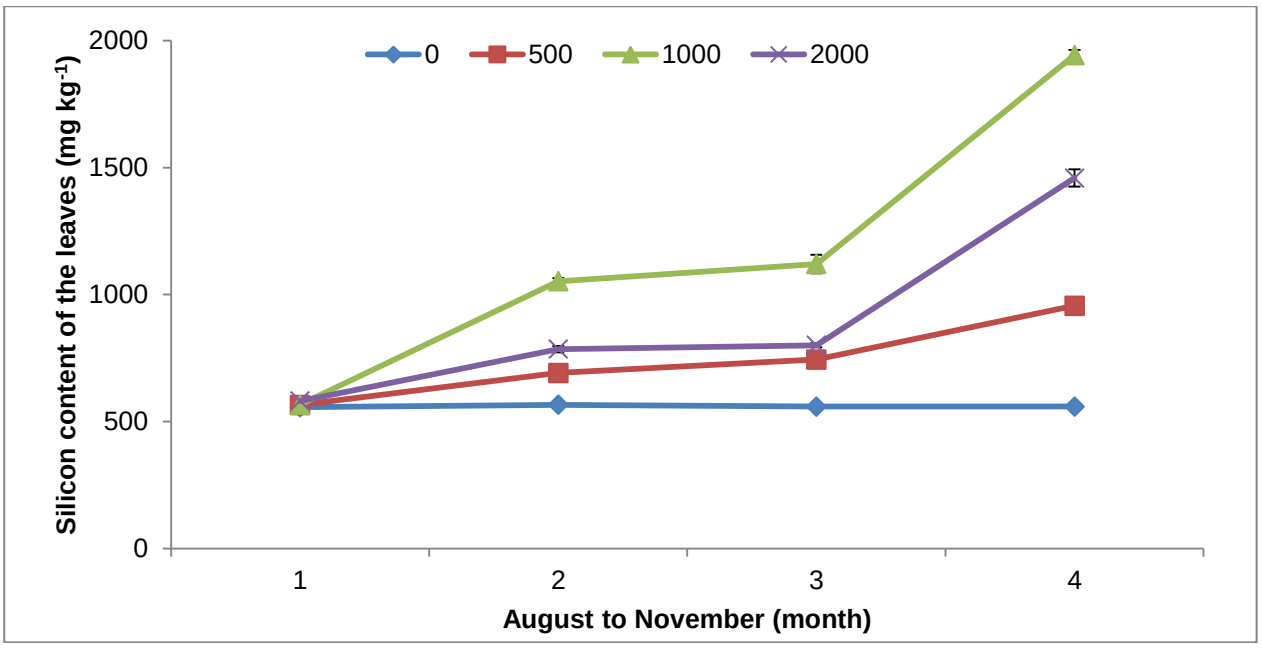

Figure 5.3.5.1. Silicon content of the leaves of 'Fairchild' mandarin.

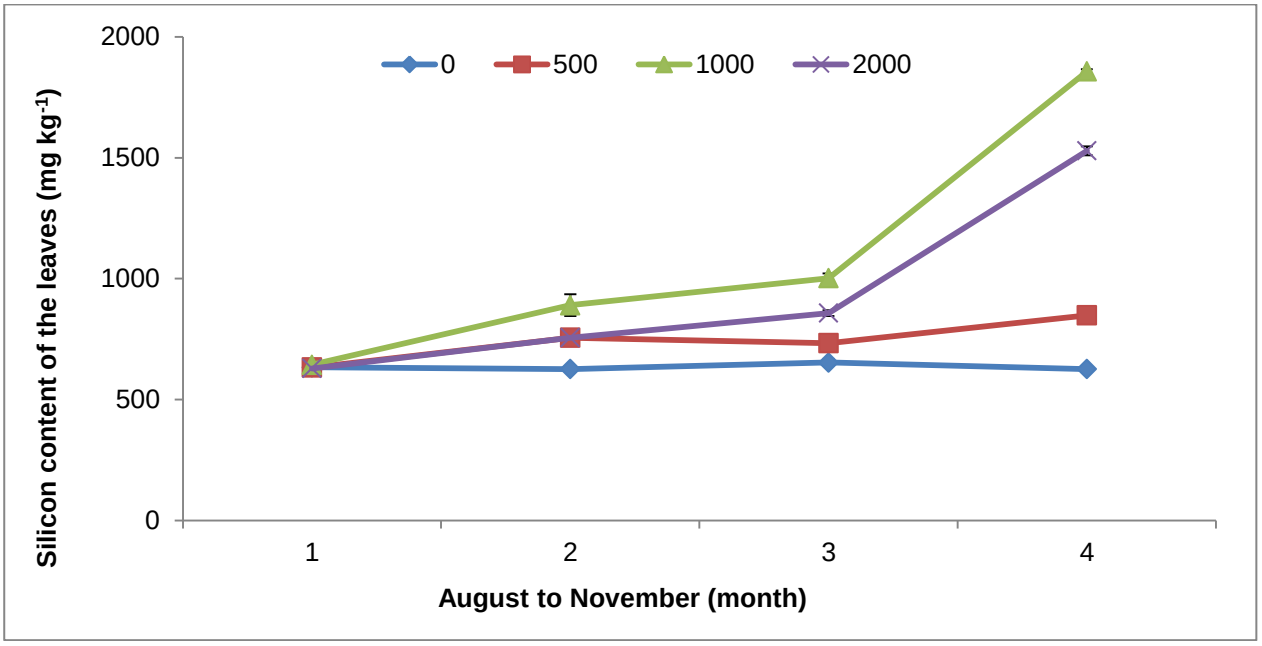

Figure 5.3.5.2. Silicon content of the leaves of 'Nova' mandarin.

<u>*Alternaria alternata* pathogenicity test</u>

In the pathogenicity trial, *Alternaria alternata* lesions were observed after three days of inoculation of the leaves and the highest disease incidence occurred after five days. The symptoms observed under controlled conditions were similar to those reported under field conditions (Figures 5.3.5.3a and 5.3.5.3b). Disease severity in both cultivars was significantly less in the trees receiving 1000 mg.L^{-1} Si than the trees receiving 0 and 500 mg.L^{-1} Si (Figures 5.3.5.4 and 5.3.5.5).

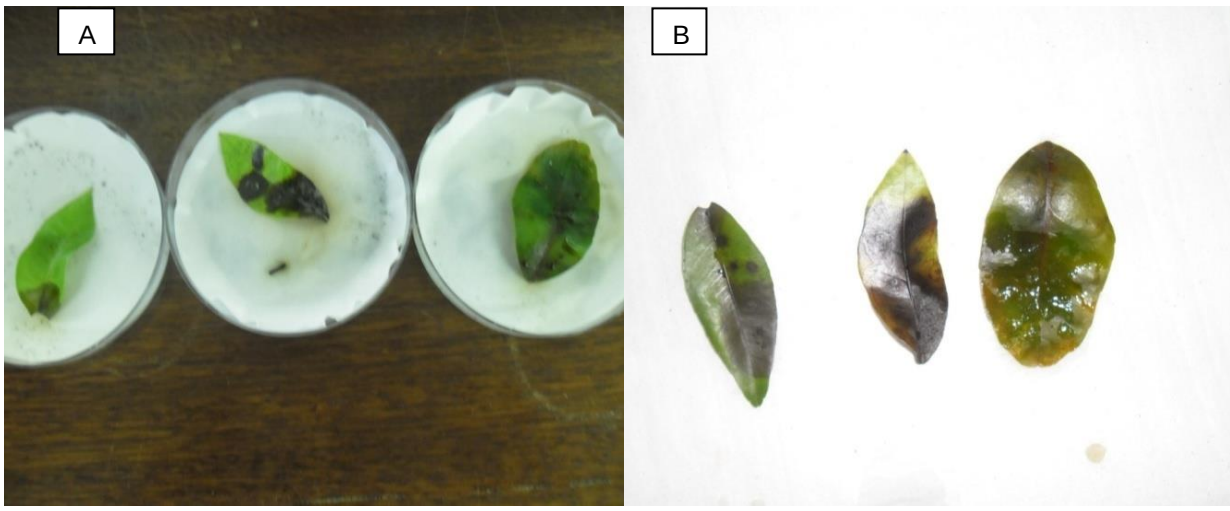

Figure 5.3.5.3. Symptoms of *Alternaria alternata* in mandarin 'Fairchild' (3A) and mandarin 'Nova' (3B) leaves.

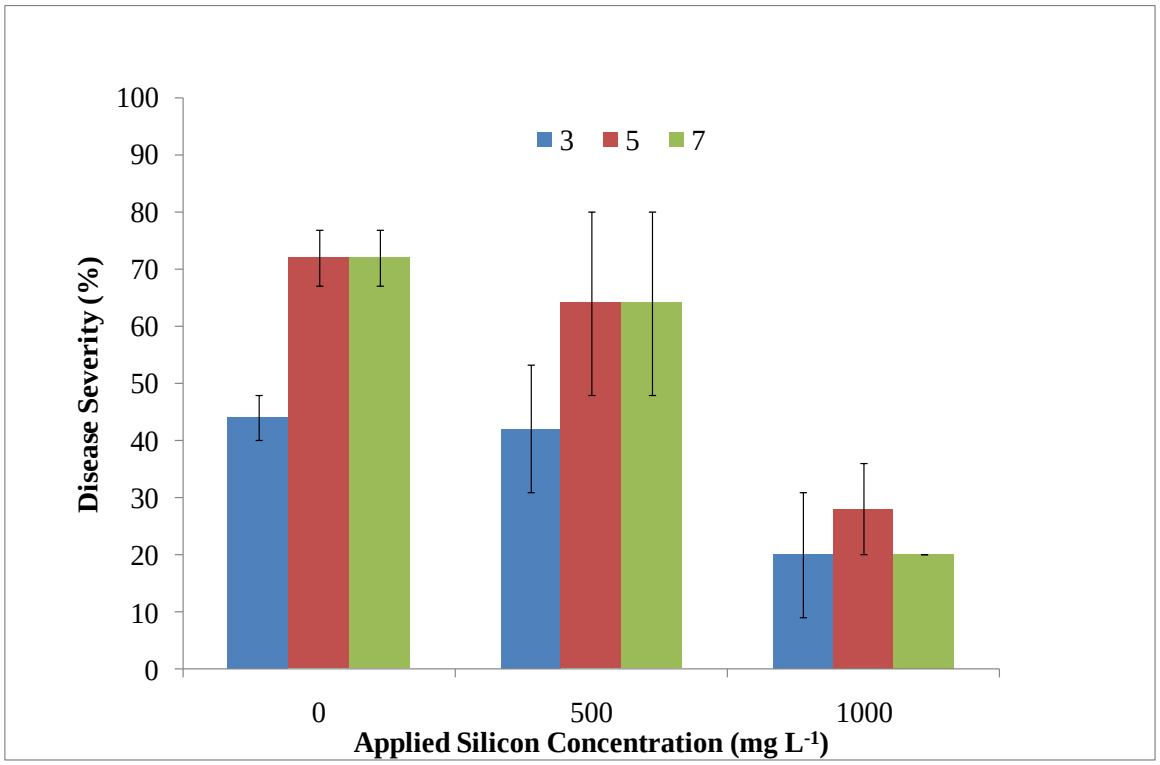

Figure 5.3.5.4. *Alternaria alternata* severity rating over 3 - 7 days in 'Fairchild' mandarin in response to root-applied potassium silicate (K_2SiO_3).

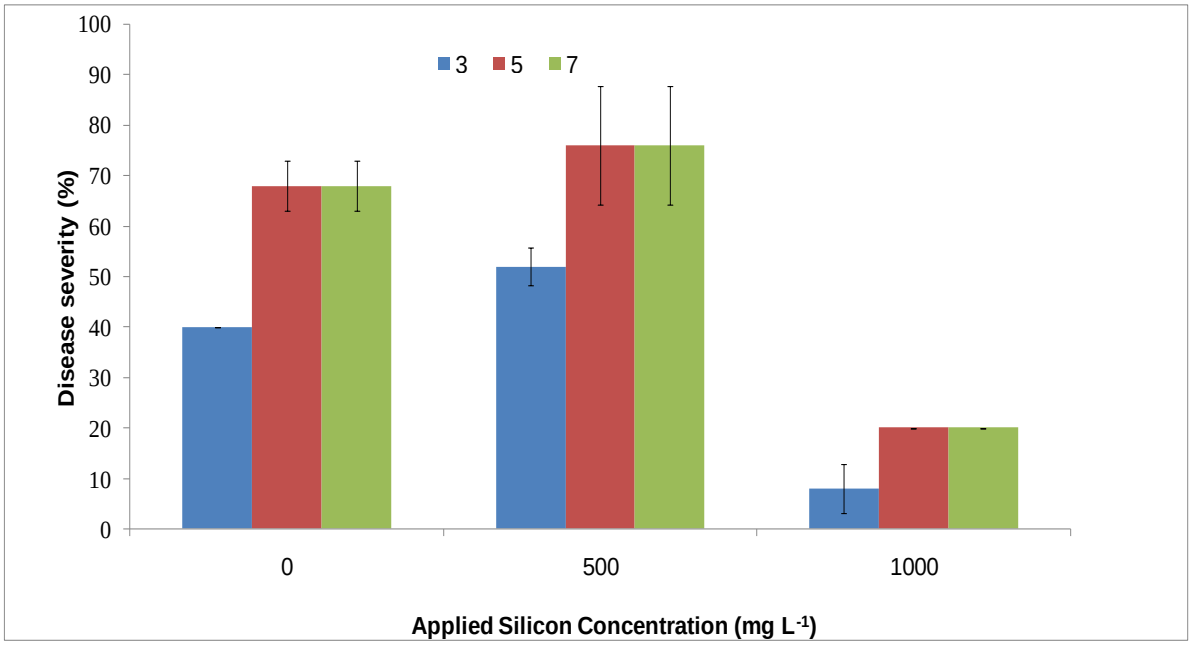

Figure 5.3.5.5. *Alternaria alternata* severity rating over 3 - 7 days in 'Nova' mandarin in response to root-applied potassium silicate (K_2SiO_3).

Field trial

Results from Figure 5.3.5.6 indicate that the Si concentration in the leaves of the lemon trees increased rapidly within the first month to stabilise at an average of 1408 mg.kg^{-1} for the root applied Si (RSi) and 1012 mg.kg^{-1} for the foliar applied Si (FSi). From these results it is also clear that the root applied Si is more effective in increasing the Si content of the leaves than the foliar applied Si. However, negligible black spot disease symptoms occurred in the orchard for the 2013/2014 season and therefore no comparison between the different treatments could be made.

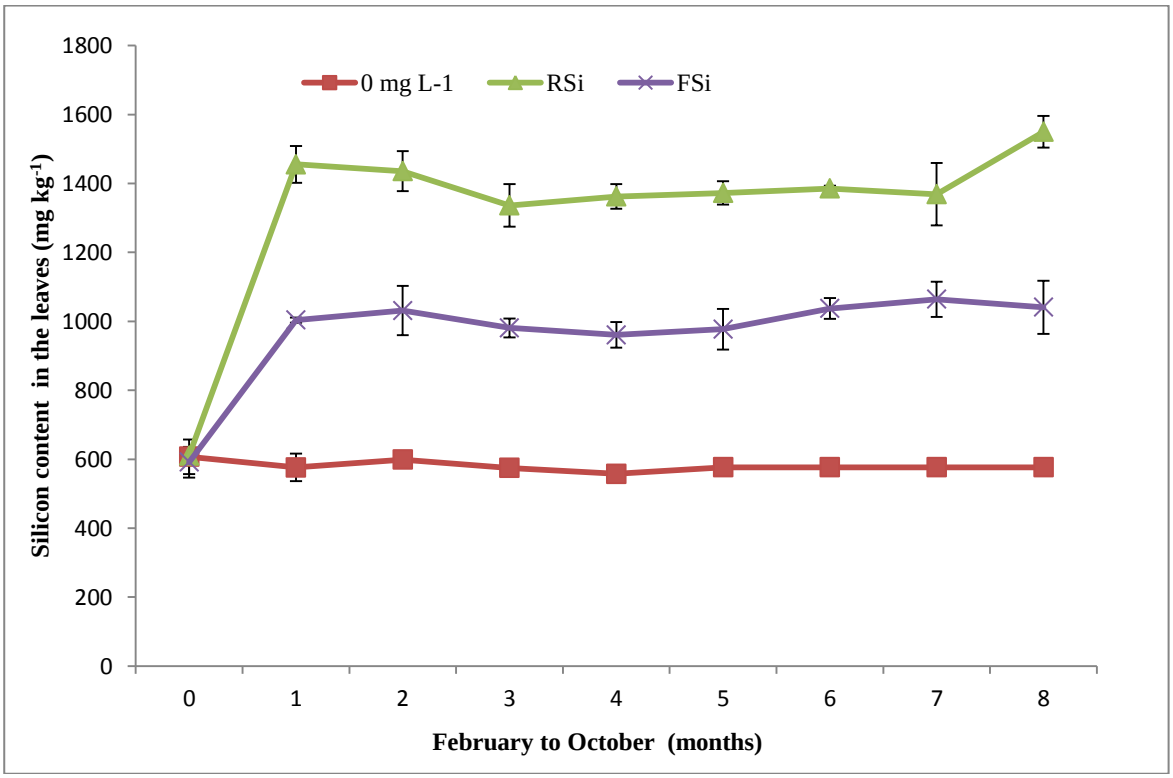

Figure 5.3.5.6. Silicon content of the leaves of 'Eureka' lemon.

Conclusions

The Si content of citrus leaves increased with an increase in the amount of Si applied. The *Alternaria alternata* isolates used were pathogenic to 'Fairchild' and 'Nova' mandarins and the results indicate that root applied Si, at a concentration of 1000 $mg.L^{-1}$, significantly reduced *Alternaria alternata* in small mandarin trees under laboratory conditions.

Technology transfer

Presentation at the Combined Congress in January 2014: Can silicon be used to treat A*lternaria alternata* in citrus trees?
Title for SAFJ article: Can silicon be used to prevent infection of A*lternaria alternata* in citrus trees?

Future research

The investigation of the influence of Si on reducing *Alternaria alternata* in citrus grown in an orchard.

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5.4 PROGRAMME: COLD CHAIN MANAGEMENT AND PACKAGING

Programme coordinator: Malcolm Dodd (SU)

5.4.1 Programme summary

During the report period two projects were completed. One used Radio Frequency Technology (RFT) to get an understanding of the storage air and fruit pulp temperatures and relative humidity in a typical South African citrus fruit export supply chain from the very beginning to very end over two seasons. This project has been completed but a full report will only be submitted next year. A full report has been provided on energy optimization of refrigerated shipping containers which involved redirection of the air flow in shipping containers.

Programopsomming

Twee projekte is tydens die verslagperiode voltooi. Een projek het Radio Frekwensie Tegnologie (RFT) aangewend om lugtemperatuur tydens opberging, vrug pulp temperature en relatiewe humiditeit in ŉ tipiese Suid-Afrikaanse vrugte uitvoerketting van begin tot einde te verstaan. Data is oor twee seisoene versamel. Hierdie projek is voltooi, maar die finale verslag sal eers volgende jaar ingedien word. ŉ Volledige verslag is verskaf aangaande energie optimisering van verkoelde skeepshouers wat die verandering in rigting van lugvloei in die skeepshouers behels het.

5.4.2 FINAL REPORT: Energy and temperature optimization in refrigerated shipping containers

Projects C1/09 and C2/13 (01/01/2010-30/04/2014) by M.C. Dodd (SU)

Summary

With the rapid adoption of twelve-meter refrigerated containers as the means of shipping citrus as well as all the challenges associated with Cold Sterilisation treatments, there has been a need to try and improve the efficacy of the forty-year-old design. The existing containers are limited in their ability to pre-cool citrus and effectively manage cold sterilisation treatment protocols. Reversed air flow technology changes the way in which the chilled and return air is channelled and managed within refrigerated shipping containers. The technology changes the direction of the chilled delivery air from vertical to horizontal. In addition, the return air is managed through a duct which limits the amount of short circuiting of the chilled air that can occur. To test the efficacy of this technology on citrus fruit temperature management and quality on a simulated shipping scale, trials comparing a standard container to one fitted with reversed air flow have been conducted. Over the past four years, eight trials on a variety of citrus types and in different packaging configurations under standard and cold sterilisation treatment conditions have been conducted. The reversed airflow technology reduces the time to pre-cool citrus packed in A15C cartons by 50%. The difference is not so marked when the fruit is packed in “Super Vent” cartons. The relative humidity in the reversed airflow container was always either the same or higher than that in the standard container. The technology had no negative impact on the fruit quality. The technology affords the citrus industry an alternative logistics strategy by way of enabling the pre-cooling of citrus in containers and managing the proposed systems sterilisation procedure by using suitably equipped containers. The reversed air flow technology has been registered by Stellenbosch University’s Innovus office as patent (PCT/IB/001811).

Opsomming

Met die snelle ingebruikneming van twaalf meter verkoelde skeepshouers vir die verskeping van sitrus, tesame met die uitdagings van koue sterilisasie behandelings, was daar ŉ behoefte om die effektiwiteit van ŉ 40 jaar oue ontwerp te verbeter. Die bestaande houers is beperk in hulle vermoë om sitrus te voor-verkoel en om effektief koue sterilisasie protokolle te hanteer. Omgekeerde lugvloei tegnologie verander die wyse waarin die verkoelde en terugvloei lug gekanaliseer en bestuur word binne die verkoelde skeepshouers. Die tegnologie verander die rigting van die verkoelde lug van vertikaal na horisontaal. Addisioneel word die terugvloei lug deur ŉ geut gestuur wat die negatiewe invloed op die verkoelde lug verminder. Om die effektiwiteit van hierdie tegnologie te toets op sitrus temperatuurbestuur en kwaliteit in ŉ nagebootste verskeping omgewing, is proewe gedoen wat ŉ standaard houer vergelyk het met een wat toegerus is met omgekeerde lugvloei. Gedurende die laaste vier jaar is agt proewe gedoen met ŉ verskeidenheid van sitrus tipes en verpakkings onder standaard en koue sterilisasie toestande. Die omgekeerde lugvloei tegnologie het die voor-verkoelingstyd van sitrus verpak in ŉ A15C karton met 50% verminder. Wanneer vrugte in ŉ “Super Vent” karton verpak is, was die verskil nie so duidelik nie. Die relatiewe humiditeit in die omgekeerde lugvloei houer was dieselfde of hoër as in die standaard houer. Die tegnologie het geen negatiewe effek op vrugkwaliteit gehad nie. Die tegnologie verskaf aan die sitrus bedryf ŉ alternatiewe strategie omdat dit voor-verkoeling van sitrus in houers moontlik maak en ook vir die bestuur van sterilisasie prosedures deur die

gebruik van toepaslik teogeruste houers. Die omgekeerde lugvloei tegnologie is deur die Innovus kantoor van die Universiteit van Stellenboschas patent (PCT/IB/001811) geregistreer.

Introduction

The citrus industry now exports over 80% of its fruit in refrigerated shipping containers. It is thus imperative that the industry ensures that the temperature and humidity management of the citrus in these containers is as good as it can be to ensure optimum quality. Refrigerated shipping containers were originally designed 40 years ago as multi-purpose units for both frozen and chilled produce. There is a vast difference in the airflow requirements for chilled (respiring product that produces vital heat) and frozen that is loaded around -25°C and does not produce any heat. In the latter case the temperature management is largely dependent upon the efficacy of the insulation keeping the external heat load away from the cargo. The internal architecture of shipping containers has not been changed or improved during the ensuing 40 years. Improvements have been made to the refrigeration units control processes and scroll compressors are now replacing reciprocating ones, but these improvements cannot compensate for the interior architecture of the container and how the chilled air is delivered. Oosthuyse, 1997, Amos and Sharp, 1998, Tanner and Amos, 2003, Punt and Huysamer, 2005, Dodd and Worthington-Smith, 2006, Kapp, 2008 and Nunes et. al. 2014 all report poor management of the pulp temperatures in shipping containers and thus compromised quality in mangoes, kiwis , plums, and berries respectively. In particular it has been shown that the pulp temperatures of various fruit kinds in shipping containers vary by as much as 2.5°C (Dodd and Worthington-Smith, 2006). This is most noticeable from the base (cooler) to the top (warmer) of the pallet and also from the front to the rear of the container. This variation has a profound effect on fruit quality, particularly the maturity of the product (Tanner and Amos, 2003, Punt and Huysamer, 2005).

Trials that have been previously conducted have shown that with relatively simple innovation the containers can be made to operate much more efficiently (Dodd and Worthington-Smith, 2006). A kit consisting of a plastic sheet which covers the width and 9m length of the floor from front to the door end, some polystyrene blocks to create an air space at the front wall and inflatable dunnage bags which are placed above the pallets to prevent short circuiting has been developed. These components redirect the chilled air horizontally through the produce. Vastly improved management of pulp temperatures result from the use of this technique. Additional benefits of higher relative humidity have also been recorded. By iteration energy costs could be lower as the refrigeration unit is operating more efficiently. Other potential benefits are that the container could be used to pre-cool certain products.
This would have enormous benefits to the fruit industry in freeing up limited cold storage space and adding flexibility to the logistics chain. The cold chain could truly become an uninterrupted link between farm and fork.

With the increasing threat of “Steri” programmes being instituted on citrus by the EU and other markets, improved pulp temperature management is essential in order to reduce the chill damage often experienced in “Steri” loads in containers and reducing the problem of hot spots in the load which leads to rejection by the importing country plant health authorities. The project aimed to reduce the temperature variation and elevate the relative humidity in shipping containers. It is also possible that the energy consumption of the refrigeration unit can be reduced. The operational costs of this project were shared by the citrus, deciduous (Hortgro Science) and grape (SATI) industries.

Stated objectives

Prove that reversed air flow technology fitted inside a reefer container works significantly better than a standard container in terms of temperature and humidity management. Prove that with the technology it would be possible to pre-cool all citrus types inside reefer containers. The benefit of this would be:

1. Improve the arrival quality of South African fruit in the market.
2. Consider using containers to pre-cool fruit, with concomitant benefits to logistics flexibility.
3. Raised relative humidity’s inside the containers with the technology which would lower the vapour deficit and thus enhance fruit quality.
4. Potentially lower energy consumption thereby reducing the carbon foot print and a possibility to negotiate reduced freight rates.
5. Easier management of fruit pulp temperatures in cold treatment programmes.

Materials and methods

Two new identical containers in terms of design and company of manufacture (CMIC in China) as well as refrigeration units (Thermoking AFAM+) were leased. The lease period was initially for a period of three

years, but this was extended to a fourth year to accommodate an additional set of trials. One of the containers was reversibly changed on the inside so that the delivery air flow was in a horizontal direction as illustrated in (Figures 5.4.2.1, 2, 3 and 4). Additionally a disposable kit that converted the control container from a vertical airflow delivery to a horizontal delivery was also tested (Figure 5.4.2.5). A series of back to back trials were conducted over four years, simulating where ever possible, the shipping time from South Africa to the EU markets. These trial involved export companies loaning either 20 pallets or 40 pallets of identical fruit in terms of fruit kind, packaging material and pallet bases. The fruit was sent to Cape Fruit Coolers in Killarney Gardens, Cape Town, where the containers were located.
Each time a trial was conducted a total of 15 Logtag® pulp temperature recorders were placed into the fruit inside the cartons in the same positions in a pallet as shown in Figure 5.4.2.6. The pallets containing the pulp temperature recorders were always placed in the same positions inside the shipping container on the left hand side (from the door end) as shown in Figure 5.4.2.7.The recorders were always set to record at three minute intervals for the duration of the trial. The trials were run for up to 14 days to simulate the sea voyage time from South Africa to the EU. The air exchange was always set in every trial at 15 cubic meters hour. On some occasions the trials were shorter (7 days) due to the need to export the fruit. At the end of the trial the fruit was unloaded and the temperature data recorders recovered for download and analysis.

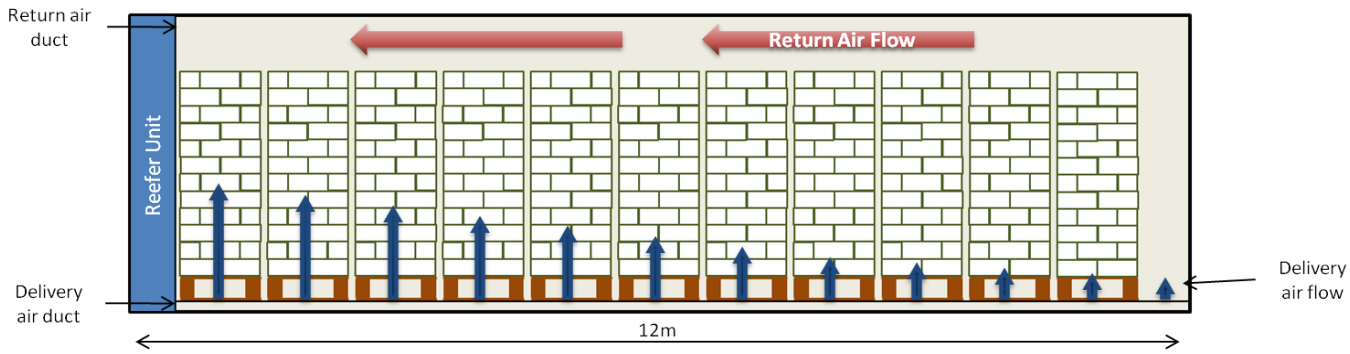

Figure 5.4.2.1. Side cut away view of a standard refrigerated container showing the air delivery system from the "T" bar floor vertically upwards.

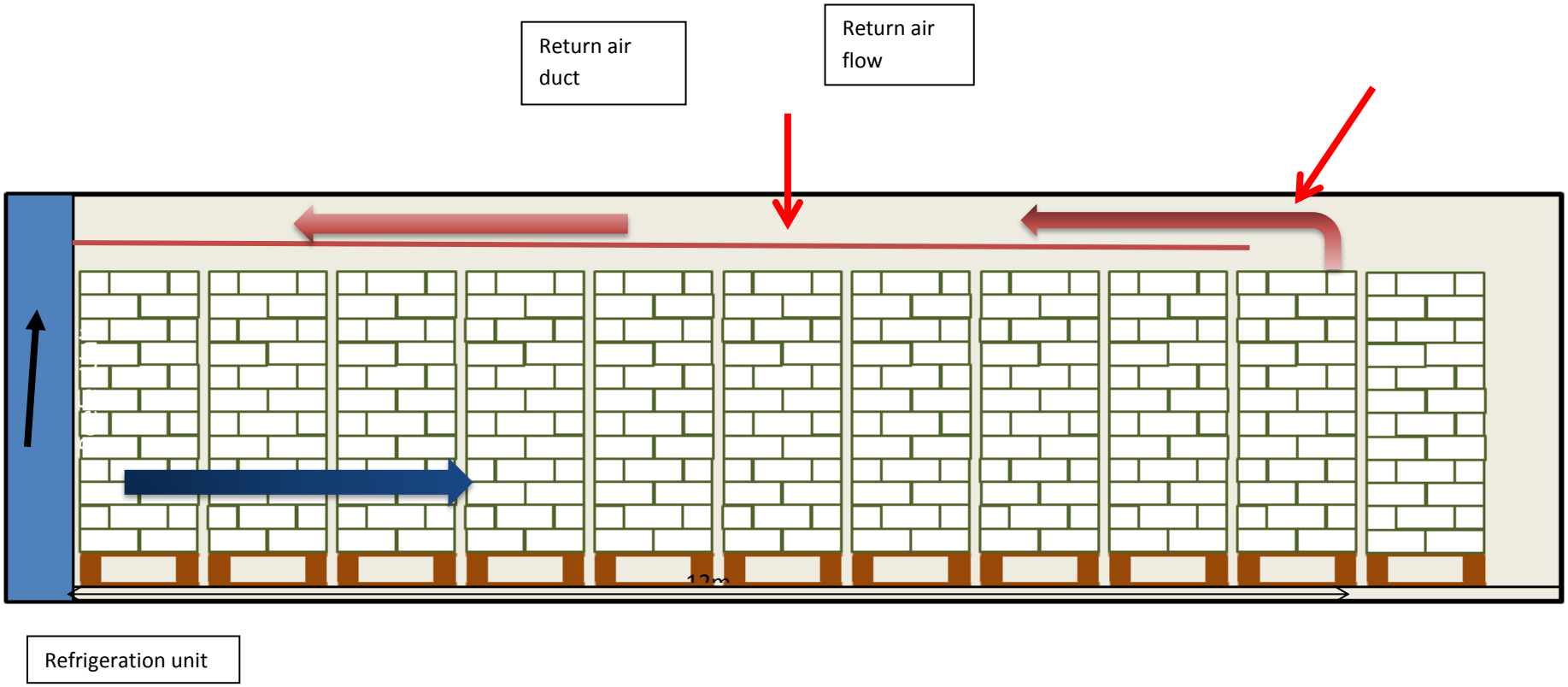

Figure 5.4.2.2. Side cut away view of a refrigerated container showing the structural modifications to achieve a reversed air delivery system. The chilled air is delivered horizontally through the pallets from the front of the container. The return air is carried from the rear of the container in a duct attached to the roof.

In addition the two containers were set up with power consumption recorders as well as airspeed recorders and relative humidity meters (HygroClip2). These were all linked via cables inside the container to a devise that used the cell phone network to transmit the data via the internet to a server.

During the first season of trials (2010) no quality samples were drawn from the fruit after the trial. The reason being that the trials were established simply to evaluate whether the airflow technology in the format installed in the container worked and whether it had any effect on the fruit temperatures. At the end of this first season when it was established that the technology was having a positive effect on the fruit temperatures and raising the relative humidity, it was decided to in future draw a sample of fruit from three positions (front, middle and rear) within the container at the end of the trial and store this for a further 4 weeks (at the appropriate temperature) followed by a shelf life period (22˚C) and then conduct a full quality evaluation. To ensure total objectivity of this quality analysis the storage life and quality evaluations were conducted by the team at Experico.

Figure 5.4.2.3. Digital image of the front interior of the container showing the structural changes made to achieve reversed air flow. The chilled air comes directly from the refrigeration unit into the front air space.

Figure 5.4.2.4. Digital image of the interior of the roof of the container showing the structural changes made to manage the return air via a duct system.

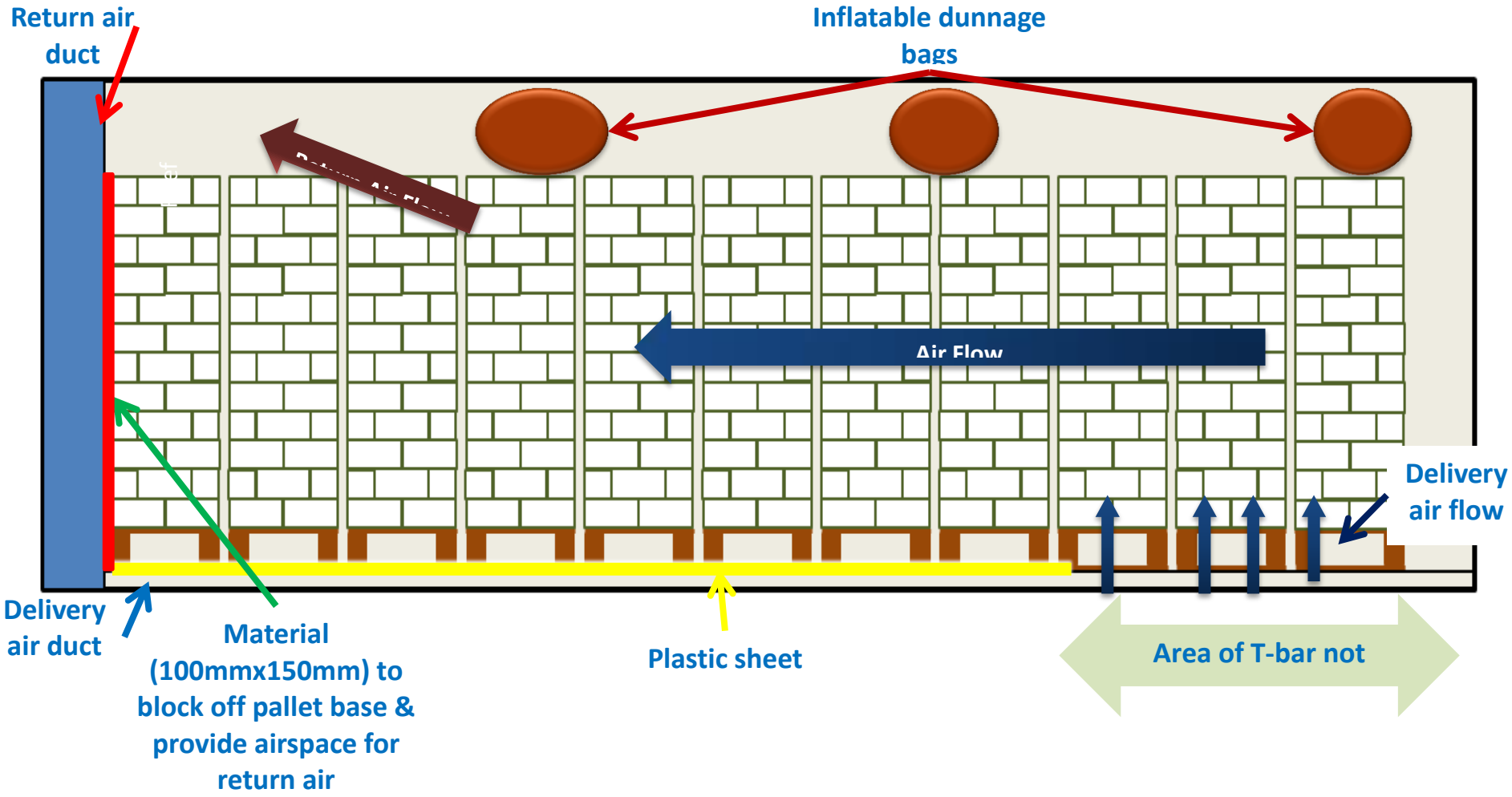

Figure 5.4.2.5. Side cut away view of a refrigerated container showing the disposable airflow kit to achieve a horizontal air delivery system. The chilled air is forced to the rear of the container and moves horizontally through the pallets.

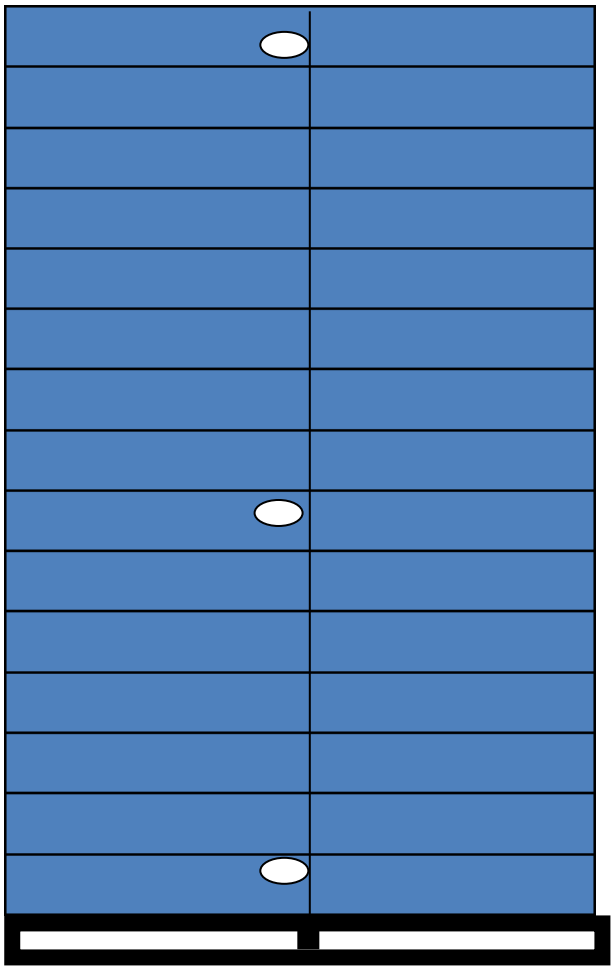

Figure 5.4.2.6. Side view of a pallet of citrus fruit showing the positions that the pulp temperature recorders were placed in fruit on the outer edge of the cartons.

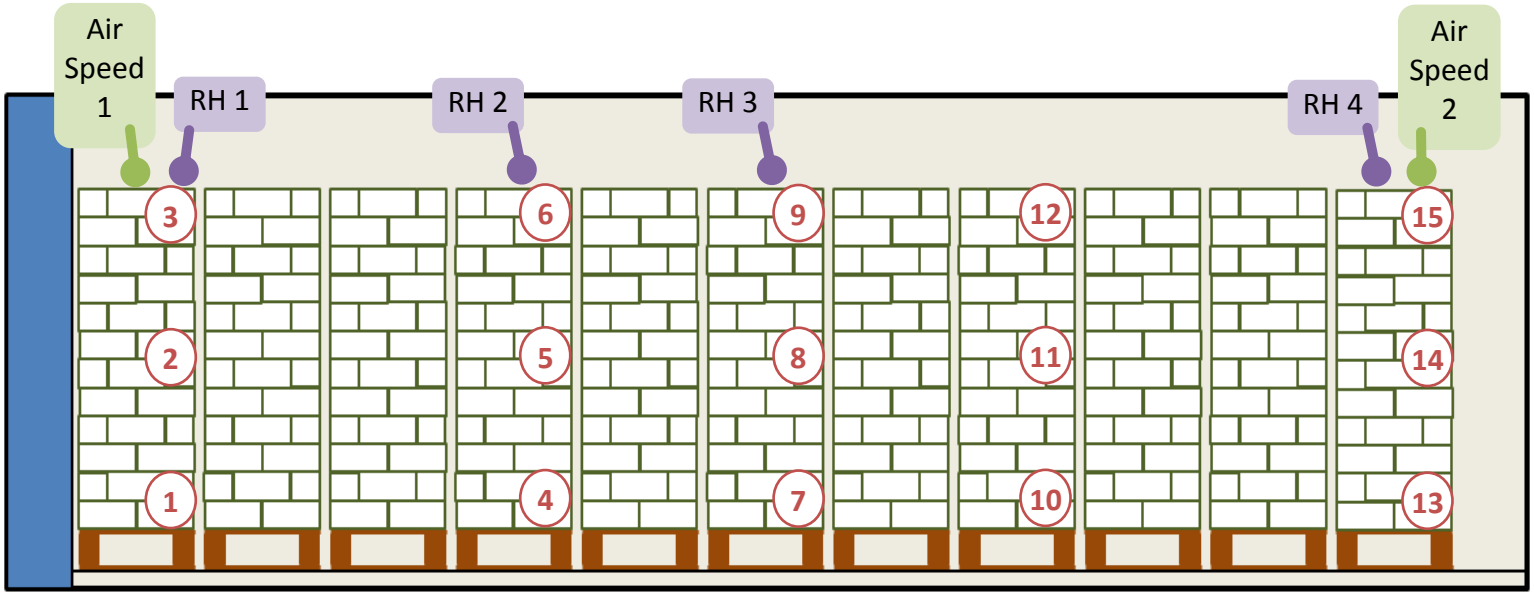

Figure 5.4.2.7. Side cut away view of a refrigerated container showing the positions that the pulp temperature recorders, relative humidity and air speed were placed during trials.

Results and discussion

Trial 1 (July 2010). This trial was conducted on Navel oranges packed in A15C telescopic cartons. The fruit, which was owned by Dole, was grown and packed in Citrusdal. There after it was trucked to Cape Fruit Coolers in Cape Town. At this point pulp temperature recorders were placed in 15 positions within each 20 pallets of fruit that were placed in a standard container and the container fitted with reversed air flow. The containers were set to run at +3.5˚C and the air exchange was set at 15 cubic meters hour. The results in Table 1 showed the modified container cooled the oranges from an average of 19.7°C to an average of 4.5°C in 4 days and 8 hours. By contrast the standard container took 7 days and 10 hours to get the fruit from an average of 20.8˚C to an average of 4.1°C. The range of temperatures in the control container at the end of the trial was 3.9˚C to 6.2˚C compared to 3.7˚C to 5.9˚C in the container fitted with the reverse air flow. So the speed of cooling to the lowest pulp temperature reached was much quicker by 3 days and 2 hours compared with the reversed airflow technology. The range of pulp temperatures was 1.8˚C in the container with reversed air flow compared to 1.9˚C in the standard container. These results can be seen in Figures 8 and 9. The length of time to pre-cool the fruit to the lowest temperature achieved in the container with the reversed airflow technology varied from 2 days to 6.5 days. In the standard container the length of time varied between 4.25 to 9.2 days. The reversed airflow technology thus cooled the fruit much faster and more consistently. In addition the relative humidity was increased by the reversed air flow technology from 89.6 to 95.1%, an improvement of 5.5% During the pre-cooling process it was found that the modified container used 2.4% less energy.

Table 5.4.2.1. Comparison of Navel orange pulp pre-cooling temperatures, storage air temperatures and relative humidity between a standard container and one fitted with reversed airflow. Set point +3.5˚C.

Measurement	Control	Reversed airflow	Difference
Fruit pulp temperature ˚C at the start of the test	20.8	19.7	1.1
Lowest pulp temperature achieved ˚C	4.1	4.5	0.4
Range of pulp temperatures ˚C (Low to high)	3.9 – 6.2 2.6	3.7 – 5.9 2.2	0.4
Average time taken to pre-cool the fruit to lowest temperature achieved (Days and hours)	7 days and 10 hrs. Range 4.25 - 9.2 days	4 days and 8 hrs. Range 2 - 6.5 days	3 days and 2 hrs.
Mean relative humidity %	89.6	95.1	5.5

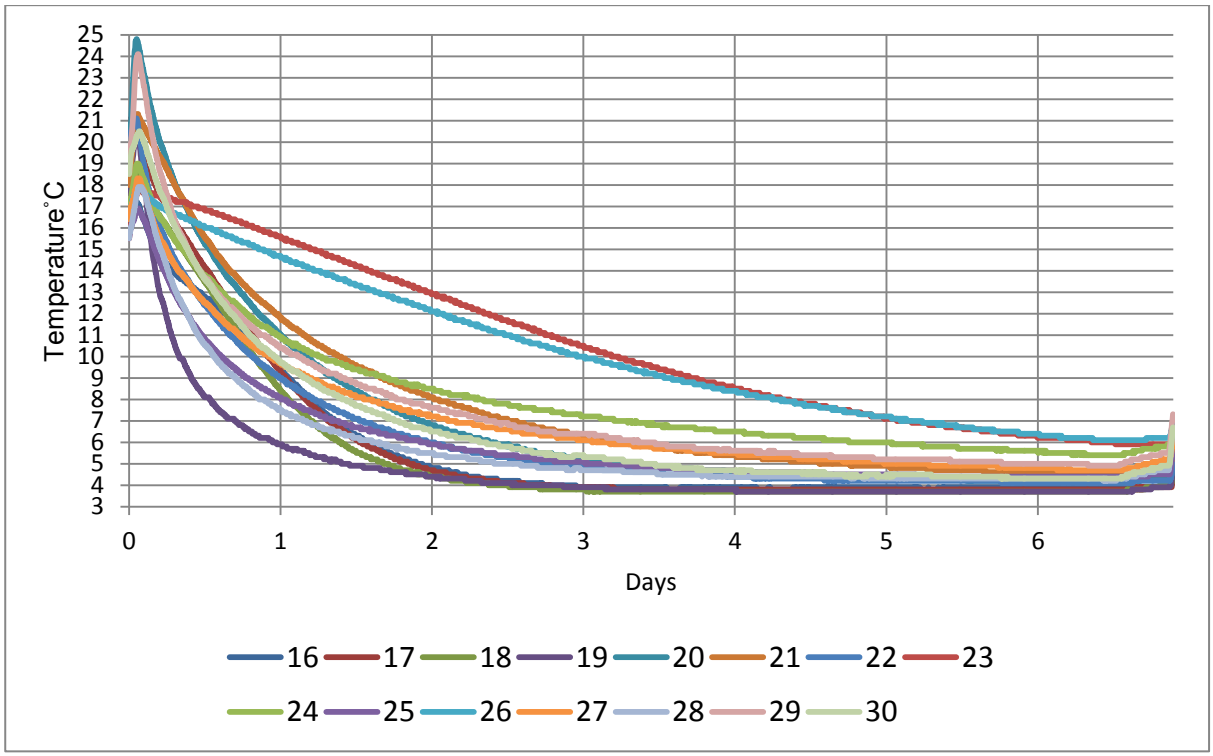

Figure 5.4.2.8. Pulp temperatures of Navel oranges in a standard container (control). Set point +3.5˚C.

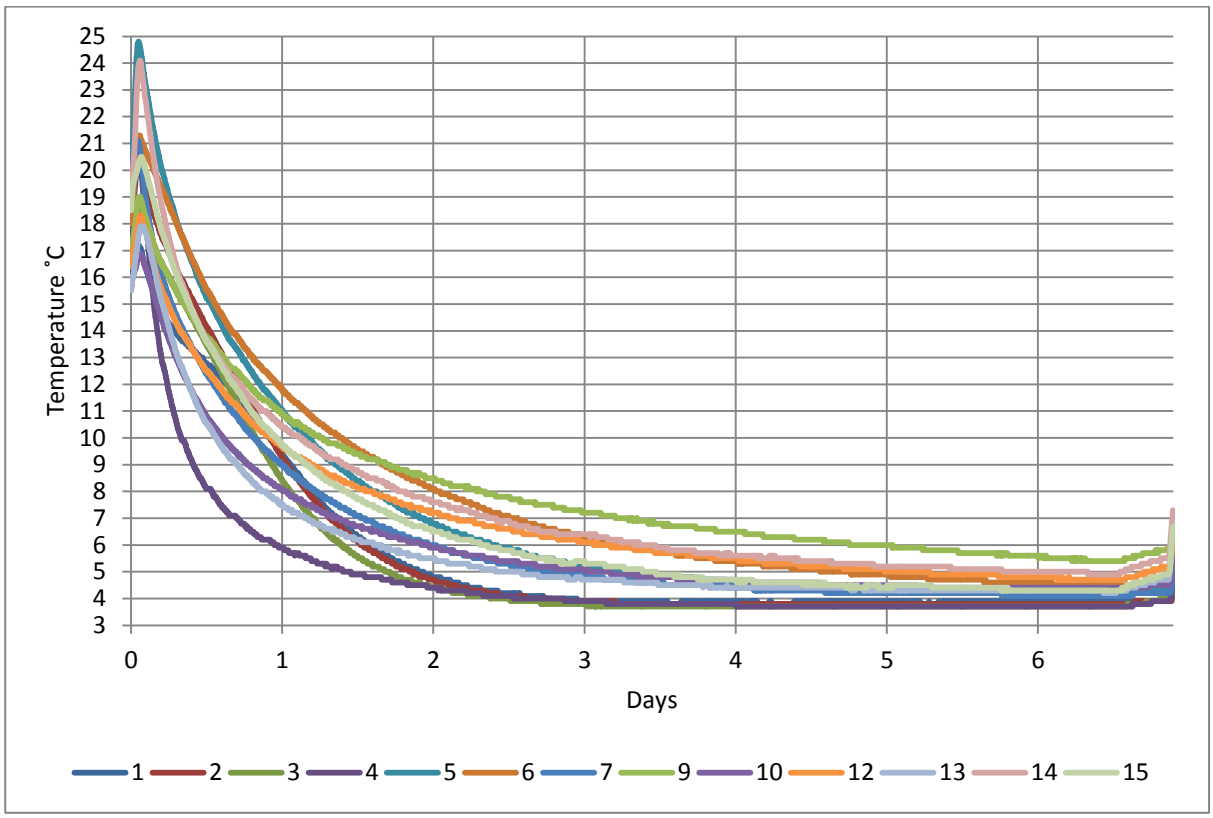

Figure 5.4.2.9. Pulp temperatures of Navel oranges in a container fitted with reversed airflow technology. Set point +3.5˚C.

Trial 2 (September 2010) simulated Chinese "Steri" protocol. Capespan had been experiencing problems managing the temperatures for Cold Sterilisation shipments. The shipping line often had to resort to a "chill blast' of air at -1.6˚C for 2 hours to keep the USDA probes below the prescribed -0.6˚C. This involves extra

cost and very importantly compromises the fruit quality. At the request of Capespan a trial was initiated on one container load of Valencia's to establish what, if any, effect the reversed airflow technology would have on the management of "Steri" temperatures. One container of citrus packed in standard A15C cartons was loaned to the project to test the ability of the modified container to manage fruit pulp temperature according to the Chinese "Steri" protocol. Initially the plan was to pre-cool the fruit to "Steri" temperature in the Cape Fruit Coolers cold store and then place it in the container. However, a logistical problem meant that there was insufficient pre-cooler tunnel space and thus it was this not possible to pre-cool the fruit to "Steri" temperature. It was decided to instead use the container to pre-cool the fruit and then set the "Steri" temperature. A set of USDA probes were also installed in the load as per the requirements shown in Figure 10. The container set point was initially + 3.5°C and after five days at this temperature it was lowered to -1.0°C. In the fruit loaded in pallet 1 at the front of the container a thermocouple devise was installed that could be read in real time via the internet. Data received via radio transmission showed that when the set point was reduced to -1.0˚C the fruit temperature nearest the delivery air duct was at -1.0°C. As the fruit would have been damaged at this temperature it was decided to raise the set point to +0.5°C and let the fruit temperature stabilize. Once the fruit temperature stabilized there was a 0.2°C difference between the three USDA probes, Figure 5.4.2.12. This narrow variation in pulp temperatures shows a very good management of cold sterilisation temperatures.

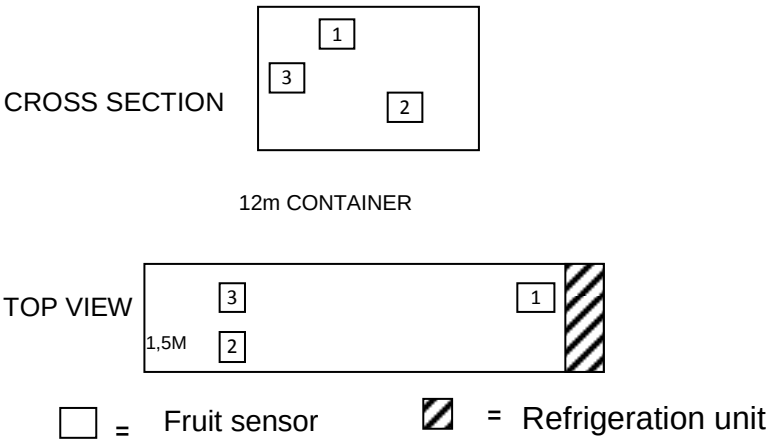

Figure 5.4.2.10. USDA Sensor position as specified by AQSIQ.

Once the trial was completed and the Logtag recorders recovered it was found that the Thermocouple reading of -1.0°C was faulty and the devise had been reading one degree too low. It had not been necessary to have elevated the set point temperature.

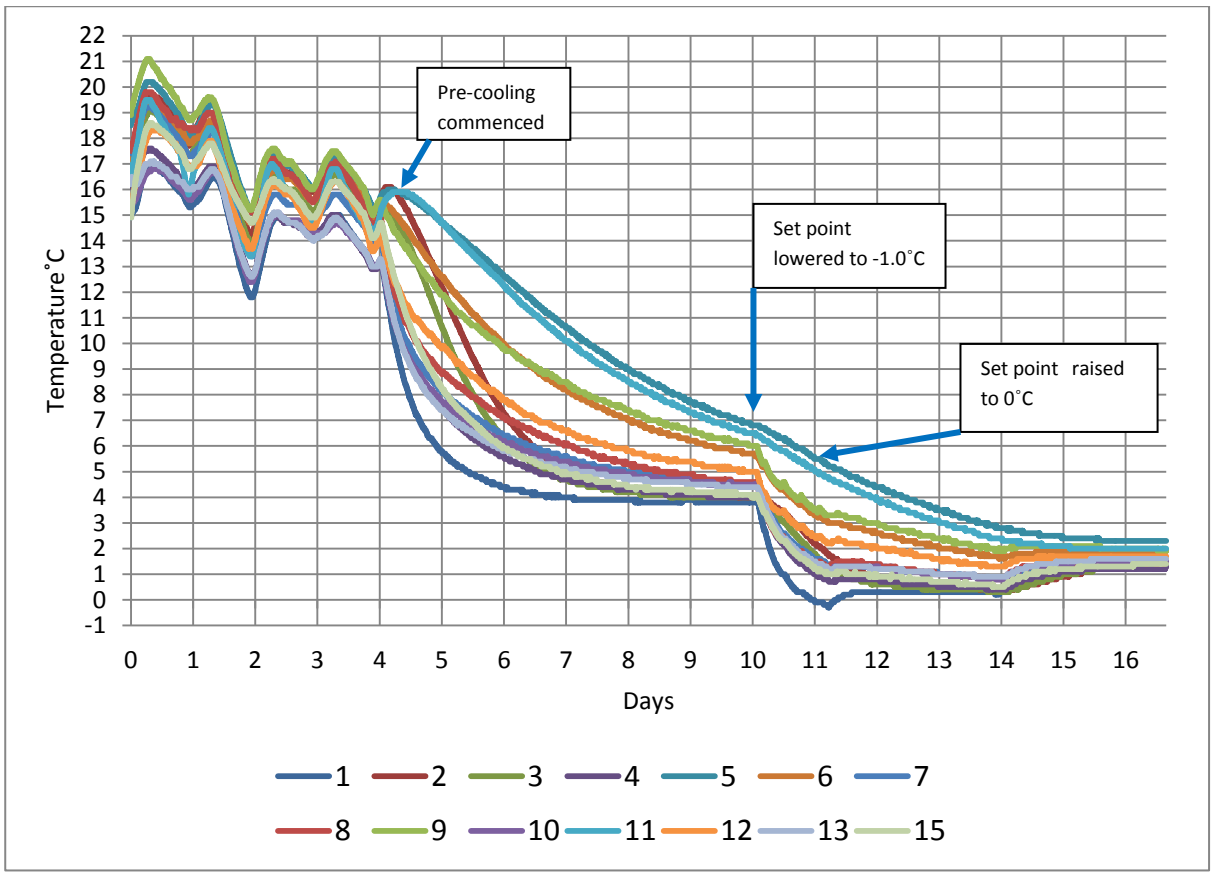

Figure 5.4.2.11. Pulp temperatures of Valencia oranges in a container fitted with reversed airflow technology. Simulated cold sterilisation procedure.

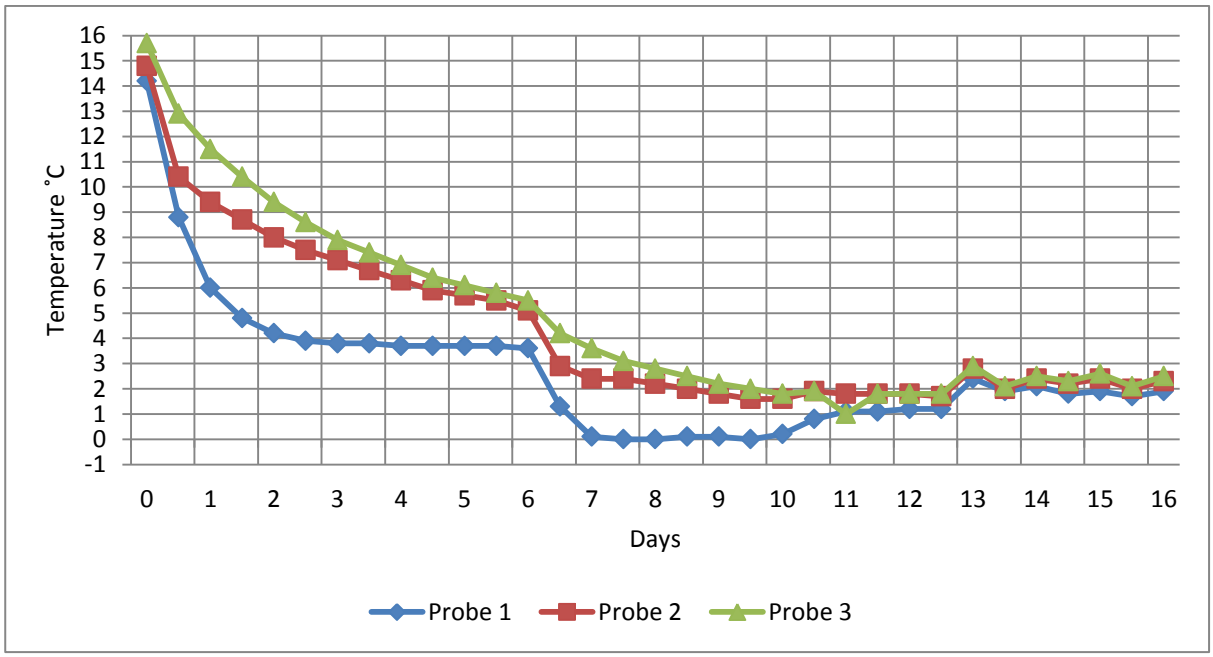

Figure 5.4.2.12. Pulp temperatures as measured by USDA pulp probes of Valencia oranges in a container fitted with reversed airflow technology under a simulated cold sterilisation procedure. Probe in positions in the fruit as shown in Figure 5.4.2.10.

Table 5.4.2.2. Results of the pre-cooling of Valencia oranges packed in A15C cartons in a simulated "Steri" trial. Pulp temperatures in ˚C measured with Logtag probes.

	Start pulp temperature ˚C	Lowest pulp temperature achieved ˚C with set point at 3.5˚C	Time (hours) taken to temperature achieved with set point of 3.5˚C	Lowest temperature achieved ˚C with set point at -1.0˚C	Time (hours) taken to temperature achieved with set point of 0˚C	Final temperature with set point of 0˚C
Mean	14.5	4.7	5.6 (days)	1.1	2.4 (days)	1.6
Range	(13.1 - 16.0)	(3.8 - 5.6)	(3 - 7)	(-0.1 - 2.5)	(1.4 - 4)	(1.1 - 2.3)

The container was able to pre-cool the fruit from 14.5˚C to 4.7˚C in an average of 4.7 days. At this time the set point of the refrigeration unit was lowered to -1.0˚C. The relative humidity averaged 93% for the period the fruit was in the container. The trial showed that it would be possible using the reversed air flow technology to pre-cool citrus packed in A15C cartons to cold steri temperature. The maintenance of the pulp temperatures recorded by the USDA probes was very accurate with there being only a 0.2˚C difference in temperature (Figure 5.4.2.12).

Trial 3. (July 2011) Lemons. A trial testing the ability of the technology to pre-cool lemons was conducted in week 28. Lemons packed in "Super vent" cartons were supplied by SRCC. This fruit was kept at ambient temperature, and trucked from the Sundays river area to Cape Fruit Coolers. Here they were fitted with pulp data recorders and loaded into a standard container and one structurally changed to Reversed Air Flow. The fruit was held in the containers (Set point +7.5°C and vent setting 15 cmh) for one week before unloading, recovering the data loggers and shipping overseas. The "Super Vent" cartons, because of their improved air flow characteristics, enabled the lemons to be cooled more quickly than had been recorded with fruit packed in standard A15C cartons. There was not as large a difference in the temperature management between the standard and modified container as seen in previous trials due to the improved airflow characteristics of the "Super vent" cartons.

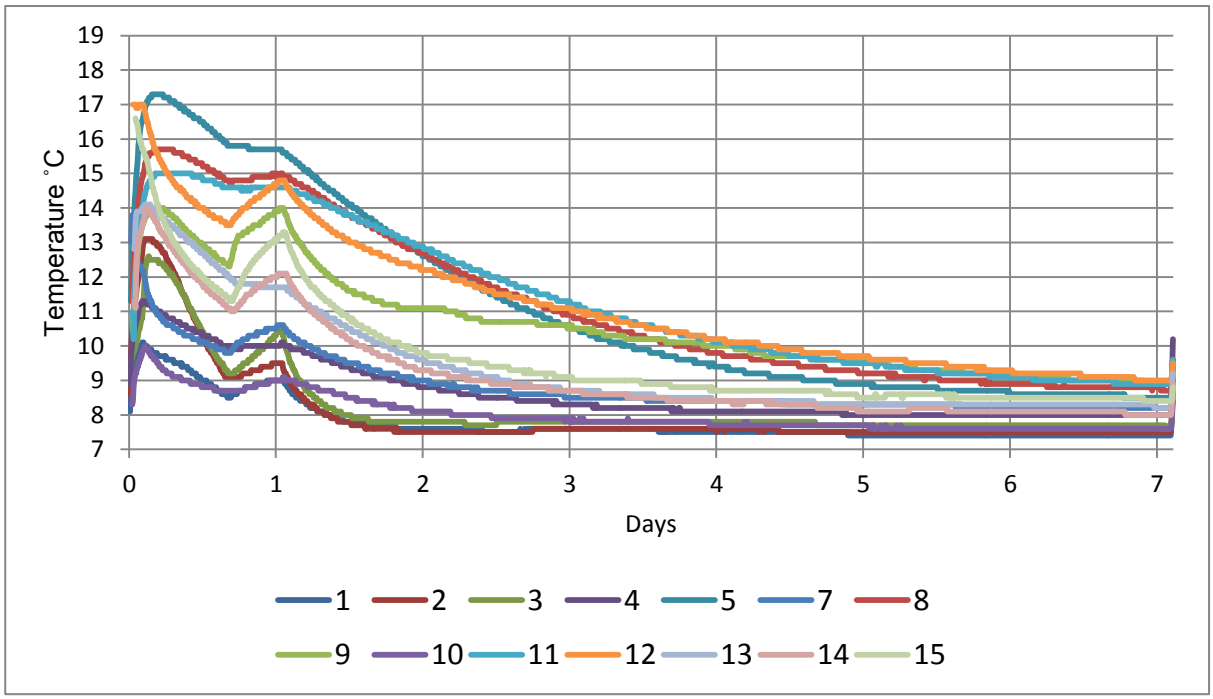

Figure 5.4.2.13. Pulp temperatures of Lemons in a container fitted with reversed airflow technology, set point 7.5˚C.

The results of this trial are shown in Figures 12 and 13. The time to pre-cool the lemons from around 12.5°C to 8.0°C was 4 days and 1 hour in the standard container compared to 4 days in the one fitted with reversed

air flow (despite there being a power interruption to the container due to an issue with the electrical distribution board at the facility). The relative humidity was measured at 94.0% and 94.5% in the standard and reversed airflow containers respectively. A small difference but once again in favour of the reversed air flow technology The reversed air flow technology was able to manage fruit pulp temperatures closer together with the standard deviation for the average fruit pulp temperature in the standard container being 2.8 compared to 2.6 in the reversed air flow one. These results are summarised in Table 5.4.2.3.

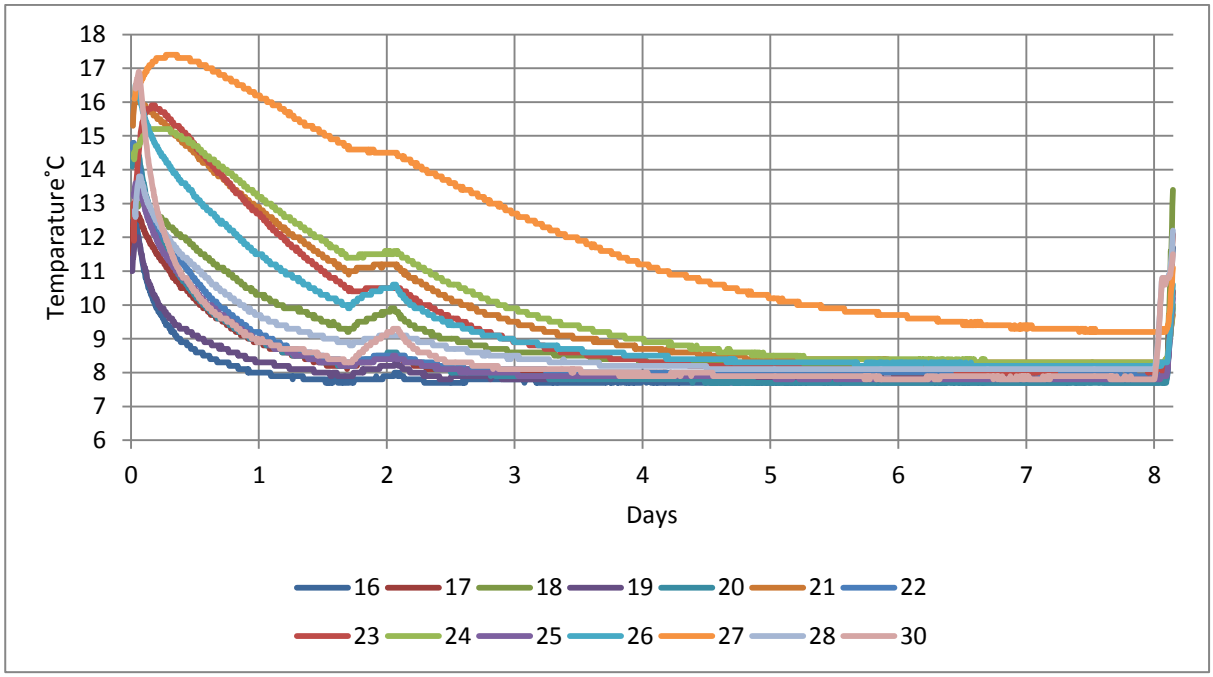

Figure 5.4.2.14. Pulp temperatures of Lemons in a standard container, set point 7.5˚C.

Table 5.4.2.3. Results of the pre-cooling of Lemons packed in "Super Vent" A15C cartons in a standard container and one with reversed airflow technology. Set point +7.5˚C.

Measurement	Result for control container	Range of temperatures recorded for control container	Result for Reversed Air Flow container	Range of temperatures recorded for Reversed Air Flow container
Average start fruit temperature	12.7˚C	Range was 11.4 -15.3˚C = 3.9 ˚C	12.5˚C	Range was 9.0 - 17.0˚C = 8.0 ˚C
Average lowest fruit temperature	8.1˚C	Range was 7.7˚C - 9.3˚C = 1.6˚C	8.4˚C	Range was 7.5˚C - 9.1˚C = 1.6˚C
Standard deviation on mean temperature	3.2	-	2.8	-
Average time to get to lowest fruit temperature	4 days and 1 hour	Range was 2 days and 7 hours - 6 days and 20 hours.	4 days	Range was 1 days and 19 hours - 6 days and 11 hours.
Relative humidity	94%	-	94.5%	-

Trial 4. (September 2011) Navels. SRCC supplied forty pallets of ambient temperature Navels packed in "Super vent" cartons. These were fitted with pulp temperature data recorders as per Fig 5.4.2.6 at Cape Fruit Coolers. They were then loaded into the standard and reversed containers and held at + 3.5°C for 14 days to simulate shipping voyage to Europe. The pre-cooling time from around 17°C to 5°C was 4.5 days in the reversed air flow container. In the standard container where the start temperature was 20˚C the time to pre-cool to the lowest temperature was as 6.5 days. The relative humidity in the standard container was 87.3% compared to 91.2%, a 3.5% improvement.

The export company needed the fruit in the reversed air flow container after 7 days to fill an export commitment so the fruit was unloaded. The fruit in the standard container was kept for sixteen days and during this time the pulp temperature continued to decrease until a power failure on the 12th day. It is of interest to note how quickly the pulp temperature of the fruit increases when the refrigeration is off. In 2.5 days the power was of due to a DVD board issue, the fruit temperature in some positions increased by 4.0˚C.

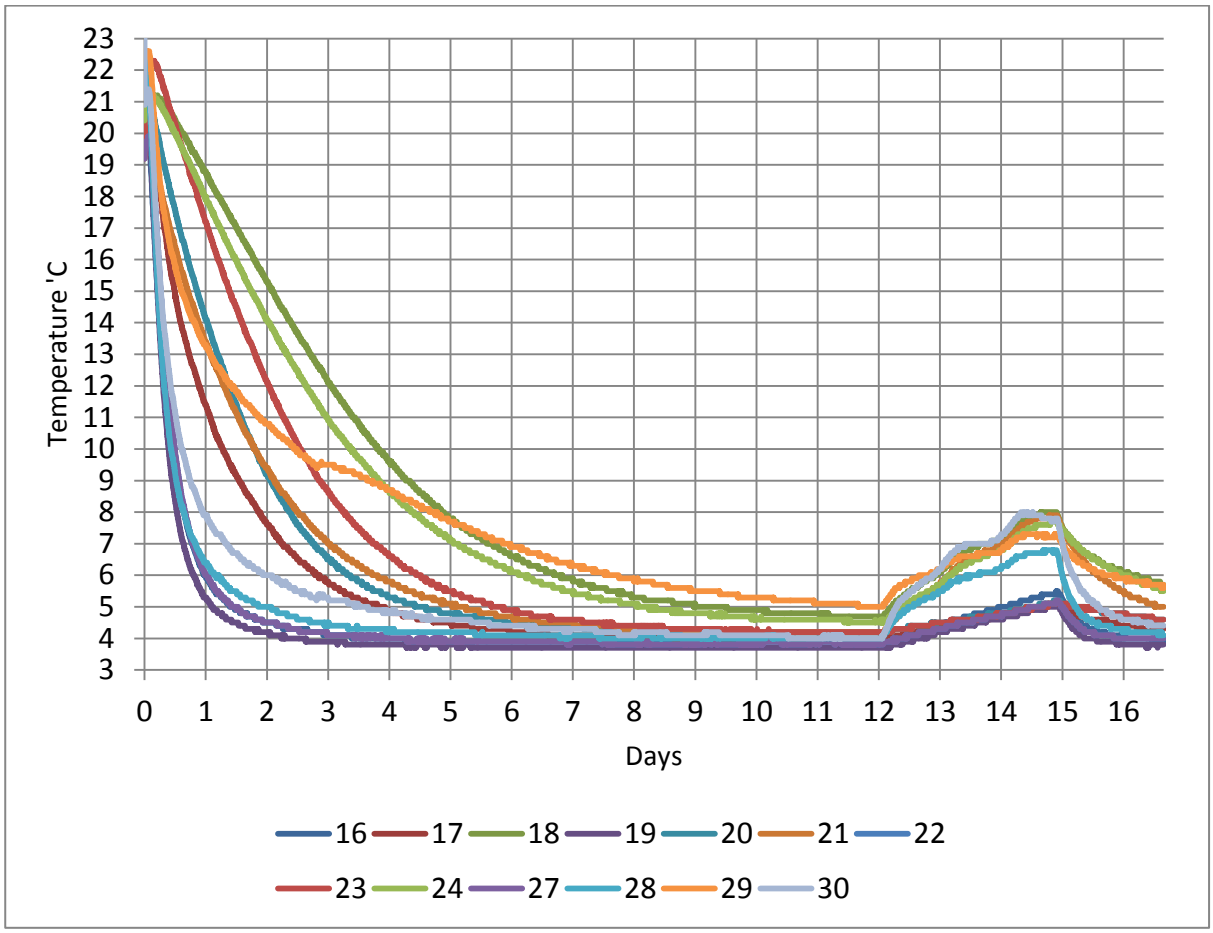

Figure 5.4.2.15. Pulp temperatures of Navels packed in "Super Vent" cartons in a standard container, set point 3.5˚C.

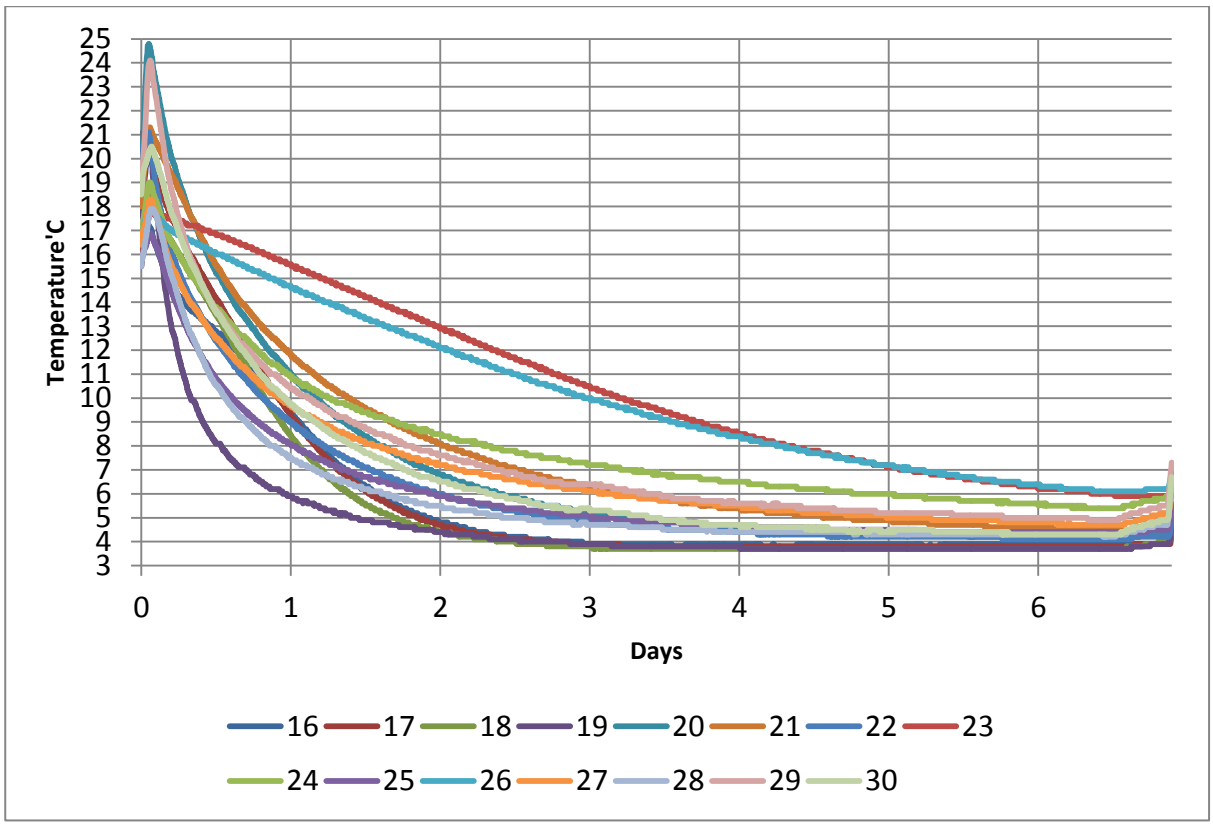

Figure 5.4.2.16. Pulp temperatures of Navels packed in "Super Vent" cartons in a standard container, set point 3.5˚C.

Table 5.4.2.4. Results of the pre-cooling analysis of Navels packed in "Super Vent" A15C cartons in a standard container and one with reversed airflow technology.

	Start Temperature ˚C	**Lowest temperature achieved ˚C**	**Range of temperatures after 7 days ˚C**	**Time to lowest temperature days and hours and hours**	**Standard deviation of average temperature**	**Relative Humidity %**
Control	20	3.8	3.8 - 6.2 Range 2.4	6.5	2.98	87.3
Reversed air flow	18.5	3.7	3.7 - 5.9 Range 2.1	4.5	2.87	91.2

The trial illustrated once again the ability of the reversed airflow technology to pre-cool the citrus faster than a control container, 4.5 days compared to 6.5 days respectively. This is a two day reduction in time to pre-cool the citrus. In addition the range of temperatures was reduced from 2.4˚C to 2.1˚C by the reversed airflow technology. The standard deviation of the mean temperature was lower at 2.87 in the reversed air flow compared to the standard at 2.98. The relative humidity was increased from 87.3% to 91.2%, a 3.9% improvement. The two probes 23 and 26 in Figure 5.4.2.16 illustrate that it is difficult to achieve totally even cooling. There were no negative effects caused by the reversed air flow technology on the quality of the citrus fruits, Table 5.4.2.5.

Table 5.4.2.5. Results of the end of shelf life quality analysis of Navels packed in "Super Vent" A15C cartons in a standard container and one with reversed airflow technology.

Quality attribute	Rind pitting %	Stem end Breakdown %	Decay %	TSS %	% total titratable acid	Rind colour. CRI chart 34
Control	2.12	0.35	0.3	10.8	1.9	1.2
Reversed air flow container	1.01	0	0	10.7	1.8	1.1

Trial 5. (July 2012) Grapefruit. The objective of this trial was to ascertain as to how the Reversed airflow technology would perform in a simulated "Partial Steri" trial. This "Systems" approach to ensuring that citrus will be free from False codling moth is being mooted by the CRI as a means of managing the possible EU steri protocol. The rationale being that if the EU tries to impose a "Steri" schedule on South African citrus exports to Europe the CRI/CGA will try and negotiate a "Systems" approach to this challenge. This will involve first sampling for False codling moth and if the fruit has less than a certain percent infection then a "Partial Steri" would be applied. This would involve pre-cooling the fruit in the shipping container and then when the desired pulp temperature is achieved the fruit would be maintained at this temperature for a period of 14 days. The desired pulp temperature of the fruit for this proposed protocol would be 2˚C (+/- 0.5˚C).To achieve this 40 pallets of local market Star Ruby grapefruit that were packed in open top E15D cartons were "loaned' by Farmsecure.

These were trucked to CFC and loaded into a standard container and one fitted with RAF technology. The set point was initially 2.0°C for seven days thereafter the set point was dropped to 1.0°C. The pulp temperature traces are shown in Figures 17 and 18. As can be seen both containers were able to pre-cool the grapefruit at an acceptable rate. The pulp temperatures stabilised and were held close to set point. The carton type has a large impact on the pre-cooling rate and temperature stabilization. The open top display cartons used provide more airflow so it is thus easier to pre-cool the fruit and maintain pulp temperatures.

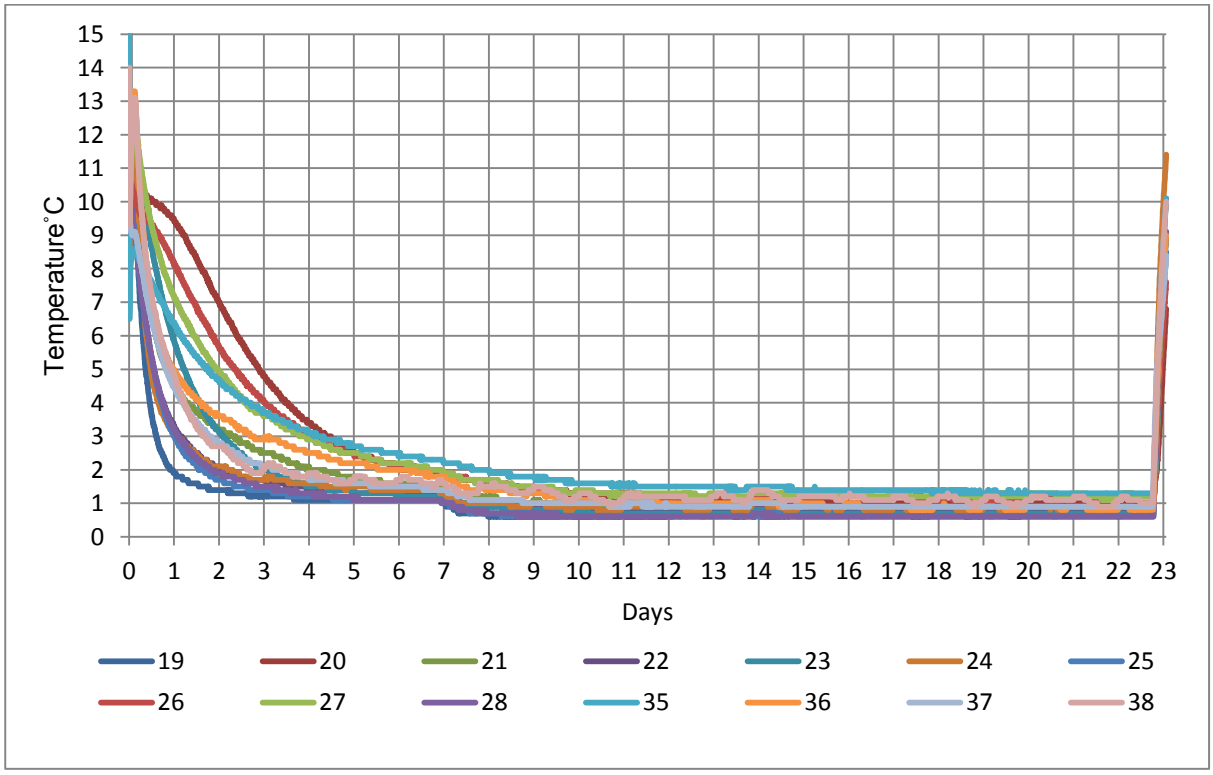

Figure 5.4.2.17. Pulp temperatures of grape fruit in a standard container. Set point +2.0˚C lowered to + 1.0˚C after seven days.

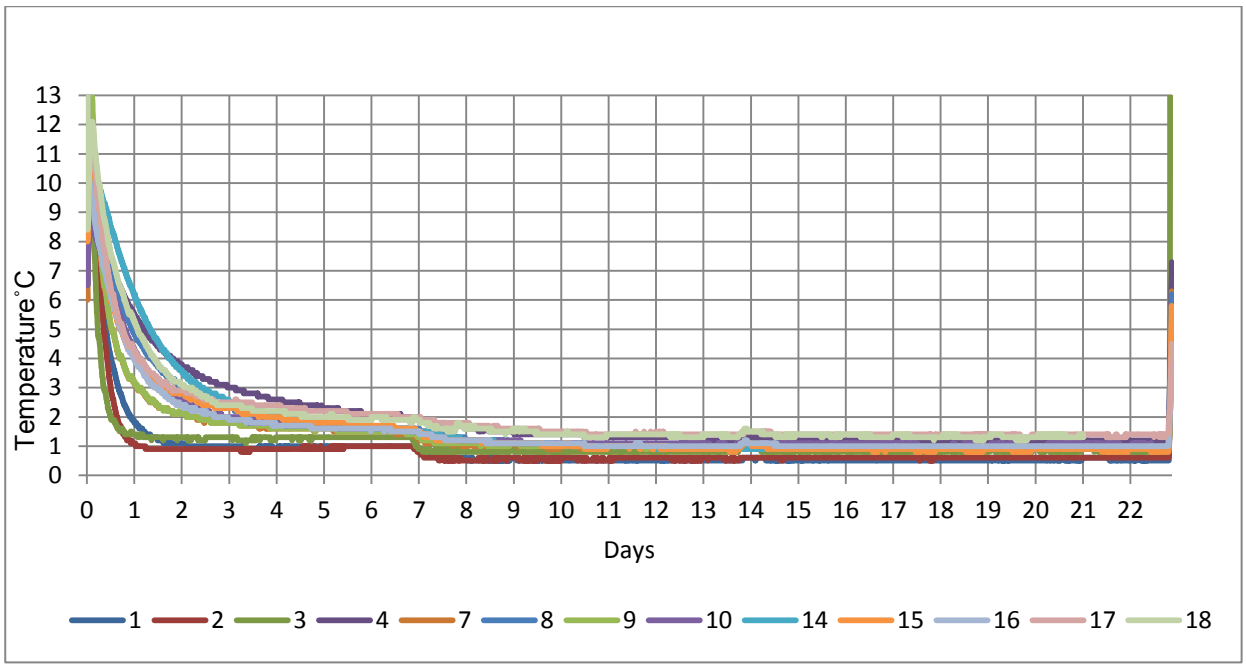

Figure 5.4.2.18. Pulp temperatures of grape fruit in a container fitted with RAF technology. Set point +2.0˚C lowered to + 1.0˚C after seven days.

Table 5.4.2.6. Results of measurements on grapefruit packed in open top cartons.

Treatment	Start temperature °C	Time to 2°C in days	Mean pulp temperature °C	Relative humidity %	SD of the mean temperature
Control container	10.5	4.0 Range 1.75 d - 7.5d	2.2	86	3.3
Reversed airflow container	10.9	2.5 Range 0.5 d - 4d	2.2	95	3.1

Table 5.4.2.7. Results of measurements on grapefruit quality after 2 weeks in the container, followed by 4 weeks in a cold store at 7˚C, followed by 7 days shelf life at 20˚C.

Quality measurement	TSS %	% Total titratable acid	Decay %	Rind colour. CRI chart 34	Puffiness %	% Chilling injury
Control container	10.1	2.3	0	1	2.3	0.4
Reversed airflow container	10.4	2.65	0	1	0	0

The summary analysis in Table 5.4.2.6 showed that the reversed airflow technology worked effectively in that it pre-cooled the grapefruit faster than in the standard container and held the fruit pulp temperatures closer to set point. Once again the relative humidity in the container fitted with reversed airflow was slightly higher than that measured in the standard container. The reversed air flow technology had no negative effect on the quality of the fruit when compared to the control fruit at the end of storage and shelf life, Table 5.4.2.7.

Trials 6 and 7 (October-November 2012). Navels and Valencia's. In order to evaluate the ability of the Horizontal airflow kit to pre-cool citrus from ambient to < 2.0˚C and then maintain the temperature during a "Systems Steri protocol" a set of two trials was initiated. The reason that two trials were conducted was that there was no data available at that time in the industry of the effect of the "Super vent" cartons on the pre-cooling speed and subsequent en route fruit pulp temperature management. The two originally identical

containers available (one was structurally modified to reversed airflow technology) were used for the trial. The control container (standard) only could be used with the disposable horizontal airflow kit. Thus a trial was conducted to compare the efficacy of the horizontal air flow kit the horizontal air flow. Thus 40 pallets of Navels packed in "Super Vent" cartons by SRCC in the Sundays River Valley were loaned for the trial. After fitting pulp temperature recorders into the pallets of fruit as shown in Figures 5.4.2.6 & 7 the pallets were placed into the two containers for two weeks.

This was then followed by a trial wherein 40 pallets of Valencia oranges that were packed in "Super vent" cartons by Goede Hoop in Citrusdal were sent to Cape Fruit Coolers. The pallets, after the fitment of pulp temperature recorders (Figures 5.4.2.6 & 7), were placed in a control container and one fitted with reversed airflow technology. This allowed for a comparison of the reversed air flow technology as fitted to a structurally modified container with a standard container and the disposable horizontal airflow kit. The data on pre cooling times is shown in Tables 5.4.2.6 and 5.4.2.8. The quality data for the end of shelf life for the two trials is shown in Tables 9 and 11. There was no negative effect of the air flow technology on either the Navel or Valencia oranges.

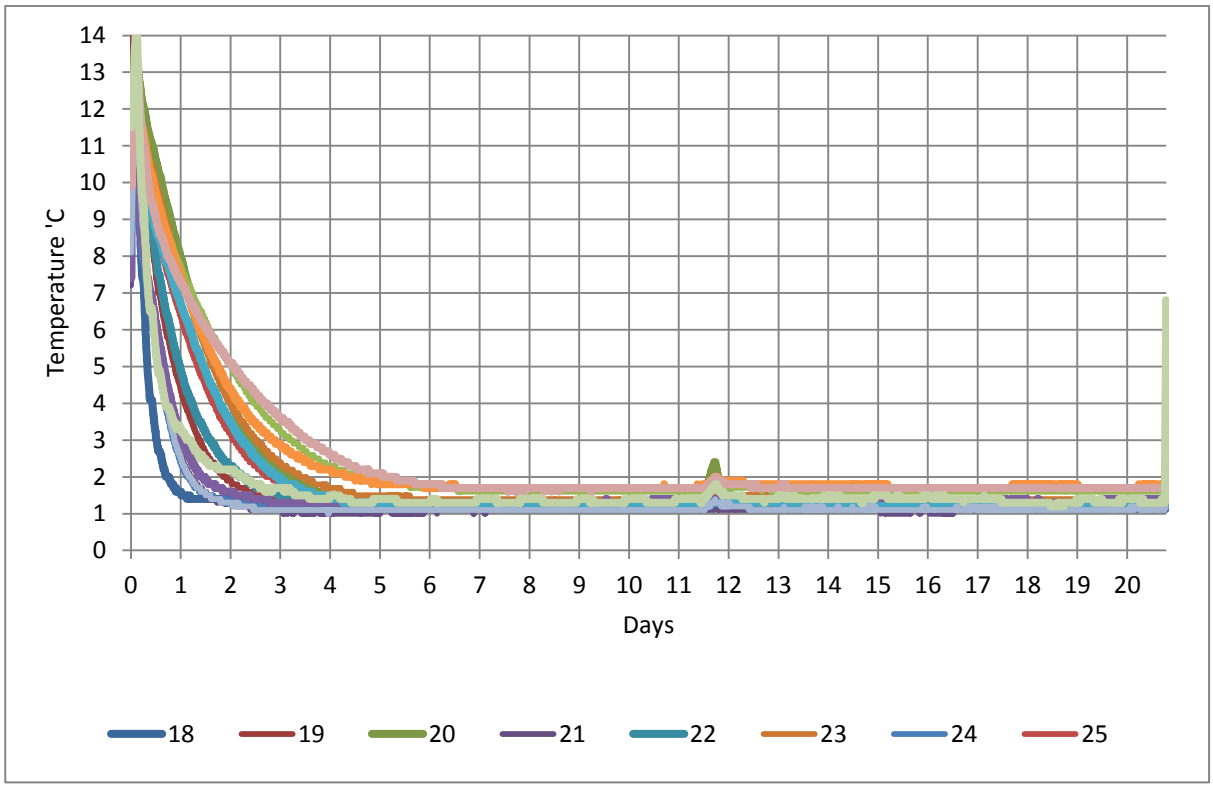

Figure 5.4.2.19. Pulp temperatures of Navels packed in "Super Vent" cartons in a standard container fitted with a disposable horizontal air flow kit. Set point +2.0˚C. Range 1.1 - 1.7C˚.

Table 5.4.2.8. Results of measurements on Navels packed in "Super Vent" cartons.

Treatment	Start ˚C	Lowest ˚C	Time to 2˚C	Relative humidity	Standard deviation of temperature
Control with Horizontal airflow kit	12.9	1.2	2.7 (0.6 – 4.3)	84	2.2
Reversed air flow	11.8	1.6	3.7 (1- 8.4)	85	1.9

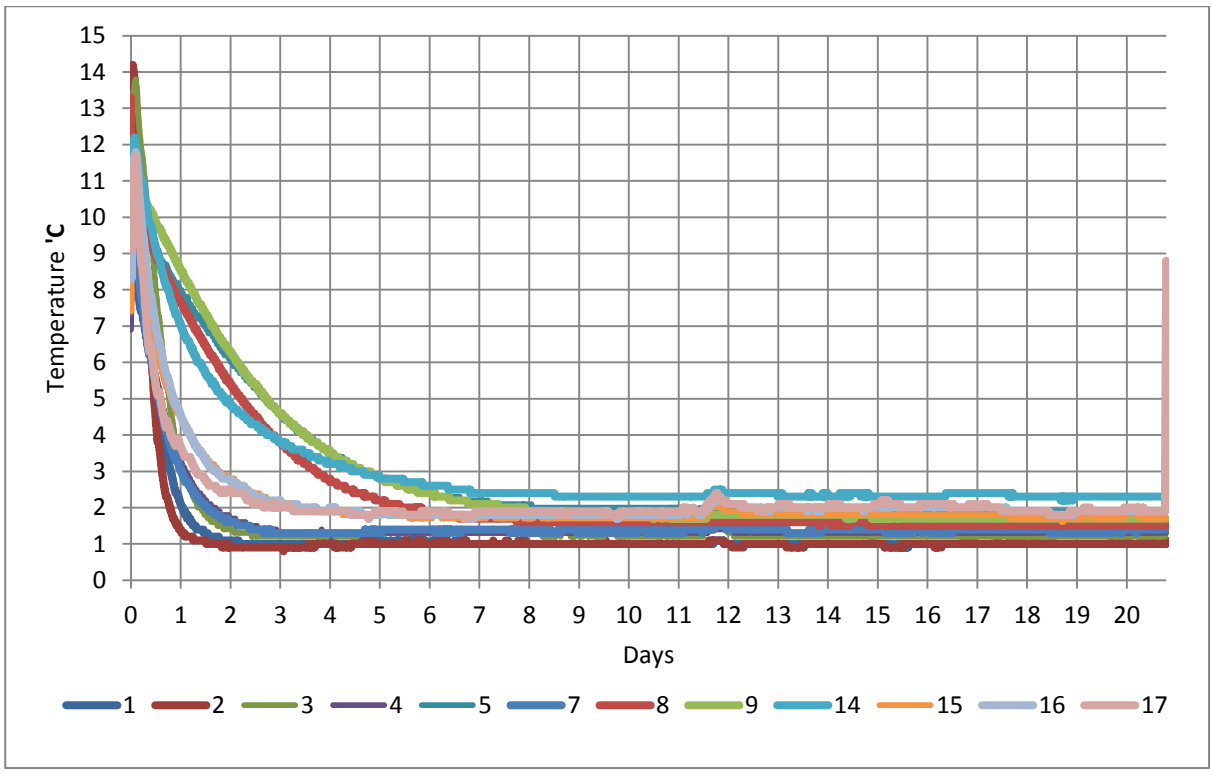

Figure 5.4.2.20. Pulp temperatures of Navels (Super vent cartons) in a container fitted with Reversed Air Flow technology. Set point +1.0˚C. 0.9 -2.4˚C.

Table 5.4.2.9. Results of quality measurements on Navels packed in "Super Vent" cartons. Stored for 3 weeks in the containers followed by one month at +3.5˚C followed by one week at ambient before end of shelf life examination.

Quality parameter	Rind colour CRI chart 34	TSS %	% Total titratable acid	% Rind pitting	% Rind breakdown	% Chilling injury	% Creasing	% Puffiness	% Disease
Control	1.32	10.8	1.7	1.32	0	0	1.02	0.8	0
Reversed air flow	1.43	10.9	1.9	0.4	0	0	2.4	0.7	0

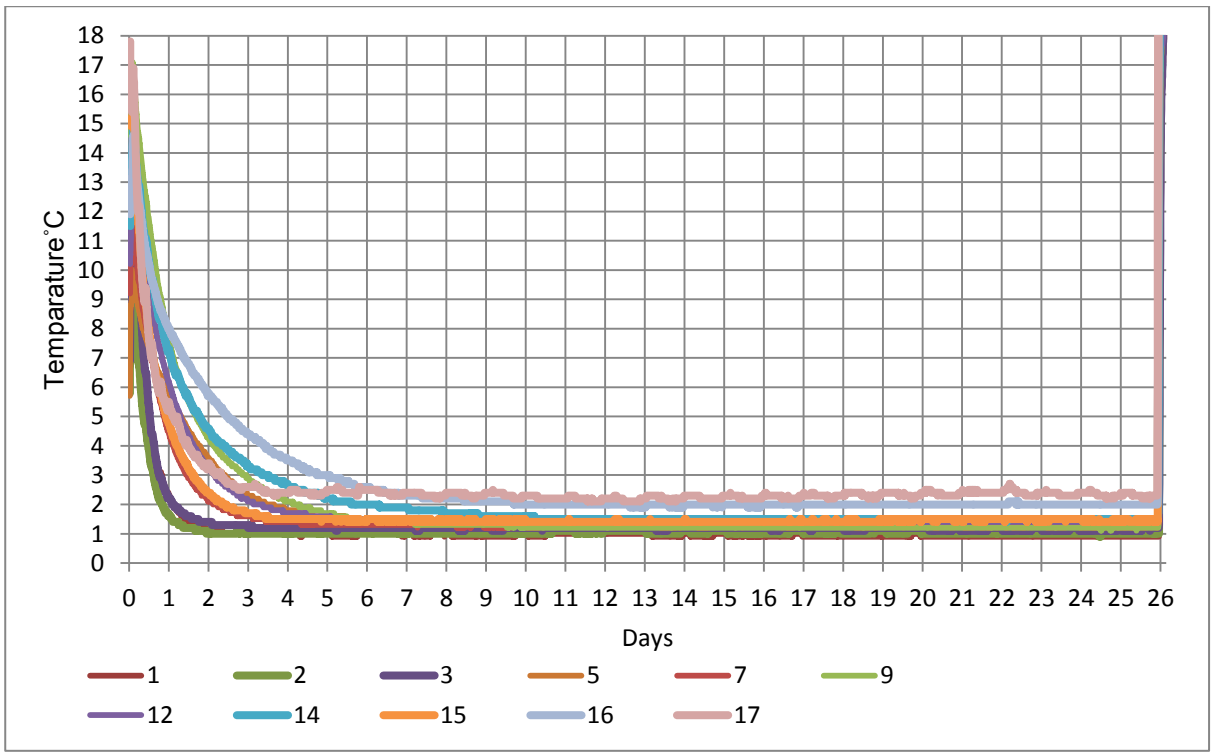

Figure 5.4.2.21. Pulp temperatures of Valencia's (Super vent cartons) in a container fitted with Reversed air flow technology. Set point +1.0˚C. Range 0.9 -2.4.

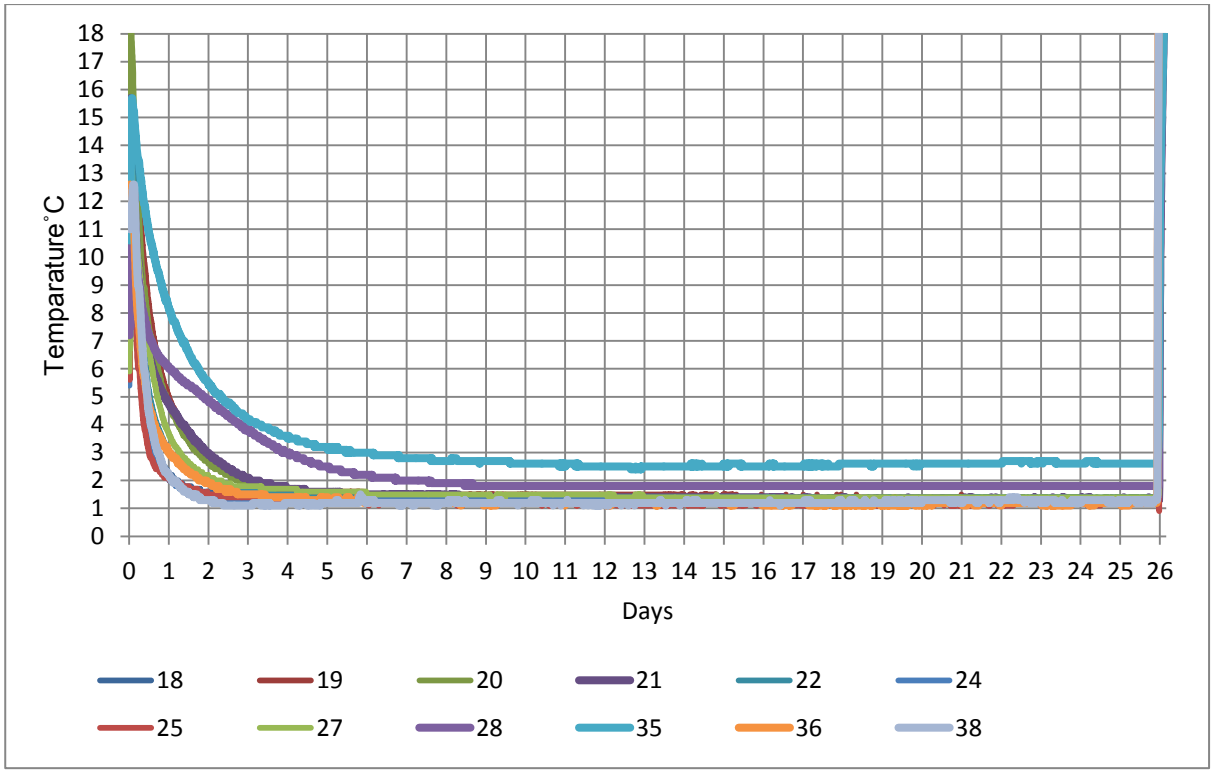

Figure 5.4.2.22. Pulp temperatures of Valencia's (Super vent cartons) in a standard container. Set point +1.0˚C. Range 0.9 -2.4.

Table 5.4.2.10. Analysis of pre-cooling time of Valencia oranges packed in "Super Vent" cartons. Set point + 1.0˚C.

Treatment	Start ˚C	Lowest ˚C achieved	Time to 2˚C Days	Relative humidity %	Standard deviation of the average temperature
Standard container	14.0	1.6	2.2 (1.1 – 6.7)	84	1.9
Container with modified air flow	13.5	1.5	3.6 (1 – 8.0)	85	2.1

A summary table enabling the comparison of the reversed air flow technology and the horizontal on the pre-cooling of Navels and Valencia's is shown in Table 5.4.2.12. It is of note to see that the horizontal air flow kit worked better than the structural modified container with the "Super Vent" cartons.

Table 5.4.2. 11. Results of quality measurements on Valencia's packed in "Super Vent" cartons. Stored for 3 weeks in the containers followed by one month at +3.5˚C, followed by one week at ambient before end of shelf life examination.

Quality parameter	Rind colour CRI chart 34	TSS %	% Total titratable acid	Rind Pitting %	Rind breakdown %	Chilling injury %	Creasing %	Puffiness %	Disease %
Control	1.7	11.1	1.6	3.8	1.5	2.03	3.6	0	0
Reversed air flow	1.6	11.1	1.4	0	0	11.24	4.0	0	0

Table 5.4.2.12. Summary of Navel and Valencia trials enabling a comparison of the standard container with the reversed air flow technology and the disposable horizontal air flow kit.

Treatment Set point + 1.0^{0}C	Start pulp temperature ˚C	Average time (days) to 2.0^{0}C	Lowest temperature achieved ˚C	Relative Humidity %	SD of the average temperature
Navels Horizontal air flow kit	12.9	2.7 (0.6 - 4.3)	1.9	85	2.2
Navels Reversed airflow	11.8	3.7 (1 - 8.4)	2.2	84	1.9
Valencia's Reversed air flow	14.0	3.6 (1.0 - 8.0)	1.5	85	2.1
Valencia's control	13.5	2.7 (1.1 - 6.7)	1.6	79	1.9

Also of note is the fact that the relative humidity was always higher with either of the airflow technologies (85%) compared to the control (79%), Table 5.4.2.12. The airflow technology in either form will enable the pre-cooling of citrus in containers for normal or systems steri procedures.

Trial 7 (October 2013) Valencias. With the prospect of some form of cold sterilisation protocol being introduced to South African citrus exports to the EU it has become strategically important to review how to achieve two important logistical steps:

1. Due to the lack of sufficient pre-cooling capacity within the citrus industry for all citrus, develop a technique enabling the pre-cooling of all citrus types in reefer containers. This procedure must be achieved within a 6 day time frame.

2. Once the required fruit pulp temperature is achieved, ensure that the cold steri pulp temperatures are maintained throughout the load for the prescribed duration of the procedure which would take place during the during the shipping procedure.

In order to establish the feasibility of achieving points 1 and 2 and what the required set point would be to achieve the pre-cooling within 6 days, without resorting to sub zero temperatures, as well as maintain the pulp temperatures of below 2˚C, a very detailed trial was initiated. This consisted of utilising the disposable horizontal airflow kit inside standard shipping container as illustrated in Figure 5.4.2.5. Twenty pallets of Valencia oranges were sourced from Goede Hoop Citrus. The fruit was packed in standard A15C cartons in Citrusdal and delivered to Cape Fruit Coolers. Each pallet was fitted with 3 pulp temperature recorders as shown in Figure 5.4.2.6, so there were a total of 60 recorders used. The numbering of the recorders was as shown in Figures 5.4.2.23 and 24. In addition there were three USDA pulp temperature probes inserted into fruit as per Figure 5.4.2.10.

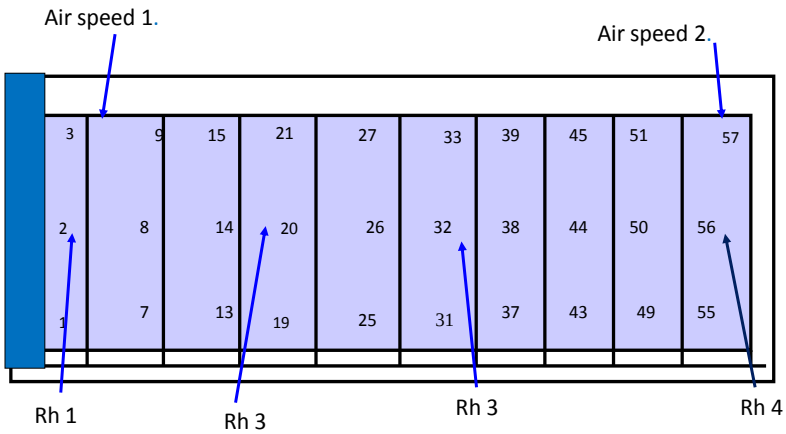

Figure 5.4.2.23. Pulp temperatures probe positions (Left hand side) of Valencia oranges in a container fitted with a horizontal air flow kit and subject to a "Systems Steri " protocol.

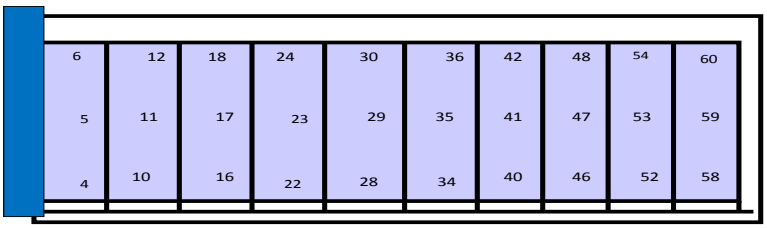

Figure 5.4.2.24. Pulp temperatures probe positions (right hand side) of Valencia oranges in a container fitted with horizontal air flow kit and subject to a "Systems Steri " protocol.

The trial was run for three weeks to ensure that there was sufficient time to pre-cool the citrus and hold the temperature at < 2.0˚C for 14 days as prescribed in the proposed "Systems Steri" procedure. Some of the results achieved from this trial are shown in Figures 5.4.2.25 - 26. The important data to note is the rate of pre- cooling of the fruit as well as the steady temperatures once the fruit has been cooled. There was a power interruption on day 7 for approximately 18 hours. Bearing in mind that the fruit was packed in standard A15C cartons the pre-cooling time with the horizontal airflow kit was acceptable.

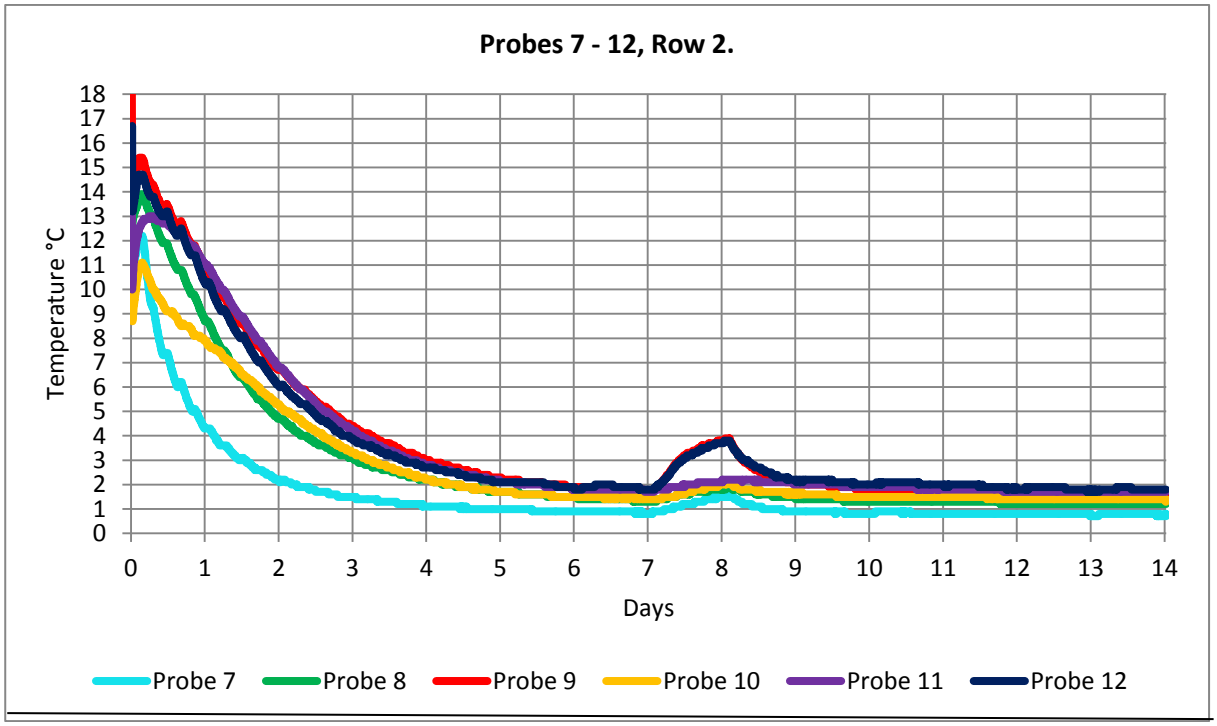

Figure 5.4.2.25. Pulp temperatures of Valencia's (A15C cartons) in a standard container fitted with a disposable horizontal airflow kit. Measurements taken in pallet row 2 from the front. Set point +0.5˚C.Temperature range 0.7 - 1.8˚C.

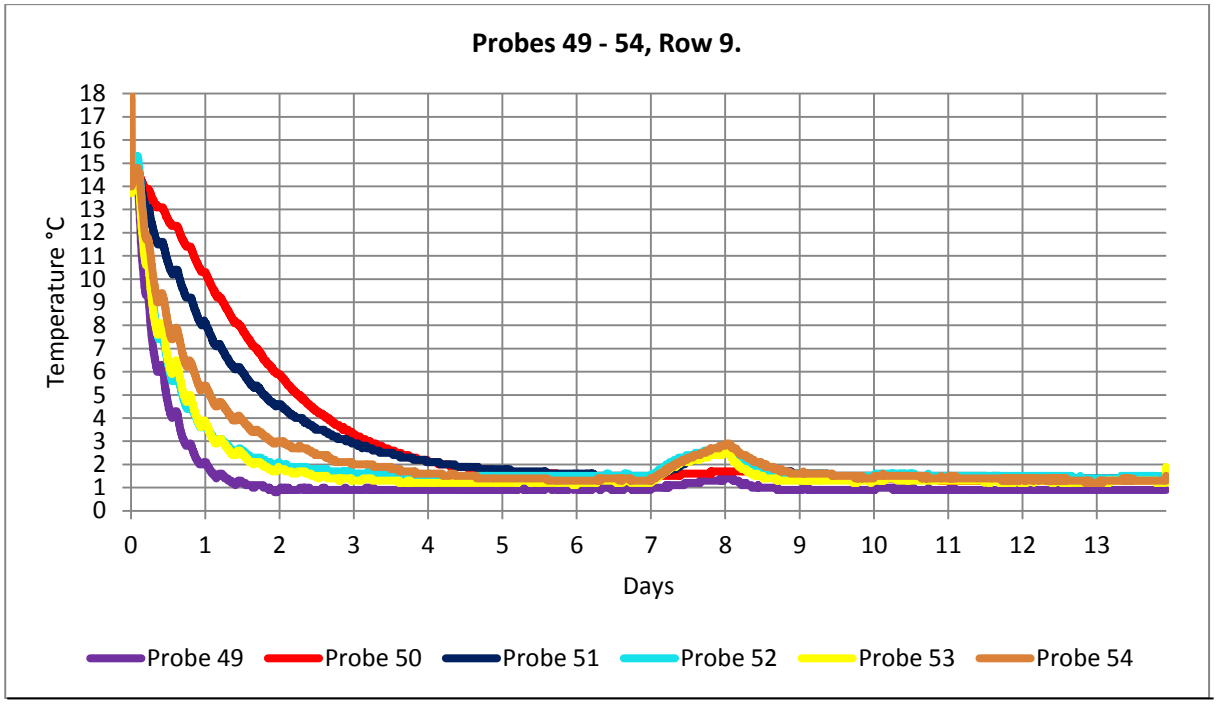

Figure 5.4.2.26. Pulp temperatures of Valencia's (A15C cartons) in a standard container fitted with a disposable horizontal airflow kit. Measurement in pallet row 9 from the front. Set point +0.5˚C. Temperature range 0.9 - 1.3˚C.

The data in Table 5.4.2.13 shows the range of time for pre-cooling from 12 hours to 144 hours. This pre-cooling time would have been much faster had the fruit been packed in the "Super Vent" cartons. The results are shown in Table 5.4.2.12, where the longest time to pre-cool to < 2˚C was 103 hours. The set point of -0.5˚C is the correct one to achieve an even pulp temperature of +2.0˚C within a time frame that would enable the application of the "Systems Steri" protocol.

The temperature profiles recorded separately by the USDA probes placed as per Figure 5.4.2.10 are shown in Figure 5.4.2.27. When the temperatures stabilise there is only a 0.3˚C difference between the recordings. This is very good management of pulp temperatures, particularly of probes 2 and 3.The thermal map in Figure 5.4.2.28 shows the pulp temperatures of the fruit once the lowest temperature was achieved. This illustrates how evenly the temperatures within the container are managed once they have been stabilised. If this fruit had been packed in "Super vent" cartons the temperatures would have been even more uniform throughout the load space.

Table 5.4.2.13. Results of analysis of the time to pre-cool the citrus from ambient (14.0˚C) to < 2˚C in a container fitted with a disposable horizontal air flow kit..

Average time in hours to cool the pulp temperature of 2˚C from 14.0˚C	Range of time in hours from fastest to slowest cooling position
87 (3.6 days)	12 - 130 (0.5 - 5.4 days)

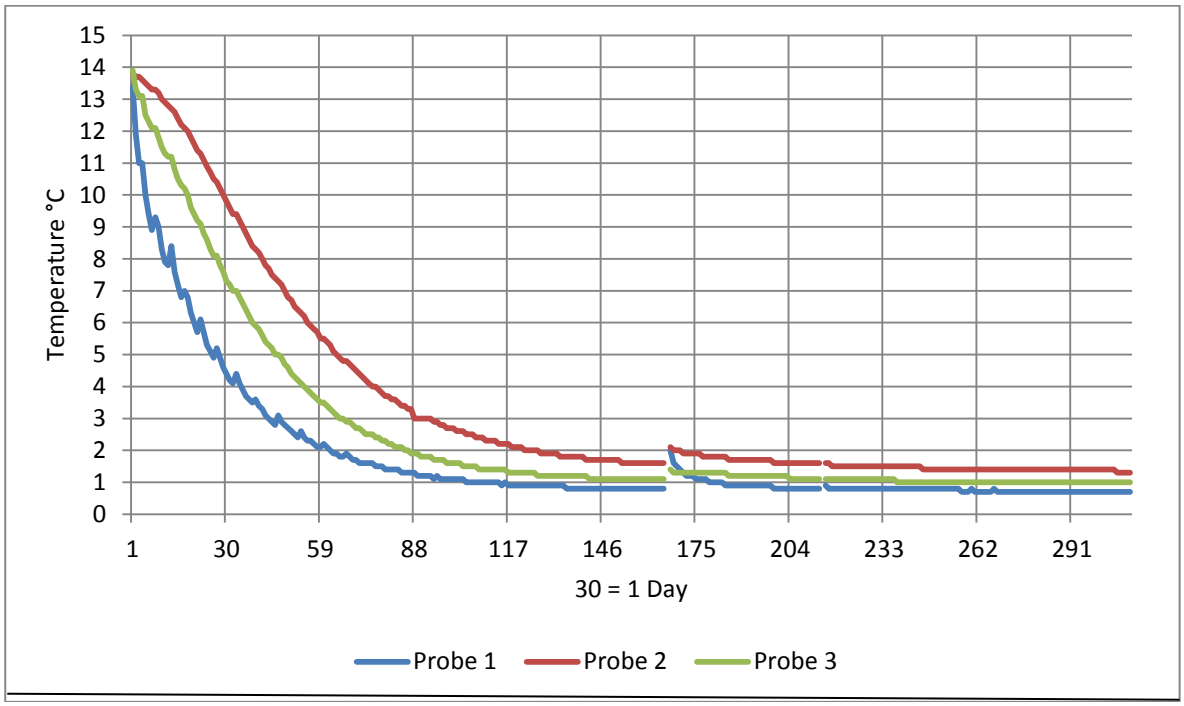

Figure 5.4.2.27. Pulp temperatures of Valencia's (A15C cartons) in a standard container fitted with a disposable horizontal airflow kit. Fruit temperature measurement by USDA probes. Set point +0.5˚C. Temperature range 0.7 - 1.0˚C.

Table 14. Results of quality measurements on Valencias packed in standard A15C cartons. Stored for 3 weeks in the container fitted with a disposable horizontal airflow followed by one month at +3.5˚C, followed by one week at ambient before end of shelf life examination.

Rind pitting %	Rind breakdown %	Creasing %	Colour CRI chart no 34.	Decay %	TSS %	% Total titratable acid
0.3	0	0.7	1.3	0	9.5	1.02

From the results achieved using a set point of +0.5˚C it will be possible to pre-cool the citrus throughout the 20 pallets inside a container within 130 hours (5.5 days) provided a disposable airflow kit is used. It would be recommended that "super vent" cartons be used which would ensure even quicker pre-cooling. This time

frame would allow for the "Systems Steri protocol" be applied within an acceptable time frame with part of the process being achieved en route on the vessel to the EU.

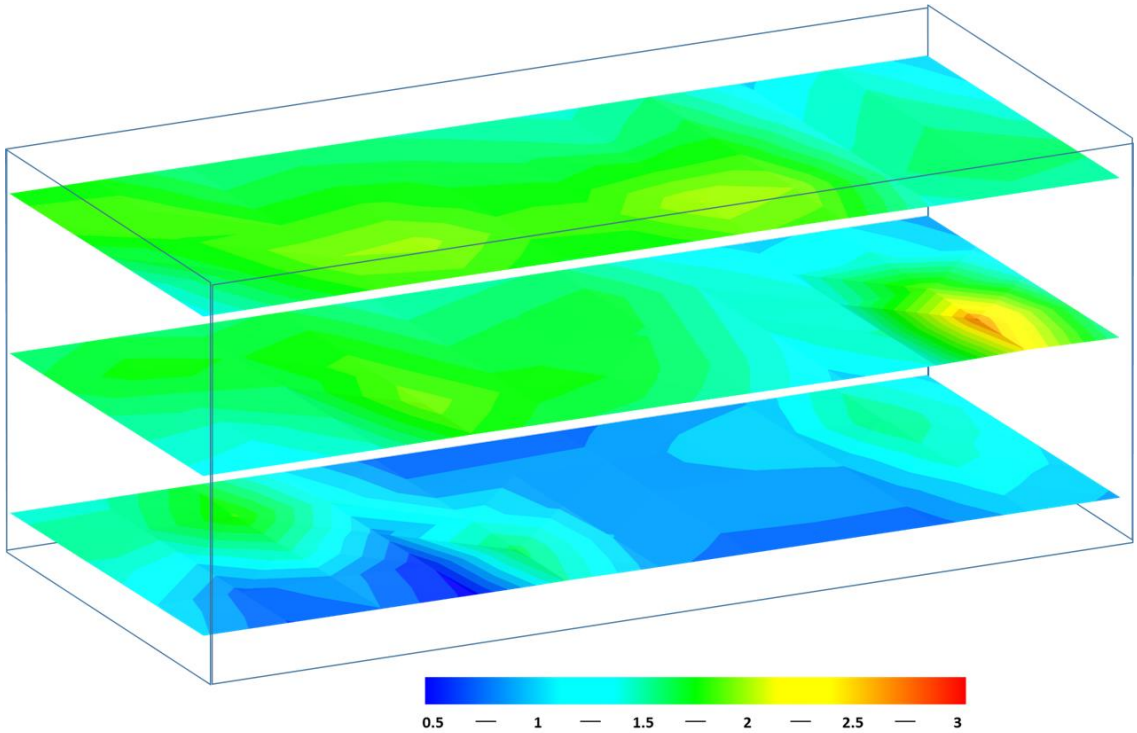

Figure 5.4.2.28. Thermal map of pulp temperatures of Valencia oranges packed in A15C cartons in a container fitted with a Horizontal air flow kit and subject to a "Systems Steri " protocol. The map is for the period from when the temperatures reached the lowest point after pre-cooling until the end of the trial. Set point +0.5˚C. Door opening on the right hand side of the drawing.

Conclusion

The reversed air flow technology shows that with fairly simple modifications to the delivery of the chilled air inside shipping containers a large difference can be made to the maintenance of fruit pulp temperatures closer to set point. In all cases where the technology was tested in back to back trials the reversed air flow resulted in temperatures closer to the set point, with less variation between the coolest and warmest fruit and an elevation of the relative humidity. The technology also enables faster and more even pre-cooling of citrus and hold the temperature at < 2.0˚C for 14 days as prescribed in the proposed "Systems Steri" procedure. There were no large differences recorded in the energy consumption in back to back trials in any of the tests conducted. Sometimes the standard container used slightly less energy than the modified one. On other occasions it was the other way around. So at this stage of development the technology cannot claim to have any impact on the energy consumption.

The technology will enable the citrus industry to pre-cool oranges, grapefruit and lemons inside a suitably modified container or with a horizontal air flow kit. The management of the pulp temperatures in cold steriisation procedures was shown to be much easier The reversed airflow technology has been registered by Stellenbosch University innovation office as an international patent (PCT/IB/001811).

Future research

The airflow technolgy can be built into new refrigerated containers. This could be done at no additional cost as there would be no need for "T" bar floors which are made of +/- 750 kg of extruded aluminium and is the second most expensive component of a container after the refrigeration unit. Grooved floors would be sufficient, provided structural strength was in built. This would also free up load space within the container which will be of benefit to commodities that are not as heavy as citrus. To maximise the benefit of the technology it will be necessary to convince the owners of shipping containers to build the technology into new containers. This will take a large amount of effort because the shipping lines collectively own 1,2 million reefer containers with an average value of around US$ 10,000.00. This is a considerable investment and

consequently the shipping lines are extra-ordinarily resistant to change because they wish to protect their current assets. Change will only come through requests and pressure from their customers. In the meantime the disposable horizontal air flow kit works almost as well as the structurally modified container. The cost of this kit is R 833.00 and it is available from Cordstrap SA.

In order to convince the owners of reefer containers that building this technology into containers is feasible it will be necessary to secure funds from an organisation such as TIA with a view to taking the frame and refrigeration unit of an existing container and rebuild it with a new floor and the correct air delivery and ducting system to enable reversed air flow technology. Once this has been achieved and live trials conducted it would be much easier to convince the owners of containers to consider the technology.

Technology transfer

1. A refereed publication; Dodd, M.C. (2012). Managing Airflow inside Reefer Containers Benefits Produce Quality. Acta Horticulturae No 1012, 1159-1167.
2. Presentation given to the CRI Postharvest Workshops 2011. What is happening in the last mile of your supply chain? M C Dodd.
3. Presentation given at the 7 th CRI Technical Symposium, August 2012. Managing air flow inside reefer containers benefits citrus fruit temperatures and relative humidity of the storage air. M C Dodd.

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Oosthuyse, S. A. 1997. In transit temperature relationships of two shipping containers loaded with mangoes. S. A. Mango Growers Association.

5.4.3 **PROGRESS REPORT: Using Radio Frequency Identification Technology (RFIT) to get an understanding of the storage air and fruit pulp temperatures and relative humidity in a typical South African citrus fruit export supply chain from the very beginning to very end over two seasons**

Project C2/12 (2012/3 – 2013/4) by M.C. Dodd (SU)

Summary

This was a co-operative project between the Department of Horticultural science, Stellenbosch University, Sainsbury's in the UK and BT9tech in Israel. The majority of the funding came from the Post Harvest Innovation Programme. The project set out to collect fruit pulp temperatures and storage air relative humidity of soft citrus (Satsuma mandarin and Nadorcott) from a packhouse in the Western Cape through to a Sainsbury's supermarket in the UK. The data were collected by the BT9tech radio enabled recorders which were installed in two pallets per container per shipment. The temperature and relative humidity information was transmitted via a previously installed radio network onto the internet. With this technology it was possible to identify from what position within the cold supply chain the data had come from. The data from eleven shipments collected over two seasons showed that, in the particular supply chain studied, there were no major temperature or relative humidity breaks or deviations from protocol and there were no quality issues recorded with any of the shipments. The conclusion was that the soft citrus cold supply chain from the Western Cape to the UK is working to the standard required.

Opsomming

Hierdie was ŉ samewerkende projek tussen die Departement Hortologie, Universiteit van Stellenbosch, Sainsbury's in die VK en BT9tech in Israel. Meeste van die befondsing was afkomstig van die Na-oes Innovasiefonds. Die doelwit van die projek was om vrug pulptemperature en relatiewe humiditeit tydens opberging te monitor vir sagte sitrus (Satsuma mandaryne en Nadorcott). Monitering is gedoen vanaf ŉ pakhuis in die Wes-Kaap tot by ŉ Sainsbury supermark in die VK. Die data is versamel deur BT9tech se radio toegeruste opnemers wat in twee palette per houer per besending geïnstalleer is. Die temperatuur en relatiewe humiditeit inligting is na die internet versend via ŉ voorheen geïnstalleerde radionetwerk. Met hierdie tegnologie was dit moontlik om die posisie binne die koueketting waar die data oorsprong was, te identifiseer. Die data van elf besendings wat oor twee seisoene versamel is het aangedui dat in die voorsieningsketting wat bestudeer is, was daar geen noemenswaardige temperatuur of relatiewe humiditeit onderbrekings of protokol afwykings nie. Daar is ook geen kwaliteitsprobleme vir enige van die besendings aangeteken nie. Die gevolgtrekking was dat die sagte sitrus voorsieningsketting van die Wes-Kaap na die VK op die verlangde standaard funksioneer.

5.5 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator (Acting): Tim G Grout (CRI)

5.5.1 Programme summary

Citrus production is becoming more specialised with market demands and climate changing over time. Consumer requirements are moving towards fruit with good taste that peels easily (clean) and preferably without seeds. The demand for Grapefruit (Star Ruby) is declining (bitter taste, peeling more difficult, bigger fruit size) with a substantial increase in Soft citrus consumption (good taste, peels better, edible portions). The escalating increase in production costs requires the best varieties for specific climate areas in combination with suitable rootstock choices that will provide optimal production and quality over a financially rewarding lifespan. The research shows an escalating focus on Soft citrus, especially late Mandarin hybrid selections to maximise the production cycle that was evaluated in most of the climate areas (5.5.4, 5.5.7, 5.5.8, 5.5.16, 5.5.17, 5.5.18, 5.5.19). Valencia production remains important for the hot-dry and hot-humid areas where evaluations were completed on existing and new selections to expand the harvest period (5.5.2, 5.5.3, 5.5.5, 5.5.6). The importance of the most suitable rootstock was emphasised with the evaluation of the 42 different rootstocks on Delta Valencia (5.5.9). Navel production is declining, but evaluations were completed in the cooler production areas to assure optimal choices for new plantings (5.5.20, 5.5.21, 5.5.22). Grapefruit evaluations were limited to the hotter and very hot production areas with excellent quality fruit. Satsuma and Clementine evaluations were limited to the cooler areas in the Western and Eastern Cape where the best quality will be produced (5.5.14 and 5.5.15). It has been possible to separate most cultivars on a genetic basis using SSR markers except for the sweet oranges. It is hoped that SRAP markers will provide enough supplementary genetic information to separate these (5.5.23).

Programopsomming

Die verbouing van sitrus word meer gespesialiseerd met mark aanvraag en klimaat wat verander oor tyd. Verbruikers voorkeure verskuif na vrugte wat makliker eet (vinnig en skoon skil) met goeie smaak en verkieslik sonder saad. Die aanvraag na Pomelo's (Star Ruby) is besig om te daal (bitter smaak, skil moeiliker, groot vrugte) met ŉ aansienlike toename in Sagtesitrus verbruik (goeie smaak, skil makliker, aanvaarbare porsies). Met die toename in produksie kostes is dit krities om die regte variteit in die geskikte klimaats area te vestig met toepaslike onderstam keuse vir optimum produksie en kwaliteit oor ŉ belonende finansiele leeftyd. Die navorsing toon ŉ toenemende klemverskuiwing na Sagtesitrus, veral laat Mandaryn hibried seleksies om die produksie termyn te verleng, wat in meeste van die klimaats areas ge-evalueer word (5.5.4, 5.5.7, 5.5.8, 5.5.16, 5.5.17,5.5.18, 5.5.19). Valencia produksie bly steeds noodsaaklik vir die warm-droe en warm-vogtige areas waar evaluasies uitgevoer is op bestaande en nuwe seleksies om die oes periode te verleng (5.5.2, 5.5.3, 5.5.5, 5.5.6). Die noodsaaklikheid van geskikte onderstamme word beklemtoon en daar was evaluasies waar tot 42 verskillende onderstam kombinasies met Delta Valencia uitgevoer is (5.5.9). Nawel produksie is aan die afneem, maar evaluasies word uitgevoer in die koeler produksie areas om optimum keuses vir nuwe aanplantings te verseker (5.5.20, 5.5.21, 5.5.22). Pomelo evaluasies word beperk tot die warmer en baie warm produksie areas wat uitstekende kwaliteit verseker. Satsuma en Clemetine evaluasies word beperk tot die koeler areas van die Wes- en Oos Kaap waar die beste kwaliteit verbou kan word (5.5.14 and 5.5.15). Dit was moontlik om meeste kultivars van mekaar te skei op ŉ genetiese basis deur die gebruik van SSR merkers behalwe vir die soetlemoene. Daar word gehoop dat SRAP merkers voldoende genetiese informasie sal byvoeg om hierdie te onderskei (5.5.23).

5.5.2 PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)

Project 75 A by J. Joubert (CRI)

Opsomming

Seleksies wat hierdie seisoen volgens optimum rypheid van vroeg tot laat goed presteer het is soos volg vir hierdie vogtige warm produksie area. Weipe (eksperimentele seleksie) wat die Limpopo SL vervang het, word eerste ryp. Hierdie seleksie kom op ŉ jong boom ouderdom in drag, wat vinniger kontant vloei kan verseker. 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 produksie en vruggrootte produseer, en kan ook aangevul word deur Bend 8A1 en 8A2 (eksperimentele seleksies) as addisionele opsies om te plant. Alpha of Midknight, aangevul deur Gusocora en McClean SL verteenwoordig die middel van die Valencia seisoen vir hierdie area, gevolg deur Henrietta (min saad) en Moosrivier Late 1 (eksperimentele seleksie). Die later seleksies wat kan bydra tot die

keuse om die seisoen te verleng, kan bestaan uit Louisa (saadloos), en dan laastens opgevolg word deur die ultra laat Lavalle 2, wat groot vrugte produseer, goeie oes verseker met belowende interne kwaliteit.

Weipe, Henrietta, Louisa, Skilderkrans, Moosrivier Late 1 en Bend 8A2 is steeds eksperimentele/semi-kommersiele seleksies wat goed presteer. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

Summary

Selections that performed well in this season, according to optimal maturity from early to late in this hot, humid production area, are as follows. Weipe (experimental selection) replaced the Limpopo SL selection, and will start the season maturing first. This selection bears fruit precociously and will start cropping good yields on young trees, generating returns for your investment sooner. Turkey will follow, but bear in mind that the selection has a sensitive rind. Do not hang the fruit too long because the optimal picking period is no longer than 4-6 weeks.

Benny 2 would follow, with good production and fruit size, supplemented by Bend 8A1 and 8A2 (experimental selections) as additional options to plant. Alpha and Midknight, with the addition of Gusocora and McClean SL represent the middle of the Valencia season for this area, followed by Henrietta (low seeded) and Moosrivier Late 1 (experimental selection). The later selections can broaden the list of choices to extend the season, commencing with Louisa (seedless), followed by the ultra-late Lavalle 2, producing large fruit, excellent yield and promising internal quality.

Weipe, Henrietta, Louisa, Skilderkrans and Bend 8A2 remain experimental/semi-commercial selections that performed well. These selections should 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, Bend 8A 1&2, Benny 2, Gusocora, Henrietta, Lavalle 2, Louisa, McClean SL, Midknight (control), Moosrivier Late 1, Nouvelle La Cotte, Skilderkrans, Turkey (control) and Weipe at Esselen Nursery, 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 2013.

Selection	Rootstock	Year Planted	No. of trees
Alpha	CC	1996	1
Bend 8A1	SC	2003	1
Bend 8A2	SC	2003	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
McClean SL	C35	2001	1
Midknight	C35	2001	1
Midknight	MxT	2001	1
Moosrivier Late 1	SC	2003	1
Nouvelle La Cotte	MxT	2006	1

Skilderkrans	MxT	2006	1
Turkey	CC	1998	1
Weipe	CC	2011 (Topwork)	1

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 2013 season.

Alpha
Alpha produced medium to large fruit (count 72-56) on the trees this season: optimal fruit size for export Valencias. The selection developed less than 0.1 seeds per fruit this season, resulting in the seedless qualities of the Alpha Valencia. The crop on the trees was similar when compared to the previous season and was estimated at between 80 to 100 kg per tree. The trees' condition was very good on Carrizo rootstock, a well known rootstock with good replant qualities. The second evaluation this season was later on 26 July 2013, with the same external colour between T1 and T3 (colour development delayed, 2 to 3 weeks later). The fruit shape was fairly round with smooth skin texture and thin rind thickness, no navel end was visible and no thorns were on the bearing branches. Good internal quality was prominent for this selection, with Brix above 11 and juice above 60 (Table 5.5.2.3), complying with minimum export standards. Based on the internal quality results in Table 5.5.2.3, estimated maturity was middle of June to end of July.

*Bend 8A1&2
The opposite scenario in regards to fruit size appeared this season, where the fruit size on Bend 8A2 was bigger (between count 40 and 64), and Bend 8A1 smaller peaking between count 72 and 36. There was a significant decrease in seed content this season, where Bend 8A1 was seedless and Bend 8A2 peaked at 1.5 seeds per fruit. This season Bend 8A1 seems earlier maturing compared to Bend 8A2, with higher juice (57.8%) and Brix (10.0) levels, as well as a higher Brix: acid ratio of 9.0. External colour was similar in both selections between T1 and T3. Both selections complied with the export minimum. Based on the internal quality results in Table 5.5.2.3 estimated maturity was mid-July.

Benny 2
The fibre strength (rag) remains soft compared to the other Valencia selections, except for Turkey being softer. The fruit size was erratic this season, measuring from count 88 to 36. 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. There was no delay in external colour this season and with the second and third evaluations, colour development was between T1 and T2. Brix: acid ratio was above 10 and Brix above 11, complying with export standards. Seed count varied from 1.5 to 3.2 seeds per fruit. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as end of June to middle of July.

Gusocora
There was a delay in external colour when compared to the internal quality maturity, between T4 and T6 with average Brix of 9 and acid below 1.0%. Gusocora was completely seedless and bore a crop of 90 kg per tree, indicating the production potential of the selection. The second evaluation was completed by 26 July 2013 and the internal quality complied with the export standards, Brix: acid ratio above 10 but external colour T4 to T6. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end of July.

*Henrietta
There was a decrease in average seed count from 5 to 1.2 seeds per fruit. Henrietta cropped lighter this year compared to the previous year and averaged 50 kg per tree, with very good tree condition on MxT rootstock. Fruit size increased this season and varied from medium to large (count 72 – 48). Fruit shape was 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. Internal quality indicated that Henrietta matures late in the Valencia season, with an acid content of 1.3% and external colour T3. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid July to end July.

Lavalle 2
Yield production increased, measuring up to 100 kg per tree; one of the qualities of the Lavalle 2 selection. Another quality is good fruit size for a Valencia selection, and the fruit size varied from count 72 to 40; excellent for Valencia production. Lavalle 2 was planted on CC, the number one rootstock recently being planted in South Africa's citrus industry, developing a medium tree size with good internal quality. The higher acid level (1.6%) tested with the second evaluation, indicated that this selection was late maturing internally. There were 0.1 seeds per fruit present in the fruit evaluated this season. The internal quality complied with

the export standards, producing Brix levels of 10 and juice of 59%. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end August.

*Louisa

The yield of the trees increased from 30 to 50 kg per tree, setting a lighter crop compared to the other selection of the same age. The fruit size tended to be bigger, possible due to the lighter crop. The seed count decreased to 0.3 seeds per fruit, and at Group 91 the fruit remained completely seedless. 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 was yellow. With the second evaluation the acid levels was fairly high, indicating a late Valencia selection, although the external colour was already at T1. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end 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 was completely seedless this year. 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 to large (count 72-40).The internal quality was good with juice levels of 60%, Brix above 10 and acceptable acid levels (above 1.0%). Based on the internal quality results in Table 5.5.2.3 maturity was mid to end July.

Midknight (Control)

C35 in combination with Midknight resulted in a medium sized tree; one third smaller compared to the tree size of Swingle. The bud union looks very good; smooth with no signs of incompatibility. There were a few indications of C35 being incompatible with other selections, Turkey being one of them. The tree produced medium to large (count 88-56) fruit size. 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 60% and Brix 11. The acid level was higher this season, but complied with the export requirements (Max 1.8 to min 0.85). Based on the internal quality results in Table 5.5.2.3 estimated maturity was end of June to middle of July.

*Moosrivier Late 1

Moos Late 1 developed a very high acid level (1.45%) when the juice (57%) and Brix (10) content were ready for harvesting, and the external colour also developed up to T1-2. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag. Moos Late 1 had less seeds this season, decreasing from 3.8 to 0.8 seeds per fruit. Based on the internal quality results in Table 5.5.2.3 estimated maturity was from the end of July to the middle of August.

*Nouvelle La Cotte

Similar to the previous season the acid (1.5%) level remained fairly high when the external colour developed to T1-2. Fruit size improved and peaked from count 72 to 56, with a light crop on the trees. There were small thorns visible on the bearing branches of the tree. Fruit shape was round, rind smooth and fairly thin, peeled easily and fair flavour. Based on the internal quality results in Table 5.5.2.3, estimated maturity was the end of July to the middle of August.

*Skilderkrans

Skilderkrans increased the fruit size from small/large (count 88-40) to medium/large (count 72-40), possibly due to the light crop on the trees. The internal quality was good, with a lower acid content compared to the previous season of 1.32% at external colour T1, below the export maximum of 1.4% by the time of the second 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 was 1.5 seeds per fruit; a slight increase from last year. Based on the internal quality results in Table 5.5.2.3 estimated maturity was the middle to end of July.

Turkey (Control)

Fruit size picked up from last season to 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, fruit peeling easily and lower seed count per fruit of 2.7 on average. The internal colour was light yellow, and externally the fruit remained yellow up to completely 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 5.5.2.3, estimated maturity was the end of May to mid-June.

•Weipe

Weipe was bearing fruit for the first time this season and only one evaluation was possible due to limited fruit numbers. The fruit size peaked between large and extra-large (count 64-40), and the internal quality was average with low juice (49%) and Brix (9) levels. The fruit tends to develop low acid levels early in the season, similar to the previous Limpopo SL selection. Weipe seems to be precocious and bears fruit at a young tree age, a very valuable characteristic. Rind texture tends to be fairly coarse and the fruit seems more susceptible to wind damage. Based on the internal quality results in Table 5.5.2.3, estimated maturity was the middle to end of May.

Conclusions

The internal quality improved this season and all the selections evaluated complied with the export standards, with the exception of the late maturing Lavalle 2, Louisa and Moosrivier Late 1, where the acid levels were above 1.4%. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selections. Where the Brix: acid ratio was below 7:1 it was often associated with later maturing selections having higher acid levels. When the acid levels decrease, the ratio will increase. There was a better colour development present with most of the selections. The average seed count for this season decreased, indicating less cross pollination in the mixed trial block. Fruit size increased on the trees, between count 88 and increasing up to count 40 on selections with lighter yields.

*This was the second evaluation of Bend 8A1&2, Henrietta, Louisa, Moosrivier 1&2, Nouvelle La Cotte and Skilderkrans at this trial site, so information is limited and future evaluations will improve recommendations on these varieties.

•This was the first evaluation of Weipe at this trial site, so information is limited and future evaluations will improve recommendations on these varieties.

Table 5.5.2.3. Internal fruit quality data for Valencia and late orange selections at Esselen Nursery (Malelane) during the 2013 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	2013/06/19	75-83	72-56	60.1	11.0	1.48	7.4	0.1	T3-4
Alpha	CC	2013/07/26	72-89	72-48	61.9	11.0	1.27	8.7	0.0	T1-3
Bend 8A1	SC	2013/07/26	76-107	72-36	57.8	10.0	1.11	9.0	0.0	T1-2
Bend 8A2	SC	2013/07/26	79-91	64-40	55.8	9.5	1.15	8.3	1.5	T1-3
Benny 2	C35	2013/04/30	70-90	88-40	53.8	8.6	1.33	6.5	1.6	T6-8
Benny 2	C35	2013/06/19	79-98	64-36	57.2	10.5	1.06	9.9	3.2	T1-2
Benny 2	C35	2013/07/26	77-93	72-40	60.2	11.1	1.06	10.5	1.5	T1
Gusocora	SC	2013/06/19	76-90	72-40	59.0	8.5	1.10	7.7	0.0	T6-8
Gusocora	SC	2013/07/26	74-89	72-48	60.7	9.2	0.95	9.7	0.0	T4-6
Henrietta	MxT	2013/06/19	77-89	72-48	57.8	9.2	1.28	7.2	1.2	T3
Lavalle 2	CC	2013/07/26	74-90	72-40	58.9	10.1	1.59	6.4	0.1	T3-5
Louisa	MxT	2013/06/19	72-96	88-40	56.6	10.6	1.71	6.2	0.0	T3-4
Louisa	MxT	2013/07/26	74-88	72-48	55.5	11.3	1.44	7.8	0.3	T1
McClean SL	C35	2013/06/19	76-88	72-48	58.1	10.0	1.15	8.7	0.0	T3-4
McClean SL	C35	2013/07/26	79-92	64-40	59.7	10.3	1.08	9.5	0.0	T1-3
Midknight	C35	2013/07/26	79-96	64-40	58.4	10.4	0.94	11.1	0.0	T1-2
Midknight	MxT	2013/06/19	68-86	88-56	60.4	12.0	1.44	8.3	0.0	T1-2
Midknight	MxT	2013/07/26	72-85	88-56	58.8	13.0	1.35	9.6	0.3	T1
Moosrivier Late 1	SC	2013/07/26	74-93	72-40	57.1	9.8	1.45	6.8	0.8	T1-2
Nouvelle La Cotte	MxT	2013/06/19	75-84	72-56	57.8	10.1	1.44	7.0	1.0	T3
Nouvelle La Cotte	MxT	2013/07/26	77-86	72-48	61.7	11.0	1.30	8.5	0.4	T1-2
Skilderkrans	MxT	2013/06/19	77-88	72-48	56.7	10.0	1.60	6.3	1.3	T3
Skilderkrans	MxT	2013/07/26	79-92	64-40	58.0	11.1	1.32	8.4	1.7	T1

Turkey	CC	2013/04/30	73-89	72-48	55.7	9.7	1.26	7.7	2.5	T6-7
Turkey	CC	2013/05/17	72-85	88-56	55.7	10.5	1.15	9.1	2.9	T5
Turkey	CC	2013/06/19	68-89	88-48	56.6	10.5	0.92	11.4	2.8	T3-4
Weipe	CC	2013/05/17	79-92	64-40	49.4	9.3	0.95	9.8	0.0	T3-4

5.5.3 **PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele)**
Project 75 B by J. Joubert (CRI)

Opsomming

Die seisoen word van vroeg tot laat rypwordende seleksies opgedeel in die warm droë produksie area en aanbevelings is soos volg. Die seisoen kan begin word met Turkey, wat groot vrugte produseer met goeie interne kwaliteit en sagte vessel. 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. Gusocora volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings bokant 11. Midknight 1 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vruggrootte, gladde skille en lae saadtellings per vrug. Lavalle 2 is tot op datum die laatste Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vruggrootte en goeie produksie op die bome.

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 Bend 8A2, hierdie seleksie kan ook as aanvulling saam met Benny 1 en 2 gebruik word. Die middel van die Valencia seisoen kan aangevul word met Jassie en Henrietta, wat goeie produksie lewer, asook goeie kwaliteit vrugte. Louisa word meer aan die einde van die Valencia seisoen ryp, is ook totaal saadloos met groot vruggroote, gevolg deur Ruby en Skilderkrans. Ruby is die enigste rooi gepigmenteerde Valencia huidiglik beskikbaar met uitstekende produksie op die bome, kleiner vruggrootte en tot vier 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 area. Recommendations have therefore been made accordingly. The season starts with Turkey, 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. Gusocora follows next with completely seedless fruit and very good Brix: acid ratio's above 11. Midknight 1 covers the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Lavalle 2 is currently the latest maturing Valencia selection that is being planted semi-commercially; developing excellent fruit size and yield.

There is a series of experimental/semi-commercial selections that have also been included in the hot production areas. The selection range will follow from early, mid, to late-maturing options. The season starts with Bend 8A2, this will be an additional selection 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 matures more towards the end of the Valencia season, is completely seedless with large fruit size, followed by Ruby and Skilderkrans. Ruby is the only red pigmented Valencia available with excellent yield production, smaller fruit size and four seeds per fruit. Late in the season you could possibly add Moosrivier Late 1 to the options, when more information becomes available from future evaluations.

5.5.3 **PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele)**
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The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production area. Recommendations have therefore been made accordingly. The season starts with Turkey, 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. Gusocora follows next with completely seedless fruit and very good Brix: acid ratio's above 11. Midknight 1 covers the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Lavalle 2 is currently the latest maturing Valencia selection that is being planted semi-commercially; developing excellent fruit size and yield.

There is a series of experimental/semi-commercial selections that have also been included in the hot production areas. The selection range will follow from early, mid, to late-maturing options. The season starts with Bend 8A2, this will be an additional selection 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 matures more towards the end of the Valencia season, is completely seedless with large fruit size, followed by Ruby and Skilderkrans. Ruby is the only red pigmented Valencia available with excellent yield production, smaller fruit size and four seeds per fruit. Late in the season you could possibly add Moosrivier Late 1 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 Bend 8A 1&2, Benny 1&2, Gusocora, Henrietta, Jassie, Lavalle 1, Louisa, Midknight 1, Moosrivier Late 1 and 2, Ruby, Skilderkrans and Turkey at Group 91, Letsitele.

Table 5.5.3.1. List of Valencia selections evaluated at Group 91 (Letsitele) during 2013.

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
Gusocora	RL		Semi-Com
Henrietta	CC/RL	2006	Semi-Com
Jassie	CC/SC	2005	10/7
Lavalle 1	CC	2005	100
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

Results and discussion

Bend 8A1

Bend 8A1 on Carrizo produced 60-70 kg per tree, compared to Swingle with 80 kg per tree and the tree condition on both combinations was average this season (dropping leaves). Carrizo develop small to large fruit (count 105 to count 40) and Swingle medium fruit size, counts 88 to 64. The internal quality improved with higher juice levels on both rootstocks (57%), Brix ranged from 11.2 up to 12.2, acids were lower after the second evaluation and Brix: acid ratios above 8. Fruit characteristics of Bend 8A1 were a fairly round fruit shape, fairly smooth rind, fruit peels easily, the fibre strength is fairly soft and the internal colour light yellow. The average seed count per fruit was 2.5 for Bend 8A1 when compared to 1.8 seeds per fruit for 8A2. Maturity seems to be mid July to beginning of August (Table 5.5.3.2).

Bend 8A2

Bend 8A2 performed well and internally produced a slightly higher juice content (58%) than 8A1, higher acids (1.42%) and average Brix:acid ratios (8.1:1). The crop on both rootstock selections was better compared to Bend 8A1. Trees on Carrizo averaged 70 to 80 kg per tree with medium to large fruit size (count 72-48), and on Swingle 80 kg per tree with medium fruit size (count 88-64). 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 was low, between 1.1 and 2.6; on average lower than 8A1. Fruit maturity was estimated at mid July and beginning of August (Table 5.5.3.2).

Benny 1 and 2

Benny 1 and 2 produced an average yield of 40 to 50 kg per tree on Carrizo and 80 kg per tree on Swingle (decrease in crop production). Fruit size on both selections peaked between count 88 and 56/48. 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 internally produced higher juice levels (avg. 57.1), acid (1.6%) and seed counts (avg. 2.5 seeds per fruit) compared to Benny 2, whereas Benny 2 produced higher Brix (avg. 11.1), and Brix:acid ratio (7.3:1). 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 July to beginning of August (Table 5.5.3.2).

Gusocora

Gusocora was completely seedless and developed a good internal quality where juice (51.6%), Brix (9.1) and acid (0.83) were on the lower side, but still complied with export requirements. The external colour varied from T1 to T4, in sequence with the internal quality and Brix: acid ratio of 11. 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 Rough lemon rootstock induces good yields but average internal quality. From the ratio on this date it is apparent that Gusocora's maturity is mid July.

Henrietta

Fruit size increased and peaked at count 88 to 56; good size for Valencia production and 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 high juice (58%), Brix (11.7) and acid (1.56%) levels; and on Rough lemon the juice (54%), Brix (9.4) and acid (1.05%) levels were average; by the second evaluation. Henrietta on Carrizo (rootstock inducing high internal quality) indicated the shelf life potential of the selection. There were 0.35 seeds per fruit counted on average, lower compared to the 2012 season. The external colour of the fruit developed into a deep orange, very favourable for the export markets. Maturity was end of July to beginning of August.

Jassie

Fruit size increased and peaked between count 88 and 48 on both rootstocks. Production on Carrizo was 60 kg per tree and on Swingle 80-90 kg. This season Carrizo produced a higher Brix (12.0) and acid (1.37%) level in combination with Jassie, where Swingle produced a higher juice (59.7%) level and seed count (3.1 seeds per fruit). Fruit shape was round with a smooth rind texture, internal colour was light yellow, juice flavour varied between very good on Carrizo to excellent on Swingle. 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 was end of July to middle of August in this area.

Lavalle 1

This season Lavalle produced 0.5 seeds per fruit compared to last year's 3. The yield on the trees averaged 100 kg; very good when you consider the tree size of 3.5 metres in height. The internal quality complied with export requirements, except for the acid level being on the higher side (1.52%), but 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. From the ratio on this date it is apparent that Lavalle 1 maturity is end of August to end of September.

Louisa

The fruit set was lighter compared to Henrietta, trees were bearing 60 kg per tree. Fruit size was optimal for Valencia production between counts 72 and 56. Internal quality complied with the requirements, although the juice content of 54% was low and the acid level of 1.5% indicated that Louisa qualified as one of the late 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 2.1 m. Acid levels and ratios indicate that this cultivar matures middle of August to middle of September.

Midknight 1

Midknight 1 on Carrizo bore a yield of 60 kg per tree and on Swingle 80 kg per tree, considering that the Swingle tree was bigger than the Carrizo. The fruit size on both rootstocks varied between count 88 and 56, juice content around 55%, Brix levels around 11.5 and acids at 1.2%. Swingle outperformed Carrizo with a slightly better Brix:acid ratio. The fruit on Carrizo was completely seedless, compared to Swingle with 0.1 seeds per fruit at the last evaluation. Fruit shape was round; rind texture was fairly smooth; fruit was raggy with a medium rind thickness and peeled moderately. Midknight 1 developed very good internal quality early in the season with ratios indicating maturity to be end-July to middle August.

Moosrivier Late 1 and 2

Moos Late 1 on both rootstocks produced medium to large fruit size (count 88-48), and Moos Late 2 on both rootstocks medium to large/very large fruit size (count 88-40). Crop production for Moos Late 1 on Carrizo was 40-60 kg per tree and on Swingle 80 kg per tree, compared to Moos Late 2 on Carrizo being 20 kg and on Swingle 30 kg per tree. Moos Late 2 decreased in crop production and increased in fruit size up to count 40. 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, except for high acids (1.7%) indicating a late maturing Valencia selection. The seed count per fruit varied from 1.2 up to 1.8 (a decrease from 2012). 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 later maturing selection, estimated maturity end-August to mid-September. Moosrivier Late 2 matured end-July to mid-August, with delayed external colour (T1-5).

Ruby

Ruby performed similarly on both rootstocks with Brix content of 11.2 up to 12, juice levels of 58% and acids around 1.5%. External colour on the fruit was between T1 and T2. Fruit size increased from 2012 (count 105-64) to 2013 (count 88-64) and produced a good crop on the trees (60-70 kg), bearing in mind the relatively small tree size (compact tree). Seeds per fruit varied from 1.8 up to 4.4. 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-July to mid-August.

Skilderkrans

Skilderkrans at Group 91 cropped well this season and improved with a yield on both rootstocks between 80 and 100 kg per tree (2012 – 20 to 30 kg per tree), with an average tree size of 3.5 m high. Fruit size varied from medium to large (count 88-56); excellent for Valencia production as well as in combination with the bigger crop on the trees. Internally the Brix content was good (11.7) and the acid level of 1.3 to 1.4% indicated a later maturing Valencia selection. Juice level increased to average 56.7; 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 indicate maturity to be mid-August to early September on both rootstocks.

Turkey

Turkey was planted on three rootstocks, Carrizo, Swingle and C35. All three combinations performed well; fruit size distribution from medium to large/very large (count 88-40), high Brix content (above 11.7), fairly high acid levels (1.4%) and lower Brix:acid ratio of 8.1:1 and average seed count per tree of 4.1 seeds (evaluation was early in the season). The external colour was similar on all three rootstocks between T1 and T2 in the beginning of June. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 produced 45 kg per tree, Carrizo produced 70 kg per tree and Swingle produced 80 kg per tree. Tree height measured 2.3 m on C35, 3 m on Swingle and 3.5 m on Carrizo. Based on the ratios, maturity was end-May for C35 and mid to end of June for Carrizo and Swingle.

Conclusion

Yield production of Bend 8A2 was higher (average 70 to 80 kg per tree) compared to Bend 8A1 (average 60 to 70 kg per tree. Bend 8A2 produced fruit with higher juice (58%) and acid levels (1.4%) as well as lower seed counts per fruit. Fruit size on 8A2 was bigger (counts 88-64), Bend 8A1 had small to large fruit (counts 105-40). Benny 1 and 2 produced similar qualities of fruit this season, as well as yield production of 40 to 80 kg per fruit on Carrizo and Swingle rootstock.

Gusocora performed well on Carrizo, meeting the export standards (acid on the lower side). Henrietta produced a bigger fruit size and peaked between count 88 and 56. The internal quality of the fruit was excellent on Carrizo and developed Brix as high as 11.7 whereas Rough lemon peaked lower at Brix 9.4.

Jassie produced an excellent internal quality on Swingle (juice of 60%); bigger fruit size (count 88-48) optimum for Valencias and very good yield (70-90 kg per tree). Lavalle 1 was ultra-late maturing in August/September (acid 1.52%). The crop production peaked at 100 kg per tree.

Louisa was completely seedless, developed the ideal fruit size (count 72-56) for Valencias, setting a better crop on the trees (60 kg per tree), with a more compact tree development (2.1 m on Carrizo).

Midknight 1 developed good yields of good fruit size with very good internal quality. 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. With the first evaluations Carrizo's acid levels were higher compared to Swingle, although by the time the final evaluations were completed, acid levels were 1.23% and 1.24% (Carrizo must have had lower acids than Swingle??). Future evaluations will confirm the conclusion, but this was the third year with these results indicating that the Swingle combination matured before Carrizo.

Moosrivier Late 1 is later maturing when compared to Moosrivier Late 2, with high juice and acid percentages, but the external fruit colour on Moos Late 2 was delayed. Moosrivier Late 2 was completely seedless.

Skilderkrans performed excellently this season compared to the previous season, bearing a good crop with good internal quality, and less seeds (0.2 per fruit).

Ruby produced very good quality fruit, excellent yields on the compact tree size and bigger fruit size from count 105 to 64. Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered.

Table 5.5.3.2. Internal fruit quality data for Valencia orange selections at Groep 91 (Letsitele) during the 2013 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Bend 8A1	CC	2013/07/10	77-87	72-48	56.6	11.5	1.22	9.4	0.8	T3-4
Bend 8A1	CC	2013/07/30	67-91	105-40	57.5	12.2	1.24	9.8	1.9	T1
Bend 8A1	SC	2013/07/10	75-84	72-64	57.1	11.2	1.40	8.0	3.8	T1
Bend 8A1	SC	2013/07/30	72-85	88-64	55.7	11.3	1.35	8.4	3.4	T1
Bend 8A2	CC	2013/07/10	77-86	72-56	58.0	11.2	1.42	7.9	1.3	T1
Bend 8A2	CC	2013/07/30	77-87	72-48	57.8	11.6	1.28	9.1	2.1	T1
Bend 8A2	SC	2013/07/10	72-82	88-64	58.7	10.8	1.54	7.0	1.1	T1-2
Bend 8A2	SC	2013/07/30	68-82	88-64	58.1	12.0	1.44	8.3	2.6	T1
Benny 1	CC	2013/05/29	78-87	64-48	53.8	9.7	1.67	5.8	2.7	T1-2
Benny 1	CC	2013/07/10	79-88	64-48	56.7	10.7	1.41	7.6	1.5	T1-2
Benny 1	CC	2013/07/30	70-81	88-56	57.2	11.4	1.44	7.9	2.8	T1
Benny 1	SC	2013/05/29	74-81	72-64	55.6	9.8	1.92	5.1	2.5	T1-3
Benny 1	SC	2013/07/10	77-89	72-48	59.2	11.5	1.60	7.2	2.5	T1
Benny 1	SC	2013/07/30	73-82	72-64	59.9	11.6	1.42	8.2	3.1	T1
Benny 2	CC	2013/05/29	78-89	64-48	51.8	9.3	1.74	5.3	2.5	T1-2
Benny 2	CC	2013/07/10	76-90	72-48	56.0	11.2	1.43	7.8	0.9	T1
Benny 2	CC	2013/07/30	71-83	88-56	57.9	11.9	1.51	7.9	1.9	T1
Benny 2	SC	2013/05/29	75-83	72-56	54.7	10.6	1.68	6.3	3.2	T1-2
Benny 2	SC	2013/07/10	76-88	72-48	58.0	11.8	1.40	8.4	2.8	T1-2
Benny 2	SC	2013/07/30	71-81	88-64	56.3	11.7	1.44	8.1	3.3	T1
Gusocora	RL	2013/07/30	70-81	88-64	51.6	9.1	0.83	11.0	0.0	T1-4
Henrietta	CC	2013/07/10	75-80	72-64	57.0	11.5	1.45	7.9	0.0	T1-3
Henrietta	CC	2013/07/30	71-84	88-56	59.8	11.8	1.56	7.6	0.4	T1
Henrietta	RL	2013/07/30	74-84	72-56	53.6	9.4	1.05	9.0	0.3	T2-4
Jassie	CC	2013/07/10	73-86	72-56	55.9	10.8	1.44	7.5	2.2	T1
Jassie	CC	2013/07/30	72-80	88-64	56.5	11.4	1.45	7.9	3.0	T1
Jassie	CC	2013/08/27	78-85	64-56	57.0	12.0	1.37	8.8	3.6	T1
Jassie	SC	2013/07/10	77-87	72-48	56.8	10.8	1.44	7.5	1.9	T1-2
Jassie	SC	2013/07/30	72-83	88-64	57.4	11.4	1.42	8.0	3.3	T1
Jassie	SC	2013/08/27	74-82	72-64	59.7	11.8	1.35	8.7	4.0	T1
Lavalle 1	CC	2013/07/10	80-87	64-48	60.8	11.1	1.49	7.4	0.0	T1-3
Lavalle 1	CC	2013/07/30	74-91	72-40	58.6	11.3	1.55	7.3	0.5	T1
Lavalle 1	CC	2013/08/27	73-87	72-48	60.3	12.2	1.52	8.0	0.0	T1
Louisa	CC	2013/07/10	76-84	72-56	51.0	11.9	1.50	7.9	0.0	T1-3
Louisa	CC	2013/07/30	73-85	72-56	54.5	12.0	1.49	8.1	0.0	T1-2
Midknight 1	CC	2013/05/29	72-91	88-64	53.3	10.2	1.56	6.5	0.0	T1-2
Midknight 1	CC	2013/07/10	72-89	88-48	54.3	11.2	1.23	9.1	0.0	T1-2
Midknight 1	CC	2013/07/30	71-80	88-64	53.9	11.8	1.23	9.6	0.0	T1
Midknight 1	SC	2013/05/29	73-83	72-56	54.8	10.0	1.25	8.0	0.0	T1-2
Midknight 1	SC	2013/07/10	74-84	72-56	56.5	11.4	1.17	9.7	0.0	T1
Midknight 1	SC	2013/07/30	73-81	72-64	52.1	11.9	1.24	9.6	0.1	T1
Moosrivier Late 1	CC	2013/07/10	73-83	72-56	57.5	10.8	1.65	6.5	1.4	T1-2
Moosrivier Late 1	CC	2013/07/30	67-89	88-48	58.7	10.0	1.47	6.8	1.8	T1
Moosrivier Late 1	SC	2013/07/10	70-84	88-56	56.2	11.3	1.7	6.6	1.4	T1
Moosrivier Late 1	SC	2013/07/30	68-81	88-64	59.0	11.7	1.7	6.9	1.2	T1
Moosrivier Late 2	CC	2013/07/10	79-89	72-48	50.4	10.3	1.4	7.4	0.0	T3-5
Moosrivier Late 2	CC	2013/07/30	73-90	72-40	49.2	10.7	1.5	7.1	0.0	T1-5
Moosrivier Late 2	CC	2013/08/27	78-88	64-48	52.6	11.4	1.2	9.5	0.0	T1-3

Moosrivier Late 2	SC	2013/07/10	80-89	64-48	45.2	9.8	1.4	7.0	0.0	T1-3
Moosrivier Late 2	SC	2013/07/30	72-88	88-48	53.0	10.3	1.27	8.1	0.0	T1-5
Moosrivier Late 2	SC	2013/08/27	80-94	64-40	53.4	10.2	1.12	9.1	0.0	T1-4
Ruby	CC	2013/07/10	73-80	72-64	58.0	11.2	1.5	7.5	2.4	T2
Ruby	CC	2013/07/30	70-80	88-64	57.0	11.8	1.42	8.3	2.1	T1-2
Ruby	SC	2013/07/10	74-81	72-64	58.7	11.5	1.49	7.7	1.8	T3
Ruby	SC	2013/07/30	71-79	88-64	57.7	12.0	1.44	8.3	4.4	T1-2
Skilderkrans	CC	2013/07/10	75-84	72-56	54.5	11.2	1.36	8.2	0.0	T1
Skilderkrans	CC	2013/07/30	68-82	88-64	56.9	11.8	1.37	8.6	0.2	T1
Skilderkrans	CC	2013/08/27	76-85	72-56	57.3	12.0	1.28	9.4	0.0	T1
Skilderkrans	SC	2013/07/10	74-85	72-56	57.0	11.2	1.45	7.7	0.0	T1
Skilderkrans	SC	2013/07/30	72-82	88-64	57.0	11.8	1.36	8.7	0.3	T1
Skilderkrans	SC	2013/08/27	72-86	88-56	57.3	12.1	1.43	8.5	0.0	T1
Turkey	C35	2013/05/29	76-91	72-40	54.1	11.1	1.33	8.3	6.3	T1-2
Turkey	CC	2013/05/29	72-92	88-40	56.7	11.7	1.48	7.9	3.6	T1-2
Turkey	SC	2013/05/29	73-84	72-56	54.9	11.4	1.44	7.9	2.3	T1-2

5.5.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele & Malelane)

Project 75 C by J. Joubert (CRI)

Opsomming

Die resultate van die 2013 seisoen vir hierdie warm produksie area het aangedui dat Tango die vroegste ryp geword het met die kleinste vruggrootte en goeie interne kwaliteit. Daarna het Tahoe Gold en Gold Nugget gevolg, met van die grootste vrugte vir hierdie seisoen. Tahoe Gold was die enigste seleksie wat saad (0.1 sade per vrug) in die vrugte ontwikkel het, asook die beste interne kwaliteit in vergelyking met die ander seleksies (Brix 11). Yosemite Gold was volgende om ryp te word nader aan die einde van die Mandaryn Hibried reeks, ge-evalueer met ŉ goeie interne kwaliteit vrug wat ook total saadloos was, asook goeie eksterne kleur ontwikkeling (T2). Shasta Gold was die laaste seleskie gereed vir oes teen einde 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 results of the 2013 season indicate that for the warm production areas Tango matures first with the smallest fruit size and good internal quality. Tahoe Gold and Gold Nugget followed, with the biggest fruit size for this season. Tahoe Gold was the only selection that developed seeds (0.1 seeds per fruit) in the fruit, as well as the best internal quality compared to the other selections (Brix 11). Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at this trial site with good internal quality, completely seedless fruit as well as good external colour (T2). Shasta Gold was the last selection to mature, at the end of July, ending 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 Bosveld Citrus (Letsitele) and Riverside (Marble Hall) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.5.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2013 season.

Selection	Rootstock	Planted
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Yosemite Gold	CC	2010
Gold Nugget	CC	2010
Tango	CC	2010

Table 5.5.4.2. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2013 season.

Selection	Rootstock	Planted
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 first time this season. The trees at Bosveld are one year older than the trees at Riverside and this had an impact on the quality and quantity of the fruit.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new 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 overmature. 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.

Shasta Gold
Shasta Gold developed fairly flat fruit on the trees at both trial sites. There was ribbing on most of the fruit, as well as sunburn. The tree size was similar to Yosemite Gold and Tango, between 1.6 and 1.8 m high. Rind texture was rough. The flavour was average with high juice (55%) and rind oil content. 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 54%, Brix above 10 and acceptable acid levels (above 1.2%). Based on the internal quality results in Table 5.5.4.3 maturity was mid to end of June at both Bosveld and Riverside.

Tahoe Gold
Tahoe Gold produced a very light crop on the trees, averaging 10 kg per tree. This selection developed the smallest tree size when compared to the other UC5 varieties with a tree height of 1.5 m (compact tree). The fruit size peaked from medium to large/very large (count 2-1XXX) and the fruit shape was similar to that of a Minneola tree. There was a delay on the external colour 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 0.1 seeds per fruit counted. The internal quality was good with juice levels of 58%, Brix average 10 and acceptable acid levels (Bosveld 0.7 and Riverside 0.8%). Based on the internal quality results in Table 5.5.4.3, estimated maturity was the end of May to middle June.

Yosemite Gold
The fruit set on Yosemite Gold was very light and additional measurements might be necessary to increase the crop on the trees (Gibb sprays or girdling). Yosemite developed a very promising soft citrus type fruit shape (similar to Minneola). The fruit was firm, rind texture was smooth and the fibre was soft, peeled very easy and was completely seedless. Yosemite developed the biggest tree size compared to the other TDE selections, measuring up to 1.8 and 2 meters in height. This aggressive growth characteristic might be the reason for the poor crop on the trees (vegetative growth), and must be generated into fruit set and crop on the trees. Fruit size varied from medium to large/very large (count 2-1XXX), similar to Tahoe Gold. The internal quality was average to good between the two sites with Riverside developing higher juice, Brix and acid levels. External colour developed with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.5.4.3, estimated maturity was the end of May to middle of June.

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 another important reason was to set more smooth textured fruit on the lower branches. Gold Nugget is familiar for its rough textured fruit with coarse rinds, but with 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 was average and developed fair juice (45%), Brix (9.5) and acid (0.8%) levels and a delayed external colour (T4-5). Keep in mind the young tree age, future evaluations will determine the feasibility of this selection in the hot areas. Based on the internal quality results in Table 5.5.4.3, estimated maturity was the middle to end of May.

Tango

Tango was completely seedless at the Bosveld trial site with a light crop (20 kg/tree) on the trees. There was no fruit on the trees at Riverside trial site (trees 2 years old). 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 and 1.6 m in height (medium growth rate) on Carrizo rootstock at Bosveld Citrus. The fruit was firm and the rind thin, fibre was soft and peeled very easy. Internally the fruit was high in juice content (55%), Brix was low for this selection (8.5), acid levels (0.6) decreased rapidly early in the season (short shelf live) and deep orange coloured fibre. Fruit size peaked at count 3 to 1XX (small to medium). Based on the internal quality results in Table 5.5.4.3, estimated maturity was the middle of May.

Conclusion

The external colour delay in the hot areas might be a problem; future evaluation will conclude the scenario. Degreening might be an option for the Gold Nugget and TDE's, but with Tango (W.Murcott selection) and Nadorcott, ethylene reacted slowly. Shasta Gold might 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 low temperatures. In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit.

Shasta Gold had the largest fruit size, followed by Yosemite- and Tahoe Gold, then Gold Nugget. The smallest fruit size was produced on Tango. There was only one seed incidence at Riverside where Tahoe Gold had 0.1 seed per fruit: all the other selections were completely seedless.

Table 5.5.4.3. Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele) and Riverside (Malelane) during the 2013 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	CC	2013/04/11	Bosveld	72-77	1XX	45.6	7.9	1.51	5.2	0.0	T8
Gold Nugget	CC	2013/04/26	Bosveld	69-80	1X-1XXX	46.1	8.6	1.24	6.9	0.0	T7-8
Gold Nugget	CC	2013/05/28	Bosveld	72-94	1XX-1XXX	44.7	9.2	0.81	11.4	0.0	T7-8
Gold Nugget	CC	2013/07/09	Bosveld	73-84	1XX-1XXX	47.9	9.7	0.76	12.8	0.0	T2-5
Gold nugget	CC	2013/04/30	Riverside	66-85	2-1XXX	50.8	8.8	1.12	7.9	0.0	T7-8
Gold nugget	CC	2013/05/17	Riverside	70-88	1X-1XXX	51.0	9.3	0.88	10.6	0.0	T6-7
Gold Nugget	CC	2013/06/19	Riverside	74-85	1XX-1XXX	46.1	10.6	0.73	14.5	0.0	T4-5
Shasta Gold	CC	2013/04/11	Bosveld	69-79	1X-1XXX	52.9	7.2	2.05	3.5	0.0	T8
Shasta Gold	CC	2013/04/26	Bosveld	69-80	1X-1XXX	52.6	8.1	1.58	5.1	0.0	T7-8
Shasta Gold	CC	2013/05/28	Bosveld	72-86	1XX-1XXX	56.2	8.9	1.39	6.4	0.0	T6-7
Shasta Gold	CC	2013/07/09	Bosveld	73-85	1XX-1XXX	55.9	10.2	1.23	8.3	0.0	T1-4
Shasta Gold	CC	2013/04/30	Riverside	69-78	1X-1XX	55.9	8.5	1.69	5.0	0.0	T5-6
Shasta Gold	CC	2013/05/17	Riverside	77-88	1XX-1XXX	56.3	9.5	1.43	6.6	0.0	T5
Shasta Gold	CC	2013/06/19	Riverside	81-90	1XXX	54.1	10.9	1.23	8.9	0.0	T1-2
Tahoe Gold	CC	2013/04/11	Bosveld	62-72	2-1XX	59.0	7.6	1.63	4.7	0.0	T7
Tahoe Gold	CC	2013/04/26	Bosveld	63-74	1-1XX	57.2	8.7	1.38	6.3	0.0	T7-8
Tahoe Gold	CC	2013/05/28	Bosveld	71-83	1X-1XXX	58.8	8.9	0.91	9.8	0.0	T6-7
Tahoe Gold	CC	2013/07/09	Bosveld	69-85	1X-1XXX	56.7	9.1	0.68	13.4	0.0	T1-6
Tahoe Gold	CC	2013/04/30	Riverside	69-80	1X-1XXX	59.9	8.8	1.24	7.1	0.0	T6-8

Tahoe Gold	CC	2013/05/17	Riverside	78-88	1XXX	56.8	9.4	1.00	9.4	0.1	T5
Tahoe Gold	CC	2013/06/19	Riverside	80-86	1XXX	56.4	11.1	0.82	13.5	0.0	T1-2
Tango	CC	2013/04/11	Bosveld	58-67	3--1	54.2	7.8	0.96	8.1	0.0	T7-8
Tango	CC	2013/04/26	Bosveld	55-65	3--1	50.9	7.6	1.58	4.8	0.0	T6-7
Tango	CC	2013/05/28	Bosveld	67-72	1-1XX	56.1	8.4	0.58	14.5	0.0	T3
Tango	CC	2013/07/09	Bosveld	60-73	2-1XX	54.8	8.6	0.56	15.4	0.0	T2-5
Yosemite Gold	CC	2013/04/11	Bosveld	61-79	2-1XXX	46.7	7.0	1.40	5.0	0.0	T8
Yosemite Gold	CC	2013/04/26	Bosveld	65-75	1-1XX	46.2	8.8	1.32	6.7	0.0	T7-8
Yosemite Gold	CC	2013/05/28	Bosveld	69-81	1X-1XXX	48.6	7.8	1.06	7.4	0.0	T7-8
Yosemite Gold	CC	2013/07/09	Bosveld	74-83	1XX-1XXX	52.8	8.6	0.90	9.6	0.0	T2-6
Yosemite Gold	CC	2013/04/30	Riverside	74-83	1XX-1XXX	51.3	8.3	1.27	6.5	0.0	T7-8
Yosemite Gold	CC	2013/05/17	Riverside	70-85	1X-1XXX	54.4	8.7	1.10	7.9	0.0	T5-6
Yosemite Gold	CC	2013/06/19	Riverside	81-92	1XXX	50.7	10.1	0.97	10.4	0.0	T2

5.5.5 PROGRESS REPORT: Evaluation of Valencia selections in the hot inland areas (Swaziland)

Project 740A by J. Joubert (CRI)

Opsomming

Die produksie area word beskou as warm in kombinasie met vogtige klimaat en maak die verbouing van Valencia en pomelo variteite baie gunstig. Turkey was eerste gereed om te oes, gevolg deur Portsgate een tot twee weke later, hulle maak die twee vroeë seleksies van hierdie proef uit. Turkey het in die kommersiële boorde in hierdie area op drie jarige ouderdom reeds swartvlek probleme getoon, hou die jong bome dus goed dop en pas spuitprogramme aan. Turkey op C35 in die koeler produksie areas (Ngonini) ontwikkel te stadig wat ŉ te klein boomgrootte tot gevolg het, in vergelyking met die warm areas, maar vir die Tambuti area was die bome gesond en in ŉ goeie kondisie, met goeie produksie. Turkey op C35 toon verseker ook meer probleme op herplant grond, waar nuwe grond goed presteer. Hoër digtheid aanplantings in kombinasie met C35 sal soortgelyke opbrengste per hektaar verseker, in vergelyke met die grootter bome op Carrizo. Die kleiner boomgrootte het verskeie voordele, bv. makliker en vinniger oes praktyke en laer bespruitings volumes met beter boom bedekking. Alpha en Jassie kwalifiseer as die mid-rypwordende seleksies van die Valencia seisoen. Alpha presteer baie goed en daar word baie nuwe aanplantings in die Letsitele omgewing gedoen, met goeie interne kwaliteit en vruggrootte. Jassie, in kombinasie met Carrizo en C35, as eksperimentele seleksie lyk baie belowend. Delta was die kontrole in hierdie proef, en pas in net na die middel van die seisoen. Dan volg McClean saadloos wat een van die later rypwordende seleksies uitmaak, met goeie produksie en interne kwaliteit, asook saadlose vrugte. McClean saadloos het wel hierdie seisoen baie wisselvallige vruggroottes geproduseer.

Summary

This production area is classified as hot and humid and the establishment of Valencia and grapefruit selections is very favourable. Turkey matured first for harvesting, followed by Portsgate one to two weeks later. These varieties represent the two early selections in this trial. However, three-year-old Turkey trees in the commercial orchards in this area already have black spot problems, so look out for this on young trees and adapt spray programmes accordingly. Turkey on C35 in the cooler production areas (Ngonini) developed too slowly and the tree size was smaller compared to the hot areas, but for the Tambuti area the trees were healthy and in good condition, bearing a good crop. Turkey performed poorly to average on C35 where replant soils were used compared to virgin soil. Planting higher density orchards in combination with C35 will result in similar yield production per hectare, compared to the bigger tree size of Carrizo. The smaller trees have numerous advantages, for example easier harvesting practices and lower spray volumes with better tree coverage. Alpha and Jassie qualify as the mid-maturing selections of the Valencia season. Alpha performed very well, and there were numerous new plantings in the Letsitele area, with good internal quality fruit and fruit size. Jassie, in combination with Carrizo and C35 rootstock, looked promising as an experimental selection. Delta was the control for this trial, and fits in just after the middle of the season. Now McClean SL follows as one of the later maturing selections, with good production and internal quality, as well as seedless fruit. McClean seedless developed erratic fruit size this season from small to large.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Delta, Jassie, McClean SL, Portsgate and Turkey at Tambuti Estate, Swaziland.

Table 5.5.5.1. Internal fruit quality data were compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brixº	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.0	0.6	1.6%	8.0:1	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
Delta SL	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Turkey – interim internal fruit quality standards

Table 5.5.5.2. List of Valencia selections evaluated at Tambuti Estate (Swaziland) during 2013.

Selection	Rootstock	Tree Age	No. of trees
Alpha	CC	2003	5
Delta	CC	2003	5
Jassie	C35/CC	2005	10/10
McClean SL	CC	2003	5
Portsgate	CC	2003	5
Turkey	C35/CC	2005	10/10

Results and discussion

These comments are based on the second evaluation date (25/7/2013). Maturity/estimated maturity was based on a Brix acid ratio of 10.0.

Alpha

There were no seeds present in the fruit tested this season, as with the previous season. Tree condition was good, considering that the trees were 11 years old this season and 6 m high on Carrizo rootstock. Due to an average yield production of 140 kg/tree, Alpha produced small to medium sized fruit (count 88-64), similar to the previous season. There was no delay in external colour development (T1-2) when the fruit was internally matured. The internal colour was deep yellow, fibre strength fairly soft, thin fairly smooth rinds, fruit peeled easily with medium rind oil. Internally the fruit quality complied with the export requirements. The estimated maturity date was middle to end of July.

Delta (control)

The tree height peaked at 4 m, and Delta produced between 100 and 120 kg per tree this season with a good to very good tree condition. The fruit size on the trees increased this season from counts 125 and 64 to counts 105 and 72 (small to medium). Delta tends to produce small fruit but the advantage of this selection is the fact that it is completely seedless. There was no sunburn, splitting or creasing noticeable on the fruit. The external colour range was better this season and peaked at T1 to T3 (2012 season T3-5). Internal quality was good with a high Brix level of 11.3 and Brix: acid ratio of 8.8, the ratio will increase later in the season when the Brix improves and the acids (1.28%) decrease. Peak maturity is estimated to be end July.

Jassie

Jassie was planted on Carrizo and C35 at this trial site to determine the impact of the semi-dwarfing C35 rootstock on external fruit colour, production, internal quality, scion/rootstock compatibility and fruit size. The external colour on C35 (T1-3) was delayed compared to Carrizo (T1-2). The yield production decreased this season on both rootstocks, peaked on C35 between 60 and 80 kg per tree, and on Carrizo at 70 kg per tree. The internal quality related to juice, acid levels and seed count per fruit evaluated, was similar on both rootstocks. Jassie on CC developed higher Brix (11.2) and on C35 higher juice levels (60.5%). The average seed count of the fruit was higher on C35 (3.9-5.0 seeds per fruit) compared to CC (1.6-2.1 seeds per fruit)

this production season. Once mature (10 years plus), tree size will be approximately one third smaller in height. Maturity is estimated to be middle to end of July.

McClean SL

Tree condition was excellent, tree height was on average 4 m and the internal fruit colour developed into a deep orange, as well as T1-2 external colour. McClean SL remained completely seedless at this trial site and the crop on the trees was lower, peaking between 100 and 120 kg per tree. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. The fruit size this season was erratic, and varied from count 105 to count 64. The Brix: acid ratio peaked above 8.5 with acid levels on the higher side, resulting in a good internal quality (Juice 58%, Brix 11.3 and acid 1.3%). The Brix: acid ratio will improve later in the season when the acid drops at peak maturity. There was no sunburn, splitting or creasing problems with the fruit, the fruit peels moderately with high rind oil content. Maturity is estimated to be the middle to the end of July.

Portsgate

Portsgate produced 100 kg per tree this season and peaked from count 105 to 72 (small to medium); slightly smaller compared to the previous season. The tree condition in combination with Carrizo was very good, developing a large tree size, measuring 5 to 6 m in height. Fruit shape was round with smooth skin texture and no thorns visible on the bearing branches. The internal colour developed to a deep yellow, with good internal quality and Brix: acid ratios above 8, due to acid level (1.18%). There were 0.1 seeds per fruit at the Tambuti trial site this season in the fruit sampled, whereas last season they were completely seedless. Maturity is estimated to be middle to end of July.

Turkey

Turkey was also planted on Carrizo and C35 to compare the impact of the two different rootstocks on the selection, as well as scion/rootstock compatibility on C35. The dwarfing effect on the C35 trees was visible, with a tree size difference between the two combinations, C35 measured 3 m high and Carrizo 4.5 m high. C35 produced 80 to 100 kg per tree, compared to Carrizo with 80 kg per tree. There was a decline in fruit set on Carrizo this year.

Both rootstock combinations produced good internal quality fruit, and peaked at a Brix reading above 12, juice content on C35 of 51.2% and on CC of 59% and an acid level of above 1.7%; very high for this time of the year. The external colour was between T1 and T3. Future evaluations will determine the extent and effects of C35 dwarfing on Turkey. Rind texture remained smooth and internal fibre strength (rag) was very soft. Based on a ratio of 10.0 and due to the high acid levels, maturity will be later towards the middle and end of June.

Conclusions

The acid levels on most of the selections were on the higher side this year. Turkey remains a very good, and currently the only, choice as an early maturing commercial Valencia type, but keep in mind that Weipe and Valearly are two new experimental varieties that are being evaluated with possible future value as early maturing Valencia selections. Jassie proved to be very promising in the other trial sites where the selection was included. There were more seeds per fruit on C35 rootstock when compared to CC where Jassie or Turkey was the scion: future evaluations will determine the value of this statement and it may only be a seasonal scenario. The performance of Jassie on Carrizo and C35 for the Tambuti trial site was very similar. Portsgate matures after Turkey and before Benny: when more information becomes available in future this will be a selection to consider for new plantings. Alpha performed well this season, but there was another drop in fruit size count: medium fruit in 2012 but more small to medium sized fruit in the 2013 season. McClean had a lighter crop on the trees and remained a completely seedless variety. The fruit size was erratic from small to medium to large.

Table 5.5.5.3. Internal fruit quality data for Valencia orange selections at Tambuti Estate (Swaziland) during the 2013 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	2013/05/22	69-76	88-72	57.2	9.1	1.69	5.4	0.0	T4-5
Alpha	CC	2013/07/25	73-80	72-64	58.7	9.7	1.35	7.2	0.0	T1-2
Delta	CC	2013/05/22	67-75	105-72	52.5	10.7	1.49	7.2	0.0	T3-5
Delta	CC	2013/07/25	68-74	88-72	58.7	11.3	1.28	8.8	0.0	T1-3
Jassie	C35	2013/05/22	70-84	88-56	60.2	9.4	1.58	5.9	3.9	T4-6

Jassie	C35	2013/07/25	71-82	88-56	60.5	10.5	1.40	7.5	5.0	T1-3
Jassie	CC	2013/05/22	73-80	72-64	58.2	9.7	1.65	5.9	1.6	T4-5
Jassie	CC	2013/07/25	72-85	88-56	59.6	11.2	1.30	8.6	2.1	T1-2
McClean SL	CC	2013/05/22	66-79	105-64	56.3	10.4	1.41	7.4	0.0	T3-4
McClean SL	CC	2013/07/25	73-79	72-64	57.7	11.3	1.32	8.6	0.0	T1-2
Portsgate	CC	2013/05/25	67-75	105-72	55.3	9.8	1.30	7.5	0.0	T4-5
Portsgate	CC	2013/07/25	72-77	88-72	59.3	9.6	1.17	8.2	0.1	T2-4
Turkey	C35	2013/05/23	65-76	105-72	51.2	12.7	1.73	7.3	5.8	T1-3
Turkey	CC	2013/05/22	70-79	88-64	59.0	12.2	1.72	7.1	1.6	T1-2

5.5.6 PROGRESS REPORT: Evaluation of Valencia selections in the intermediate areas (Tom Burke)

Project 899 A by J. Joubert (CRI)

Opsomming

Die seisoen word van vroeg tot laat rypwordende seleksies opgedeel in die warm produksie area en aanbevelings is soos volg. Du Roi was eerste gereed om geoes te word met goeie eksterne kleur en klein tot medium vruggrootte. Daar was wel saad in hierdie seleksie wat goeie opbrengste in die toekoms gaan verseker. Du Roi op Swingle het beter vrug kwaliteit opgelewer in vergelyking met Growweskil. McClean saadloos sal later in die Valencia seisoen volg, met totaal saadlose vrugte en beter vruggrootte as Du Roi, tussen telling 72 tot 48. Inligting is op hierdie stadium baie beperk weens die eerste oes op die bome. Opvolg evaluasies sal gedoen word wanneer meer seleksies in produksie kom.

Summary

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production area. Recommendations have therefore been made accordingly. Du Roi matured first and was ready to harvest with good external colour development and small to medium fruit size. There were seeds in the fruit that will guarantee good crops on the trees in the future. The fruit quality was better with Du Roi on Swingle compared to Rough lemon. McClean seedless will follow later in the Valencia season, with completely seedless fruit and bigger fruit size compared to Du Roi, ranging from counts 72 to 48. Information at this stage was limited due to it being the first crop on the trees. Future evaluations will be conducted when more selections come into production.

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 Du Roi and McClean SL at Rolemsha, Tom Burke.

Table 5.5.6.1. List of Valencia selections evaluated at Rolemsha (Tom Burke) during 2013.

Selection	Rootstock	Topwork	No. of trees
Du Roi	RL/SC	2011	1 / 4
McClean SL	RL/SC	2011	1 / 5

Results and discussion

<u>*Du Roi</u>

Du Roi on Rough lemon produced a better crop on the trees compared to Swingle. The tree condition was good and the fruit size on Rough lemon was medium to large (count 88-56), outperforming Swingle with small to medium fruit (count 125-72). Internally the juice, Brix and acid levels on Swingle were higher and complied with the minimum export requirements, unlike on Rough lemon. The seed count per fruit was similar on both rootstocks, averaging 2.2 to 2.3 seeds per fruit. There was a delay on external colour with Rough lemon (T2-5). Maturity will be determined when trees grow older for this specific production area.

McClean SL
Tree condition was good and the internal fruit colour developed into a deep orange with delayed external colour from T3-6. McClean SL remained completely seedless at this trial site. There was a good crop on the young trees. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. The fruit size on both rootstocks varied from count 72 to count 48. The Brix: acid ratio peaked above 8.9 on Swingle with an acid level of 1.13%, Brix: acid ratio of 8 (low). There was no sunburn, splitting or creasing problems with the fruit, the fruit peels moderately with high rind oil content. Maturity will be determined when trees grow older for this specific production area.

*This was the first evaluation of Du Roi and McClean SL at this trial site, so information is limited and future evaluations will improve recommendations on these varieties.

Conclusions

Du Roi will mature more in the middle of the Valencia season and McClean will be one of the later selections for this area. Du Roi on both rootstocks developed smaller to medium fruit size, whereas McClean ranged from medium to large. The external colour on Du Roi was better and more advanced, but there was a delay on McClean SL. Du Roi on Swingle performed well with the best internal quality for this trial. Hopefully more selections will come into production next season; at this stage information was limited due to young tree age.

Table 5.5.6.2. Internal fruit quality data for Valencia orange selections at Rolemsha (Tom Burke) during the 2013 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Du Roi	RL	2013/09/19	72-83	88-56	44.3	8.9	1.08	8.2	2.3	T2-5
Du Roi	SC	2013/09/20	63-75	125-72	54.3	11.5	1.20	9.6	2.2	T1-4
McClean SL	RL	2013/09/07	76-90	72-48	44.4	7.5	0.86	8.7	0.0	T3-6
McClean SL	SC	2013/09/07	74-83	72-56	48.7	8.9	1.13	7.9	0.0	T3-6

5.5.7 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tom Burke & Tshipise)**
Project 899 B by J. Joubert (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die twee produksie areas, wat ŉ baie belangrike punt uitlig wanneer dit gaan kom by die keuse van die kultivars vir aanplanting, sowel as die onderstam wat gebruik gaan word. Die resultate van die 2013 seisoen vir hierdie warm produksie areas het aangedui dat Tango weereens die vroegste ryp geword het met die kleinste vruggrootte en goeie interne kwaliteit. Daarna het Gold Nugget en Tahoe Gold, met van die grootter vrugte vir hierdie seisoen. Daar was saad in al drie van die TDE seleksies by Rolemsha gewees (kruisbestuiwing druk). Die interne kwaliteit van die vrugte oor die algemeen was beter gewees by Rolemsha in vergelyking met Alicedale. Yosemite Gold was volgende om ryp te word, nader aan die einde van die Mandaryn Hibried reeks, met ŉ goeie interne kwaliteit, asook goeie eksterne kleur ontwikkeling (T2-3). Shasta Gold was die laatste seleskie gereed vir oes gewees teen middel tot einde 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 very different, indicating the crucial scenario when it comes to deciding what variety to plant where, as well as the suitable rootstock for that area. The results of the 2013 season indicate that for the warm production areas Tango matures first with the smallest fruit size and good internal quality. Gold Nugget and Tahoe Gold followed, with the biggest fruit size for this season. There were seeds in all three TDE selections at Rolemsha trial site (pressure of cross pollination). The internal quality of the fruit at Rolemsha was better compared to Alicedale. Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at this trial site with good internal quality, as well as good external colour development (T2-3). Shasta Gold was the last selection

to mature, the middle to end 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 hot dry production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Alicedale (Tshipise) and Rolemsha (Tom Burke) from the Limpopo region. The following varieties were evaluated: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango.

Table 5.5.7.1. List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2013 season.

Selection	Rootstock	Topwork
Shasta Gold	RL	2010
Tahoe Gold	RL/X639	2010
Yosemite Gold	RL	2010
Gold Nugget	RL	2010
Tango	RL	2010

Table 5.5.7.2. List of Mandarin Hybrid selections evaluated at Rolemsha (Tom Burke) during the 2013 season.

Selection	Rootstock	Topwork
Shasta Gold	CC/RL/SC	2011
Tahoe Gold	CC/SC	2011
Yosemite Gold	CC/RL/SC	2011
Gold Nugget	CC/RL/SC	2011
Tango	CC/RL/SC	2011

Results and discussion

All the selections were bearing fruit for the first time this season. The trees at Alicedale (2010) are one year older compared to the trees at Rolemsha (2011), also having an impact on the quality and quantity of the fruit.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new 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 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>Shasta Gold</u>

The crop on the Shasta Gold trees at Alicedale peaked at 60 kg per tree on Rough lemon, where the crop at Rolemsha was lighter due to smaller and younger trees. Shasta Gold developed fairly round fruit (Minneola type) on the trees at both trial sites. There was ribbing on most of the fruit, as well as sunburn (severe at Alicedale). The tree size at Alicedale was between 1.6 and 1.8 m high on Rough lemon, similar to Tahoe Gold. There were a lot of thorns on the bearing branches of the trees. Rind texture was rough (scale 4-5). The flavour was fair with high juice (up to 59%) and rind oil content. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless, except for one evaluation at Rolemsha on Carrizo rootstock (1.0 seed per fruit). The fruit size peaked from large to very large (count 1X-1XXX). The internal quality was good with high juice levels, Brix above 10 and acceptable acid levels (above

1.4%). Based on the internal quality results in Table 5.5.7.3 maturity was mid to end of June depending on rootstock choice (Rough lemon middle to end May).

Tahoe Gold

Tahoe Gold produced a very light crop at Alicedale averaging 15 kg per tree (rough lemon). This selection developed a small tree size when compared to the other UC5 varieties with a tree height of 1.8 m on Rough lemon at Alicedale (compact tree). The tree bears fruit in a similar way to Grapefruit (bundles). The fruit size peaked from medium to large/very large (count 3-1XXX) and the fruit shape was similar to that of a Minneola tree. There was a slight delay on the external colour when the internal quality was optimum. Rough lemon developed very low acid levels early in the season, compromising fruit quality, flavour and shelf life. Tahoe produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated was completely seedless, except for two evaluations at Rolemsha on Swingle (0.5 and 0.7 seeds per fruit). The internal quality was good with juice levels reaching 60%, Brix averaged 10 and acid levels were acceptable (1.0% and higher). Based on the internal quality results in Table 5.5.7.3, estimated maturity was the middle to end of May (Alicedale) and middle to end of June (Rolemsha).

Yosemite Gold

The fruit set on Yosemite Gold was very light (40 kg per tree 3 m high) and additional measures might be necessary to increase the crop on the trees (Gibb sprays or girdling). Yosemite developed a very promising soft citrus type fruit shape (similar to Minneola). The fruit was firm, rind texture was smooth and the fibre was soft, peeled very easily and was completely seedless, except for evaluations at Rolemsha on Carrizo and Swingle (0.3 and 0.5 seeds per fruit). Yosemite developed the biggest tree size compared to the other TDE selections, measuring up to 3 m in height at Alicedale on Rough lemon. This aggressive growth characteristic might be the reason for the poor crop on the trees (vegetative growth), and must be generated into fruit set and crop on the trees. Fruit size varied from large to very large (count 1-1XXX), similar to Tahoe Gold. The internal quality was average to good between the two sites with Carrizo and Swingle at Rolemsha developing higher Brix and acid levels. External colour developed with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.5.7.3, estimated maturity was the end of May (Rough lemon) to end of June (Carrizo and Swingle).

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 another important reason was to set more smooth textured fruit on the lower branches. Gold Nugget is familiar for its rough textured fruit with coarse rinds, but with 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 were completely seedless, fruit size was smaller at Rolemsha (counts 5 to 1XX) compared to Alicedale (counts 1 to 1XXX). The internal quality of the fruit was average and developed fair juice (avg 51.5%) with the exception of the one evaluation on Swingle at Rolemsha (68%), Brix Alicedale (8.0) / Brix Rolemsha (11 on Carrizo and Swingle) and acid levels above 1.0% and a delayed external colour (T3-5). The young tree age contributed to the average fruit quality, future evaluations will determine the feasibility of Gold Nugget in the hot areas. Based on the internal quality results in Table 5.5.7.3, estimated maturity was the middle to end of May to end middle of June (external colour delay).

Tango

Tango remained completely seedless at Alicedale and Rolemsha as well as the Bosveld trial site. There was a light crop (20 kg/tree) on the trees at Alicedale and 10 kg per tree due to smaller tree size at Rolemsha. 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 2.3 m in height (medium growth rate) on Rough lemon rootstock at Alicedale (Tshipise), whereas at Rolemsha on Carrizo and Swingle between 1.5 and 1.8 m. The fruit was firm and the rind thin, fibre was soft and peeled very easy. Internally the fruit was high in juice content (55 to 60%), Brix was low for this selection (8.5) at Alicedale, but at Rolemsha on Carrizo the Brix peaked at 12.1, acid levels (below 1.0) decreased rapidly early in the season at Alicedale (short shelf life), but was on average higher at Rolemsha on Carrizo and Swingle (above 1.2%) and deep orange coloured fibre. Fruit size peaked at count 3 to 1XXX (small to medium) except for one evaluation on Carrizo at Rolemsha (count 5 to 4). Based on the internal quality results in Table 5.5.7.3, estimated maturity at Alicedale was the middle to end of April, and at Rolemsha end of May to middle of June.

Conclusion

The external colour delay in the hotter areas might be a problem; future evaluation will conclude the scenario. Degreening might be an option for the Gold Nugget and TDEs, but with Tango (W. Murcott

selection) and Nadorcott, ethylene reacted slowly or not at all. Shasta Gold might 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 the Shasta Gold's fruit in the Tshipise area (hot) might 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 of the fruit (Shasta had severe sunburn at Alicedale). Gold Nugget improved considerably in the Tom Burke area, compared to the Tshipise area, although the trees were on Carrizo and Swingle (good internal quality rootstocks) versus Rough lemon (good production and size, average internal quality, lower acid). Shasta Gold had the largest fruit size, followed by Yosemite- and Tahoe Gold, then Gold Nugget. The smallest fruit size was produced on Tango. There were five incidences of seed in the fruit at Rolemsha, one on Shasta Gold, two on Tahoe Gold and two on Yosemite Gold (the three TDEs), and the other selections were completely seedless.

Table 5.5.7.4. Internal fruit quality data for Mandarin hybrid selections at Rolemsha (Tom Burke) and Alicedale (Tshipise) during the 2013 season.

Selection	Root-	Date	Site	Size	Count	Juice	Brix º	Acid	Ratio	Avg.	Colour
	stock	harvested		mm		(%)		(%)		seed	
Gold Nugget	RL	2013/04/10	Alicedale	69-76	1X-1XX	51.6	7.5	1.01	7.4	0.0	T7-8
Gold Nugget	RL	2013/04/24	Alicedale	65-82	1-1XXX	51.6	8.2	0.94	8.7	0.0	T6-7
Gold Nugget	RL	2013/05/30	Alicedale	76-107	1XX-1XXX	44.9	8.4	0.67	12.5	0.0	T4-6
Gold Nugget	CC	2013/04/25	Rolemsha	57-70	3-1X	49.3	9.5	1.47	6.5	0.0	T7-8
Gold Nugget	CC	2013/04/25	Rolemsha	53-69	4-1X	50.8	9.7	1.18	8.2	0.0	T5-6
Gold Nugget	CC	2013/05/23	Rolemsha	50-72	5-1X	51.1	11.2	1.16	9.7	0.0	T3-4
Gold Nugget	RL	2013/05/23	Rolemsha	70-75	1X-1XX	44.3	9.3	1.01	9.2	0.0	T6
Gold Nugget	SC	2013/05/23	Rolemsha	55-81	3-1XX	68.1	11.3	1.14	9.9	0.0	T4-5
Shasta Gold	RL	2013/04/10	Alicedale	73-81	1XX-1XXX	58.6	8.6	1.70	5.1	0.0	T7-8
Shasta Gold	RL	2013/04/24	Alicedale	75-83	1XX-1XXX	55.3	8.3	1.42	5.8	0.0	T4-6
Shasta Gold	RL	2013/05/30	Alicedale	92-107	1XXX	52.1	8.1	1.00	8.1	0.0	T1-3
Shasta Gold	CC	2013/04/25	Rolemsha	70-79	1X-1XXX	55.5	9.2	2.16	4.3	0.0	T7-8
Shasta Gold	CC	2013/05/23	Rolemsha	69-88	1X-1XXX	59.4	9.4	1.77	5.3	0.0	T2-3
Shasta Gold	CC	2013/06/26	Rolemsha	71-89	1X-1XXX	55.9	10.7	1.44	7.4	0.0	T1-4
Shasta Gold	RL	2013/05/23	Rolemsha	93-102	1XXX	50.7	8.4	1.44	5.8	1.0	T4
Shasta Gold	SC	2013/04/25	Rolemsha	70-79	1X-1XXX	55.5	9.2	2.24	4.1	0.0	T6-7
Shasta Gold	SC	2013/05/23	Rolemsha	73-90	1XX-1XXX	56.7	11.2	1.60	7.0	0.0	T2-4
Shasta Gold	SC	2013/06/26	Rolemsha	73-90	1XX-1XXX	54.6	10.6	1.40	7.6	0.0	T1-3
Tahoe Gold	RL	2013/04/10	Alicedale	75-84	1XX-1XXX	55.0	7.8	1.09	7.2	0.0	T7-8
Tahoe Gold	RL	2013/04/24	Alicedale	69-80	1X-1XXX	57.8	7.7	0.99	7.8	0.0	T6-7
Tahoe Gold	RL	2013/05/31	Alicedale	80-105	1XXX	49.5	7.4	0.68	10.9	0.0	T2-4
Tahoe Gold	CC	2013/04/25	Rolemsha	66-76	1-1XX	61.3	9.7	1.67	5.8	0.0	T4-5
Tahoe Gold	CC	2013/05/23	Rolemsha	75-88	1XX-1XXX	57.3	11.1	1.42	7.8	0.0	T1-4
Tahoe Gold	SC	2013/04/25	Rolemsha	62-77	1-1XX	60.3	10.0	1.94	5.2	0.5	T7-8
Tahoe Gold	SC	2013/05/23	Rolemsha	66-81	1-1XXX	60.2	9.8	1.24	7.9	0.0	T2-3
Tahoe Gold	SC	2013/06/26	Rolemsha	54-90	3-1XXX	53.7	9.9	1.00	9.9	0.7	T1-5
Tango	RL	2013/04/10	Alicedale	67-73	1X-1XX	55.4	7.6	0.72	10.6	0.0	T6-8
Tango	RL	2013/04/24	Alicedale	55-75	3-1XX	55.2	8.3	0.73	11.4	0.0	T4-5
Tango	X639	2013/04/09	Alicedale	60-73	2-1XX	55.1	8.2	0.94	8.7	0.0	T7
Tango	X639	2013/04/24	Alicedale	60-74	2-1XX	55.5	8.6	0.85	10.1	0.0	T4-5
Tango	CC	2013/04/25	Rolemsha	60-68	2-1X	54.3	9.4	1.04	9.0	0.0	T5-6
Tango	CC	2013/05/23	Rolemsha	40-54	5--4	60.0	12.1	1.50	8.1	0.0	T2-3
Tango	RL	2013/05/23	Rolemsha	63-81	1-1XXX	48.2	8.4	0.67	12.5	0.0	T5-6
Tango	SC	2013/04/25	Rolemsha	57-77	3-1XX	53.5	8.9	0.98	9.1	0.0	T7-8
Tango	SC	2013/05/23	Rolemsha	48-65	3--1	58.8	9.1	1.21	7.5	0.0	T5-6
Yosemite Gold	RL	2013/04/10	Alicedale	76-81	1XX-1XXX	55.0	8.0	0.93	8.6	0.0	T7-8
Yosemite Gold	RL	2013/04/24	Alicedale	71-80	1X-1XXX	53.0	7.9	0.93	8.5	0.0	T6-7
Yosemite Gold	RL	2013/05/27	Alicedale	81-104	1XXX	48.2	7.9	0.74	10.7	0.0	T2-3

Yosemite Gold	CC	2013/04/24	Rolemsha	65-78	1-1XX	51.0	9.2	1.34	6.9	0.0	T7-8
Yosemite Gold	CC	2013/05/23	Rolemsha	72-88	1XX-1XXX	50.5	9.5	1.14	8.3	0.0	T3-4
Yosemite Gold	CC	2013/06/26	Rolemsha	70-88	1X-1XXX	48.6	9.1	1.08	8.4	0.3	T2-7
Yosemite Gold	RL	2013/05/23	Rolemsha	68-93	1X-1XXX	36.9	7.3	0.84	8.7	0.0	T4-5
Yosemite Gold	SC	2013/04/25	Rolemsha	69-78	1X-1XX	52.5	9.6	1.68	5.7	0.5	T5-6
Yosemite Gold	SC	2013/05/23	Rolemsha	63-84	1-1XXX	51.5	10.3	1.28	8.0	0.0	T2-3

5.5.8 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool inland areas (Burgersfort)

Project 990 by J. Joubert (CRI)

Opsomming

Die resultate van die 2013 seisoen vir hierdie koel binnelandse produksie area het aangedui dat Tango steeds die vroegste (middle Junie tot einde Junie) ryp geword het (stem ooreen met warm areas) met die kleinste vruggrootte en baie goeie interne kwaliteit en smaak. Daarna het Yosemite Gold en Gold Nugget (einde Julie tot middle Augustus) gevolg, met van die grootste vrugte vir hierdie seisoen. Gold Nugget het die beste interne kwaliteit vir die seisoen gelewer met hoë Brix en goeie suur vlakke wat belowende rakleeftyd sal verseker. Die sap inhoud moet noukeurig met opvolg evaluasies gekontroleer word, effens aan die laer kant. Daar was saad in Gold Nugget, Shasta Gold en Yosemite Gold gewees in ŉ ge-isoleerde proefblok. Tahoe Gold was volgende om ryp te word (middle tot einde Augustus), 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 teen September, 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 optimum internal fruit quality. The results of the 2013 season indicated that Tango matures first (middle June to end June) for the cool inland production area with the smallest fruit size and very good internal quality and flavour. Yosemite Gold and Gold Nugget followed (end of July to middle of August), with one of the biggest fruit sizes for this season. Gold Nugget produced the best internal quality for the season with high Brix and good acid levels extending the shelf life of the fruit. Monitor the juice levels closely on this selection, because they tend to be on the low side. There were seeds present in Gold Nugget, Shasta Gold and Yosemite Gold, despite the isolated trial orchard location. Tahoe Gold matured next (middle to end August) towards the end of the Mandarin Hybrid range, evaluated at this trial site with good internal quality resulting in high juice levels in the fruit. Shasta Gold was the last selection to mature, in September, ending of 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 Fraaihesto (Burgersfort) 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 Fraaihesto (Burgersfort) during the 2013 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 first time this season.
When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new 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 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.

Shasta Gold
Shasta Gold developed fairly flat fruit on the trees at the trial sites. There was less ribbing compared to the hot production trial sites (Tshipise, Tom Burke, Letsitele and Malelane) on most of the fruit, and barely any sunburn. The tree size was similar to Yosemite Gold and Tango, between 1.5 and 1.7 m high. Rind texture was rough. The fruit size peaked from large to very large (count 1X-1XXX), similar to hot production areas. The internal quality was excellent with juice levels of 58%, Brix as high as 13 and good acid levels of 1.36 when the colour peaked at T1. The higher acid might delay harvest time (Brix: acid ratio of 9.6), 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, except for one evaluation with 0.1 seeds per fruit. Based on the internal quality results in Table 5.5.8.2 maturity was end of August to middle of September (ultra late).

Tahoe Gold
Tahoe Gold developed the smallest tree size when compared to the other UC5 varieties with a tree height of 1.2 to 1.5 m (compact round tree shape). The fruit size peaked from medium to large/very large (count 2-1XXX) 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 1.0%. Tahoe produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless. Tahoe developed an excellent internal quality with a Brix: acid ratio of 12.0. With the second last evaluation the juice levels were above 50%, Brix average 10.3 and good acid levels of 1.2%. Based on the internal quality results in Table 5.5.8.2, estimated maturity was the middle to end August.

Yosemite Gold
The fruit set on Yosemite Gold was very light and additional measurements might be necessary to increase the crop on the trees (Gibb sprays or girdling). The fruit was firm, rind texture was smooth and the fibre was soft, peeled very easy and was completely seedless, except for 0.1 seeds per fruit with the 2nd of July's evaluation. Yosemite developed the biggest tree size compared to the other TDE selections, measuring up to 1.8 and 2 m in height. This aggressive growth characteristic might be the reason for the poor crop on the trees (vegetative growth), and must be generated into fruit set and crop on the trees. Fruit size varied from medium to large/very large (count 2-1XXX), similar to Tahoe Gold, and the hot production areas. The internal quality was excellent and the Brix (13) levels very high, the acid above 1.0% and the juice content still above 50% with evaluations completed on the 26 of August and 3rd of September (Brix: acid ratio 12.5). External colour developed with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.5.8.2, estimated maturity was the end of July to middle of August.

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 another important reason is 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 superior internal quality (Brix: acid ratio 13), the highest Brix (14 to 14.8) content of all the UC5 trials, and

good acid (1.14%) levels for longer shelf life at optimal external colour (T1). Juice levels throughout all the evaluations at the Burgersfort site remained in the higher 40s (range from 46 to 49.5%), although low to compete in the export market demands. Keep in mind the young tree age, future evaluations will determine the optimal internal quality of this selection in the cooler areas. There were 0.2 seeds present in the sample fruit with the second evaluation. Based on the internal quality results in Table 5.5.8.2, estimated maturity was the end of July to middle of August.

Tango

Tango was completely seedless at the Fraaihesto trial site in Burgersfort and the trees developed an average crop (20 to 30 kg/tree). 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 and 1.6 m in height (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 2 selections evaluated in this trial, high in juice content (52%), Brix was very good for this selection (range from 11.9 to 13.2) and excellent acid level (range from 1.05 to 1.18%) at optimal external colour (T1). Brix: acid ratio peaked between 11.3 and 11.5 and the fruit displayed a deep orange fibre colour. Fruit size peaked from count 5 to 1XX (small to medium/large). Based on the internal quality results in Table 5.5.8.2, estimated maturity was the middle of June to the end of June.

Conclusion

There was no external colour delay in the cooler area and the optimal colour development was in sequence with a very good to excellent internal quality; future evaluation will support this statement. In the cooler areas it might be necessary to cover the mandarin orchards with shade net, to minimise possible hail damage and sunburn to improve packout percentage of the fruit.

The best internal quality fruit was on Gold Nugget, developing Brix content of up to 14, Shasta Gold will mature late in the Mandarin hybrid season due to high acid levels (1.4% with last evaluation). Shasta Gold had the largest fruit size, followed by Yosemite- and Tahoe Gold, then Gold Nugget. The smallest fruit size was produced on Tango. There were seeds present in the fruit on Gold Nugget (0.2), Shasta Gold (0.1) and Yosemite Gold (0.1) and all the other selections were completely seedless.

Table 5.5.8.2. Internal fruit quality data for Mandarin hybrid selections at Fraaihesto (Burgersfort) during the 2013 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	CC	2013/04/22	60-69	2-1X	47.9	10.1	2.04	5.0	0.0	T6-7
Gold Nugget	CC	2013/05/27	60-83	2-1XXX	47.8	10.8	1.44	7.5	0.2	T2-3
Gold Nugget	CC	2013/07/02	66-82	1-1XXX	49.5	12.0	1.21	9.9	0.0	T2
Gold Nugget	CC	2013/08/26	58-79	3-1XXX	48.6	14.0	1.06	13.2	0.0	T1
Gold Nugget	CC	2013/09/03	62-78	1-1XX	45.6	14.8	1.14	13.0	0.0	T1
Shasta Gold	CC	2013/04/22	68-75	1X-1XX	53.3	9.1	2.48	3.7	0.0	T4-5
Shasta Gold	CC	2013/05/27	72-84	1XX-1XXX	52.7	9.7	1.91	5.1	0.0	T1-3
Shasta Gold	CC	2013/07/02	71-84	1XX-1XXX	54.9	11.1	1.48	7.5	0.1	T1-3
Shasta Gold	CC	2013/08/26	72-99	1XX-1XXX	50.7	12.6	1.30	9.7	0.0	T1
Shasta Gold	CC	2013/09/03	68-94	1X-1XXX	57.8	13.1	1.36	9.6	0.0	T1
Tahoe Gold	CC	2013/04/22	65-75	1-1XX	58.2	9.0	2.09	4.3	0.0	T4-5
Tahoe Gold	CC	2013/05/27	61-77	2-1XX	58.7	10.1	1.52	6.6	0.0	T1-4
Tahoe Gold	CC	2013/07/02	69-92	1X-1XXX	55.0	10.1	1.42	7.1	0.0	T1-4
Tahoe Gold	CC	2013/08/26	65-82	1-1XXX	50.3	11.9	1.16	10.3	0.0	T1
Tahoe Gold	CC	2013/09/03	64-90	1-1XXX	49.7	12.6	1.05	12.0	0.0	T1
Tango	CC	2013/04/22	63-78	2-1XX	52.3	8.8	1.15	7.7	0.0	T5-7
Tango	CC	2013/05/27	67-88	1-1XXX	50.5	9.7	0.92	10.5	0.0	T1-3
Tango	CC	2013/07/02	64-77	1-1XX	54.3	11.9	1.05	11.3	0.0	T1
Tango	CC	2013/08/26	54-75	4-1XX	52.6	13.2	1.15	11.5	0.0	T1
Tango	CC	2013/09/03	50-70	5-1X	48.9	12.5	1.18	10.6	0.0	T1-2
Yosemite Gold	CC	2013/04/22	61-73	2-1XX	46.0	9.1	1.81	5.0	0.0	T4-5

Yosemite Gold	CC	2013/05/27	70-82	1X-1XXX	48.1	10.1	1.34	7.5	0.0	T2-4
Yosemite Gold	CC	2013/07/02	67-85	1-1XXX	44.5	11.0	1.17	9.4	0.1	T1-4
Yosemite Gold	CC	2013/08/26	68-83	1X-1XXX	52.4	12.4	0.99	12.5	0.0	T1
Yosemite Gold	CC	2013/09/03	58-83	2-1XXX	50.0	13.0	1.03	12.6	0.0	T1

5.5.9 PROGRESS REPORT: Evaluation of Delta Valencia on new imported rootstocks in the Marble Hall area

Project 94 by J. Joubert (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde was oor ŉ agt jaar termyn in die Marble Hall omgewing geëvalueer, tot in 2007. Daarna is elk tweede boom afgesaag om meer spasie in die rye te verseker. Die bome het stadig herstel, maar was in 2013 weer gereed om ge-evalueer te word (opvold evaluasie). Geen tekens van onverenigbaarheid was sigbaar op enige van die bostam: onderstam kombinasies nie. Die bome se interne kwaliteit het baie verbeter met ŉ indrukwekkende toename in produksie. Vruggrootte het gepiek by telling 105/125, tipies vir Delta wat kleiner vrugte produseer. Citrange 32, gevolg deur Flying dragon x Sunki 1112 en F80 citrumelo 3 het die beste oes produksie op die bome verseker. Die tradisionele onderstamme wat in die sitrus bedryf gebruik word, het heelwat swakker gevaar. Die Sunki hibried onderstamme vorm ŉ heelwat kleiner boomgrootte, maar die boomvorm (oop kroon gedeelte met take wat afhang) kan moontlik probleme inhou (sonbrand, takke breek ens).

Summary

The performance of Delta Valencia on 42 different rootstocks in replant soils was evaluated over an eight-year production cycle in the Marble Hall production area up to 2007. Every second tree was removed in 2008 to allow better tree spacing in the rows. The trees recovered slowly and a follow-up evaluation was only possible in 2013. No signs of incompatibility were visible on any of the scion: rootstock combinations. The internal quality of the trees improved markedly with impressive crop production. Fruit size peaked at count 105/125, typical smaller fruit size of Delta Valencia. Citrange C32, followed by Flying dragon x Sunki 1112 and F80 citrumelo 3 produced excellent yields on the trees. The traditional rootstocks being used in the citrus industry performed moderately. The Sunki hybrid rootstocks developed smaller trees (dwarfing effect) but the tree shape (more open with long bearing branches) might be problematic (sunburn and weaker branches).

Objectives

- To investigate the performance of Delta Valencia on 42 new, imported rootstocks on replant soils over an eight-year production cycle.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

A randomised block design was used for 22 rootstocks with two replicates of five trees each; the other 20 rootstocks were planted in a non-randomised design comprising 10 trees per rootstock. All 42 rootstocks were selected and evaluated in the 2013 season, after only 30 had been evaluated the previous time in the 2007 season (best combinations selected).

Every second tree had been removed from the orchard in 2008, due to limited tree spacing in the rows. Trees recovered slowly; this been the reason for harvesting the trees only in 2013.

Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, X639, RL-C, RL-S, RL-W, PT, HRS812, RxT, Sunki 1113, CM, CC, TC, Volk, KC, TB, ML, RC, JT, RT, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116, AT, K, C, SCS, GT, OT, CA, JT, ChM, N, CLM, CO, RT, JC. The trees were planted in 1998. Trees were evaluated at Moosrivier Estates (Marble Hall), in Mpumalanga during the 2007 (final evaluation) season. Full names for these abbreviations appear below.

Table 5.5.9.1. Number of trees per rootstock in the Delta Valencia trial at Marble Hall.

No	Abbreviation	Rootstock	No of trees
1	F80/8	F80 citrumelo 8	5
2	PT	Pomeroy trifoliate	5
3	C32	Citrange 32	5
4	AT	Australian trifoliate	5
5	HRS 812	Sunki 812	5
6	K	Konejime	5
7	CM	C. macrophylla	5
8	C35	Citrange 35	5
9	C	Calamandarin	5
10	SCS	Sun chu sha	5
11	X639	Cleopatra x Trifoliate	5
12	GT	Gou Tou	5
13	ML	Milan Lemon	5
14	OT	Orlando tangelo	5
15	CA	C. amblycarpa	5
16	RC	Rusk citrange	5
17	JT	Jacobsen trifoliate	2
18	RL-S	Rough lemon schaub	5
19	SC	Swingle citrumelo	5
20	RP	Rangpur lime	5
21	SM	Shekwasha manderin	5
22	ChM	Changsa mandarin	5
23	N	Natsudaidai	5
24	RxT	Rangpur x Troyer	5
25	RL-C	Rough lemon cairn	5
26	CLM	Cleopatra mandarin	5
27	Sunki 1113	Flying dragon x Sunki(1113)	5
28	CO	C.obovoideae	5
29	CC	Carrizo citrange	5
30	TC	Troyer citrange	5
31	Volk	Volkameriana	5
32	KC	Koethen citrange	5
33	TB	Terrabella citrumelo	5
34	RT	Rubidoux trifoliate	5
35	JC	Japanese citron	5
36	BC	Benton citrange	5
37	F80/3	F80 citrumelo 3	5
38	Sunki 1112	Flying dragon x Sunki(1112)	5
39	ST	Sampson tangelo	5
40	SFS	Smooth flat seville	5
41	Sunki 1116	Flying dragon x Sunki(1116)	5
42	RL-W	Rough lemon wallace	5

Results and discussion

Internal fruit quality analysis (Table 5.5.9.2)

- Juice%: The highest juice content was produced by Volkameriana (62.3%), followed by Sunki 1116 (59.4%) and RxT as well as Swingle with 58.1%. Only Rough lemon cairn of the rootstocks evaluated, tested below 52%, not complying with the minimum export standards, and had the lowest juice content of 50%.

- Brix°: This season the Brix content increased, with 41 of the 42 rootstock combinations evaluated producing a Brix higher than 9.5 (export minimum requirement for Delta), except for Konejime with 9.3. Rusk citrange produced the highest Brix content of 13.3, followed by Rubidoux trifoliate with 13.1 and Australian trifoliate with 12.4.

- Acid: Terrabella rootstock provided the highest acid content (1.8%) for this season, followed by RT, JC, Sinki 1112, PT and Sunki 1113 with 1.7%. The lowest acid content was produced on RL-S (1.0%) and complied with the minimum export standards (above 0.85%).

Fruit size distribution (Table 5.5.9.3)

- The fruit size of Delta in combination with 41 of the 42 rootstocks peaked at count 105/125 except for Rangpur x Troyer at count 144 (60.8% of the fruit). The next fruit size peak was at count 144 with 28 of the rootstocks and count 88 with 13 of the remaining rootstock combinations.

Production per tree (Table 5.5.9.4)

- Citrange 32 produced the best crop on the trees with an excellent yield of 310 kg per tree (115 kg per tree on SFS in 2007). Flying dragon x Sunki 1112 bore the second highest crop on the trees, peaking at 205.7 kg per tree. F80/3 was next with 204 kg per tree, outperforming all the prominent rootstocks used currently in the citrus industry (C35, X639, RL-C, CC, TC, VOLK, TB, and BC which ranged from 144 to 188 kg per tree).

Conclusions

The internal quality of the fruit in general was very good to excellent for the 2013 season; and Rough lemon cairn was the only rootstock below the minimum level required for juice content with 50.0% (Delta). Rusk citrange was below the 9.5 requirement for Brix levels on Delta with 9.3 (41 rootstocks complied with minimum. Rough lemon schaub developed the lowest acid level for this trial (1.9%). Two of the Sunki cross selections (Sunki 1112 and 1113) developed high acid levels with maturity, this will be valuable information for the lower acid varieties as well as areas where low acid levels in the fruit occur (Marble Hall and Groblersdal area).

Fruit size peaked at count 105/125 on 41 of the scion: rootstock combinations: typical Delta Valencia fruit size (smaller fruit size). Crop production on the trees increased from 115 kg per tree in 2007 (SFS) to 310 kg per tree on Citrange 32, followed by Flying dragon x Sunki 1112 with 205.7 kg per tree and F80 citrumelo 3 with 204 kg per tree.

Table 5.5.9.2. Internal fruit quality of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) on 20 August 2013.

Root-stock	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
F80/8	72-56	57.5	11.2	1.3	8.68	0.0	T1
PT	88-72	56.4	11.8	1.7	7.11	0.0	T1
C32	88-72	54.8	11.7	1.5	8.07	0.0	T1
AT	105-72	54.2	12.4	1.5	8.44	0.0	T1
HRS 812	105-88	57.2	11.7	1.4	8.18	0.0	T1
K	105-72	54.3	9.3	1.3	7.10	0.0	T1
CM	105-88	55.6	10.4	1.4	7.54	0.0	T1
C35	105-88	58.0	12.0	1.5	8.05	0.0	T1
C	88-72	56.3	11.2	1.2	9.33	0.0	T1
SCS	105-88	54.9	10.9	1.6	6.94	0.0	T1
X639	105-88	55.5	11.3	1.3	8.76	0.0	T1
GT	88-72	54.2	10.7	1.4	7.54	0.0	T1
ML	88-72	56.1	11.9	1.4	8.56	0.0	T1
OT	105-88	56.2	11.1	1.3	8.35	0.0	T1
CA	105-72	57.5	10.7	1.3	8.36	0.0	T1
RC	105-72	54.2	13.3	1.6	8.53	0.0	T1

JT	105-72	56.8	11.5	1.2	9.50	0.0	T1
RL-S	88-72	58.0	10.1	1.0	10.52	0.0	T1
SC	88-72	58.1	11.3	1.5	7.69	0.0	T1
RP	105-88	57.3	10.3	1.2	8.44	0.0	T1
SM	105-72	52.3	9.9	1.4	6.88	0.0	T1
ChM	105-88	54.7	10.6	1.4	7.74	0.0	T1
N	88-72	57.3	10.9	1.4	7.90	0.0	T1
RxT	105-72	58.1	11.5	1.3	9.13	0.0	T1
RL-C	105-88	50.0	10.5	1.2	9.05	0.0	T1
CLM	88-72	57.9	11.1	1.5	7.21	0.0	T1
Sunki 1113	105-72	57.1	12.1	1.7	7.25	0.0	T1
CO	88-72	54.9	11.2	1.4	8.06	0.0	T1
CC	88-72	57.8	12.0	1.4	8.33	0.0	T1
TC	105-72	54.5	11.8	1.2	9.52	0.0	T1
Volk	88-72	62.3	10.3	1.2	8.80	0.0	T1
KC	105-88	56.8	12.2	1.5	8.19	0.0	T1
TB	88-72	54.1	11.1	1.8	6.13	0.0	T1
RT	88-72	56.3	13.1	1.7	7.62	0.0	T1
JC	88-72	55.8	10.5	1.7	6.36	0.0	T1
BC	105-88	56.5	11.2	1.5	7.52	0.0	T1
F80/3	105-88	57.4	9.8	1.4	7.21	0.0	T1
Sunki 1112	105-88	56.3	12.0	1.7	7.19	0.0	T1
ST	105-88	57.0	11.9	1.5	7.93	0.0	T1
SFS	105-72	55.5	11.2	1.4	7.78	0.0	T1
Sunki 1116	105-72	59.4	11.5	1.4	8.46	0.0	T1
RL-W	88-72	56.0	11.9	1.4	8.50	0.0	T1

Table 5.5.9.3. Fruit size distribution of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2013 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/8	48	0.4	CA	48	0.6	CC	48	0.2
F80/8	56	3.0	CA	56	4.5	CC	56	2.2
F80/8	72	8.4	CA	72	12.5	CC	72	8.8
F80/8	88	19.0	CA	88	28.5	CC	88	20.5
F80/8	105/125	48.3	CA	105/125	31.7	CC	105/125	49.6
F80/8	144	20.8	CA	144	22.2	CC	144	18.8
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
PT	48	0.2	RC	48	0.2	TC	48	0.1
PT	56	1.7	RC	56	2.1	TC	56	1.2
PT	72	5.3	RC	72	6.4	TC	72	6.1
PT	88	15.0	RC	88	14.9	TC	88	15.3
PT	105/125	49.2	RC	105/125	38.3	TC	105/125	50.1
PT	144	28.6	RC	144	38.1	TC	144	27.1
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C32	48	0.4	JT	48	0.1	Volk	48	0.3
C32	56	2.2	JT	56	0.9	Volk	56	2.3
C32	72	7.4	JT	72	4.9	Volk	72	6.9
C32	88	16.8	JT	88	15.3	Volk	88	16.3
C32	105/125	52.5	JT	105/125	55.1	Volk	105/125	49.4

C32	144	20.8	JT	144	23.7	Volk	144	24.9
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
AT	48	0.1	RL-S	48	0.1	KC	48	0.1
AT	56	0.4	RL-S	56	0.7	KC	56	0.8
AT	72	2.5	RL-S	72	3.7	KC	72	2.9
AT	88	9.6	RL-S	88	12.7	KC	88	12.1
AT	105/125	48.9	RL-S	105/125	53.6	KC	105/125	52.2
AT	144	38.3	RL-S	144	29.2	KC	144	31.9
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
HRS812	48	0.3	SC	48	0.6	TB	48	0.1
HRS812	56	1.5	SC	56	3.0	TB	56	0.9
HRS812	72	6.5	SC	72	7.7	TB	72	3.9
HRS812	88	17.9	SC	88	15.9	TB	88	12.3
HRS812	105/125	49.9	SC	105/125	47.8	TB	105/125	43.9
HRS812	144	23.9	SC	144	25.1	TB	144	39.0
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
K	48	0.8	RP	48	0.3	RT	48	0.1
K	56	4.7	RP	56	1.5	RT	56	0.6
K	72	11.6	RP	72	6.7	RT	72	2.7
K	88	21.0	RP	88	18.1	RT	88	9.7
K	105/125	46.6	RP	105/125	51.5	RT	105/125	49.4
K	144	15.4	RP	144	21.8	RT	144	37.6
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
CM	48	0.1	SM	48	0.3	JC	48	0.4
CM	56	1.0	SM	56	3.7	JC	56	2.6
CM	72	3.4	SM	72	11.6	JC	72	8.8
CM	88	11.4	SM	88	23.8	JC	88	18.7
CM	105/125	49.7	SM	105/125	50.2	JC	105/125	54.7
CM	144	34.4	SM	144	10.3	JC	144	14.8
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C35	48	0.4	ChM	48	0.2	BC	48	0.1
C35	56	2.1	ChM	56	1.4	BC	56	0.8
C35	72	7.4	ChM	72	5.9	BC	72	3.7
C35	88	16.8	ChM	88	13.1	BC	88	12.6
C35	105/125	52.6	ChM	105/125	48.0	BC	105/125	51.2
C35	144	20.8	ChM	144	31.5	BC	144	31.7
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
C	48	1.1	N	48	0.3	F80/3	48	0.4
C	56	5.1	N	56	3.8	F80/3	56	1.4
C	72	12.8	N	72	13.8	F80/3	72	6.1
C	88	23.0	N	88	30.2	F80/3	88	14.6
C	105/125	45.8	N	105/125	39.5	F80/3	105/125	45.1
C	144	12.3	N	144	12.5	F80/3	144	32.4
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
SCS	48	0.3	RxT	48	0.0	Sunki 1112	48	0.2

SCS	56	1.7	RxT	56	0.4	Sunki 1112	56	1.7
SCS	72	5.7	RxT	72	1.6	Sunki 1112	72	5.3
SCS	88	13.7	RxT	88	8.6	Sunki 1112	88	14.7
SCS	105/125	46.5	RxT	105/125	28.4	Sunki 1112	105/125	50.8
SCS	144	32.1	RxT	144	60.8	Sunki 1112	144	27.3
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
X639	48	0.1	RL-C	48	0.1	ST	48	0.2
X639	56	1.4	RL-C	56	0.7	ST	56	1.3
X639	72	5.0	RL-C	72	3.7	ST	72	5.7
X639	88	14.6	RL-C	88	11.8	ST	88	6.2
X639	105/125	46.6	RL-C	105/125	53.0	ST	105/125	59.1
X639	144	32.3	RL-C	144	30.6	ST	144	27.6
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
GT	48	0.7	CLM	48	0.6	SFS	48	0.6
GT	56	3.6	CLM	56	4.2	SFS	56	3.5
GT	72	12.4	CLM	72	12.5	SFS	72	11.5
GT	88	19.9	CLM	88	23.5	SFS	88	25.9
GT	105/125	44.9	CLM	105/125	45.9	SFS	105/125	32.8
GT	144	18.5	CLM	144	13.4	SFS	144	25.7
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
ML	48	0.1	Sunki 1113	48	0.2	Sunki 1116	48	0.7
ML	56	1.2	Sunki 1113	56	1.2	Sunki 1116	56	4.0
ML	72	4.9	Sunki 1113	72	6.4	Sunki 1116	72	11.2
ML	88	17.0	Sunki 1113	88	17.2	Sunki 1116	88	21.2
ML	105/125	56.0	Sunki 1113	105/125	38.6	Sunki 1116	105/125	46.3
ML	144	20.9	Sunki 1113	144	36.5	Sunki 1116	144	16.6
Rootstock	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**	**Rootstock**	**Size**	**% Fruit**
OT	48	0.1	CO	48	0.5	RL-W	48	0.2
OT	56	1.8	CO	56	2.4	RL-W	56	1.0
OT	72	7.5	CO	72	10.6	RL-W	72	4.7
OT	88	18.5	CO	88	23.7	RL-W	88	14.0
OT	105/125	56.0	CO	105/125	52.8	RL-W	105/125	51.4
OT	144	16.1	CO	144	10.1	RL-W	144	28.7

Table 5.5.9.4. Production of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2013 season.

Rootstock	Kg/tree	
	2007	2013
F80/8	75.5	142.0
PT	66.9	135.9
C32	61.0	310.0
AT	0.0	133.0
HRS 812	75.5	175.1
K	0.0	135.9
CM	104.6	129.0
C35	61.1	144.4
C	0.0	120.1

SCS	0.0	127.8
X639	72.5	157.7
GT	0.0	139.7
ML	87.3	135.2
OT	0.0	107.3
CA	0.0	84.1
RC	72.9	103.7
JT	35.8	136.8
RL-S	75.2	140.5
SC	79.3	160.9
RP	89.0	151.1
SM	86.3	130.2
ChM	0.0	170.7
N	0.0	134.7
RxT	57.0	100.8
RL-C	77.6	165.9
CLM	0.0	112.7
Sunki 1113	78.5	149.1
CO	77.6	124.3
CC	77.6	159.0
TC	101.1	146.0
VOLK	49.5	155.9
KC	72.7	136.8
TB	56.9	168.3
RT	0.0	144.3
JC	0.0	172.1
BC	80.1	188.5
F80/3	65.0	204.0
Sunki 1112	88.0	205.7
ST	73.7	128.3
SFS	115.4	135.5
Sunki1116	92.4	169.2
RL-W	44.8	172.2

5.5.10 **PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area**
Project 416 A by J. Joubert (CRI)

Opsomming

Midknight, met ŉ gesonde entlas verbinding, het bewys dis verenigbaar met Sunki x Beneke 812, ŉ hibried onderstam kruising tussen Sunki mandaryn en Beneke trifoliaat. Die boomgrootte van hierdie kombinasie word as medium beskou (vergelyk met Carrizo boomgrootte en groeikragtigheid), alhoewel Sunki onderstam as boom op sy eie baie groeikragtig is en ŉ groot boom oplewer. Die produksie hierdie seisoen het met twee derdes toegeneem, met ŉ indrukwekkende toename in vruggrootte met pieke by telling 56.

Delta toon vereenigbaarheid met Sunki x Beneke 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 uitstekende toename in produksie op die bome gewees hierdie seisoen, met FF6 wat to meer as 220% verbeter het. Vruggrootte het ook toegeneem en onderstam kombinasies het by telling 105/125, 88 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. Sunki 812 is a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliate. The tree size of this combination was described as medium (similar to Carrizo tree size and growth rate), although Sunki rootstock as a tree on its own is aggressive and develops into a fairly large tree. Yield production this season was excellent and increased by more than two thirds, with a corresponding increase in fruit size, peaking at count 56.

Delta seems to be compatible with Sunki x Beneke 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 an impressive increase on crop production this season with FF-6 producing more than 220% additional fruit on the trees. Fruit size on all three rootstock combinations increased and peaked at count 105/125, 88 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 HRS 802, HRS 812, HRS 809 and C61 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), HRS 812 (Sunki mandarin x Beneke trifoliate) and FF-6 (Sunki x MTO trifoliate orange). Midknight Valencia was budded onto HRS 812 (Sunki x Beneke). The trees were planted at Esselen Nursery in March 1999.

Table 5.5.10.1. 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 Sunki x Beneke 812 in South Africa. Internally fruit quality was excellent with high Brix (11.3) and juice levels higer compared to 2012 (56.8%) with 61.7% (Table 5.5.10.2). The acid content was lower this season (1.4%) similar compared to the 2011 season (1.3%), keeping the Brix: acid ratio well below 10 (8.31). There was a significant increase in crop production for the 2013 season from 47.6 to 72.0 kg/tree (50%) in comparison with the previous season (Table 5.5.10.4), and the 8 year mean was 55.2 kg/tree (Table 5.5.10.4). Delta produced a 37% better crop on Sunki Beneke 812 over the 8 year period compared to Midknight. 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 88 (15%), producing a bigger fruit size on the trees for this season, despite of the higher crop (excellent scenario).

Delta Valencia

Delta on HRS 802 produced the best juice content (60%) and highest acid level (1.14), as well as a Brix:acid ratio of 10:1, followed by Sunki 812 with the highest Brix level (11.5) and FF6 the lowest Brix:acid ratio of 9:1, (Table 5.5.10.2). There was a delay on the external colour development on both Sunki 812 and FF6 (T2-4). Fruit size on all three rootstocks peaked at count 88 (2012-105/125), followed by count 105/125 on HRS 812 and FF6 (2012-72) and count 72 on 803. All three scion: rootstock combinations increased crop production this season, with FF6 more than 220% from 33 kg per tree to 106.1 kg per tree. Sunki 812 increased from 57 kg per tree to 114 kg per tree (100% increase), and 802 from 51 kg per tree to 112 kg per tree (120% increase).

Conclusions

There was an impressive increase in crop production on all the combinations this season due to excellent fruitset, less theft (better security) as well as trees in peak production at this trial site.

Midknight on Sunki 812 performed well, producing an excellent crop compared to the previous season (from 34 to 48 kg per tree), with bigger fruit size despite the better yield, and good internal qualities. The acid levels (1.3%) decreased this season compared to 2012 (1.5%), and were similar to the 2011 (1.3%) levels. The Brix: acid ratio was higher due to the lower acid level, slightly delaying the maturity of the selection with external colour at T1-2.

Delta was evaluated on three hybrid rootstocks, HRS Sunki 812, HRS 802 and FF-6. The more important combination of the above mentioned was Sunki 812. Sunki 812 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 bigger fruit size. There was an excellent crop increase on all three combinations, with FF6 producing well over 200% more fruit on the trees, HRS 802 internally performed well with the best internal quality, as well as the biggest fruit size (peaked at counts 88an 72) for this trial site.

The FF-6 rootstock was also selected for high pH soils, future trials in the Musina and Kakamas areas must be conducted to determine these characteristics.

Table 5.5.10.2. Internal fruit quality of Midknight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 26 July 2013.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Midknight	Sunki 812	61.7	11.3	1.36	8.31	0.0	T1-4
Delta	Sunki 812	59.1	11.5	1.14	10.09	0.1	T1-3
Delta	HRS 802	59.9	9.6	1.00	9.60	0.0	T2-4
Delta	FF-6	57.8	9.6	1.02	9.41	0.0	T2-4

Table 5.5.10.3. Fruit size distribution at Esselen nursery during the 2013 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	12.09	Delta	HRS 802	48	1.45
Midknight	Sunki 812	56	35.93	Delta	HRS 802	56	14.31
Midknight	Sunki 812	72	27.73	Delta	HRS 802	72	28.18
Midknight	Sunki 812	88	14.98	Delta	HRS 802	88	28.27
Midknight	Sunki 812	105/125	8.53	Delta	HRS 802	105/125	24.04
Midknight	Sunki 812	144	0.75	Delta	HRS 802	144	3.74
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	Sunki 812	48	1.95	Delta	FF-6	48	1.05
Delta	Sunki 812	56	12.42	Delta	FF-6	56	11.12
Delta	Sunki 812	72	24.80	Delta	FF-6	72	27.03
Delta	Sunki 812	88	29.41	Delta	FF-6	88	30.20
Delta	Sunki 812	105/125	28.39	Delta	FF-6	105/125	28.05
Delta	Sunki 812	144	3.56	Delta	FF-6	144	2.54

Table 5.5.10.4. Production per tree of Midknight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2013 season.

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)	Kg/tree (2008)	Kg/tree (2009)	Kg/tree* (2010)	Kg/tree* (2011)	Kg/tree* (2012)	Kg/tree (2013)	8 Year Total	8 Year Mean
Mid-knight	Sunki 812	46.8	98.7	50.4	51.7	40.2	34.1	47.6	72.0	441.5	55.2
Delta	Sunki 812	65.4	120.5	66.4	78.6	58.7	42.7	56.5	114.0	602.8	75.4
Delta	HRS 802	58.5	120.6	102.3	102.4	81.4	74.7	51.1	112.0	703.0	87.9
Delta	FF-6	62.9	134.1	97.2	80.7	79.8	61.4	33.3	106.1	655.5	81.9

*Note: Heat wave experienced during fruit set period (fruit also stolen from this specific section)

5.5.11 **PROGRESS REPORT: Evaluation of Grapefruit varieties on different rootstocks in the Swaziland area**
Project 416 B by J. Joubert (CRI)

Opsomming

Die gemiddelde produksie van Marsh, Star Ruby en Nelruby op al vier onderstam kombinasies het toegeneem teenoor die vorige seisoen, behalwe vir Marsh op Swingle en X639, asook Star Ruby op C35. Star Ruby op C35 en MxT bly ŉ uitstekende kombinasie, veral as die boomgrootte van C35 (derde kleiner as ander bome) in ag geneem word, en die feit dat beide onderstamme (C35 en MxT) vir hierdie seisoen in totaal ŉ hoër opbrengs per boom as Swingle geproduseer het. Hierdie jaar het Nelruby op C35 die hoogste Brix: suur verhouding vir hierdie proef behaal met die tweede hoogste Brix (10.1) en laagste suur (1.17), maar Marsh het die hoogste sap persentasie (59.5%) gelewer. Die algemene vrug kwaliteit vir hierdie seisoen was beter in vergelyking met die 2012 seisoen. Marsh, en meer spesifiek in kombinasie met X639, het die laagste Brix: suur verhouding van 5.53: 1 opgelewer.

Die algemene tendens wat vruggrootte aanbetref was kleiner vrugte, met ŉ hoër obrengs op die bome. Hierdie seisoen was die scenario omgekeerd met heelwat meer vrugte op die bome, sowel as grootter vruggrootte oor die algemeen. Al drie kultivars het op telling 48 gepiek, behalwe Nelruby in kombinasie met C35.

Nota: Die bome in hierdie proef is oor die algemeen kleiner as normal, alhoewel die boomgrootte al heelwat toegeneem het, a.g.v. probleme wat met die besproeiings skema ondervind was.

Summary

The average yield production on Marsh, Star Ruby and Nelruby on all four rootstock combinations was better compared to the previous season, except for Marsh on Swingle and X639, as well as Star Ruby on C35. Star Ruby on C35 and MxT remains an excellent combination, when tree size of C35 (a third smaller than other trees) were taken into account, and the fact that both rootstocks (C35 and MxT) for this season produced a better yield compared to Swingle. Nelruby on C35 produced the highest Brix: acid ratio for this trial with second highest Brix (10.1) and lowest acids (1.17%), but Marsh developed the highest juice percentage (59.5%) levels. The average fruit quality for this season was better compared to the 2012 season. Marsh, and more specifically in combination with X639, developed the lowest Brix: acid ratio of 5.55: 1.

The general tendency for fruit size was towards bigger fruit, and a heavier crop on the trees. This season the typical scenario was different with more fruit on the trees, as well as larger fruit size. All three cultivars peaked at count 48, except for Nelruby in combination with C35.

Note: The trees in this trial are generally smaller than expected, although there were increases in tree canopy, due to problems encountered with the irrigation scheme.

Objective

- Investigate the performance of grapefruit cultivars on different rootstocks on heavy, replant soils.
- Improve production, fruit size, internal quality and rind colour.

Materials and methods

Trees were planted in 2003, 10 trees Marsh, Nelruby and Star Ruby all on C35, MxT, SC, and X639.

Table 5.5.11.1. Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

Planted 2003		
Selection	**Rootstock**	**No. of trees**
Marsh	C35	10
Marsh	MxT	9
Marsh	SC	10
Marsh	X639	10
Nelruby	C35	10
Nelruby	MxT	10
Nelruby	SC	10
Nelruby	X639	10
Star Ruby	C35	10
Star Ruby	MxT	10
Star Ruby	SC	8
Star Ruby	X639	8

Results and discussion

Internal quality and colour (Table 5.5.11.2.)

Marsh gave the highest juice percentages (mean 58.3%) followed by Star Ruby (mean 52.4%) and Nelruby (mean 49.7%); Juice percentages on two of the Nelruby combinations (SC and X639) were below 50%, but acceptable (export standards) for all cultivars and rootstocks. This season Star Ruby gave the lowest internal quality with Brix levels from 8.3 on MxT/SC to 9.6 on C35 (mean 8.7) with acid levels and ratios averaging 1.4% and 6.23:1 respectively. Star Ruby ratios were below 7.0 on X639, MxT and Swingle rootstocks, due to the high acid levels delaying peak maturity as well as harvest time.

Nelruby gave the highest Brix levels from 10.1 on C35 to 9.3 on MxT (mean 9.6), the lowest acid levels (mean 1.3%) and highest ratios (mean 7.5:1) followed by Marsh with Brix levels of 8.3 on SC to 10.4 on C35 (mean 9.1) with acid levels and ratios averaging 1.48% and 6.2 respectively. Marsh seed counts were the lowest at 0.01 seeds per fruit followed by Star Ruby at 1.6 seeds per fruit and Nelruby at 4.0 seeds per fruit. External colour was T1 for Star Ruby, T1-2 for Nelruby and T1-5 for Marsh.

Fruit size (Table 5.5.11.3.)

The fruit size increased this season on Marsh, Nelruby and Star Ruby; where all three selections on all four rootstock combinations peaked at count 48 (except for Nelruby on C35 at count 64 (43.2%)), followed by count 64, except for Marsh on SC with 22% at count 40 and Star Ruby on C35 (18.9%), MxT (21.8%) and SC (20.5%), all at count 40. The average fruit size on all the combinations was very similar to that in 2012.

Yield (Table 5.5.11.4.)

Star Ruby produced the highest yield on MxT with 120.4 kg/tree, followed by Nelruby on Swingle with 104.2 kg/tree and Marsh on C35 with 102.8 kg/tree. The only yield decrease this season was Marsh on Swingle and X639 with 95.4 and 68.0 kg per tree respectively, as well as Star Ruby on C35 only dropping 1.2 kg per tree from 113.9 to 112.7 kg per tree. All the other rootstock: scion combinations produced more fruit on the trees.

Taking the total yield production of the trial into consideration per rootstock, MxT bore the most fruit (548.4 kg/tree) followed by Swingle (546.6 kg/tree), C35 (495.4 kg/tree) and X639 (406.4 kg/tree).

Conclusions

The crop production on the trees increased this season; except for Marsh on Swingle and X639; and Star Ruby on C35 (only by 1.2 kg per tree). All the problems were addressed with regards to red scale and irrigation. When compared to the lighter crops of the 2012 season, the 2013 season was good to very good. The best crop production remained with Star Ruby on all four rootstock combinations (94.3 kg per tree), and

the lowest was Nelruby (40.7 kg per tree). The lowest crop for this trial was Marsh on MxT with 46.4 kg per tree.

Marsh on C35 developed the highest Brix (10.4) and juice (59.5%) content for this trial, with Marsh on X639 the highest acid (1.59%). C35 outperformed all the scion: rootstock combinations with the best average internal quality. Fruit size on all three cultivars peaked at count 48, except for Nelruby on C35 (peaked at count 64), developing larger fruit size this season, as well as a improved yields on most combinations (crop decrease limited to 3 combinations).

Nelruby on C35 developed small trees when compared to Marsh and Star Ruby. This might be a specific characteristic of this combination. No incompatibility has been detected at the bud-union, but future evaluations will determine if there might be a problem. External colour of the Nelruby was paler than Star Ruby with fewer blushes on the rind. Internal pigmentation of Nelruby was also slightly less than Star Ruby.

Note: The trees in this trial are generally smaller than expected due to problems encountered with the irrigation scheme.

Table 5.5.11.2. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 21 May 2013.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Marsh	C35	59.5	10.4	1.40	7.43	0.0	T1-3
Marsh	MxT	58.2	8.9	1.55	5.74	0.1	T1-3
Marsh	SC	58.6	8.3	1.36	6.10	0.0	T4-5
Marsh	X639	57.0	8.8	1.59	5.53	0.2	T1-3
Nelruby	C35	52.5	10.1	1.17	8.63	2.0	T1-2
Nelruby	MxT	51.6	9.3	1.40	6.64	3.8	T2
Nelruby	SC	47.8	9.6	1.30	7.38	4.6	T2
Nelruby	X639	46.8	9.5	1.28	7.42	5.6	T1-2
Star Ruby	C35	51.6	9.6	1.32	7.27	1.6	T1
Star Ruby	MxT	51.8	8.3	1.35	6.15	2.1	T1
Star Ruby	SC	52.1	8.3	1.47	5.65	1.8	T1
Star Ruby	X639	54.0	8.4	1.44	5.83	0.8	T1

Table 5.5.11.3. Fruit size distribution per rootstock at Tambuti Estate during the 2013 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	C 35	27	1.38	Nelruby	SC	27	0.77
Marsh	C 35	32	1.85	Nelruby	SC	32	1.03
Marsh	C 35	36	7.52	Nelruby	SC	36	5.87
Marsh	C 35	40	15.19	Nelruby	SC	40	15.73
Marsh	C 35	48	40.70	Nelruby	SC	48	43.11
Marsh	C 35	64	33.35	Nelruby	SC	64	33.49
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	MxT	27	2.25	Nelruby	X639	27	0.22
Marsh	MxT	32	1.60	Nelruby	X639	32	2.11
Marsh	MxT	36	6.84	Nelruby	X639	36	4.70
Marsh	MxT	40	15.85	Nelruby	X639	40	13.55
Marsh	MxT	48	40.87	Nelruby	X639	48	41.32
Marsh	MxT	64	32.58	Nelruby	X639	64	39.53
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	SC	27	9.97	Star Ruby	C 35	27	8.90
Marsh	SC	32	7.31	Star Ruby	C 35	32	6.22
Marsh	SC	36	19.30	Star Ruby	C 35	36	17.21
Marsh	SC	40	22.22	Star Ruby	C 35	40	18.90

Marsh	SC	48	30.54	Star Ruby	C 35	48	33.55
Marsh	SC	64	10.68	Star Ruby	C 35	64	15.24
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Marsh	X639	27	0.21	Star Ruby	MxT	27	5.20
Marsh	X639	32	1.33	Star Ruby	MxT	32	5.31
Marsh	X639	36	6.00	Star Ruby	MxT	36	13.67
Marsh	X639	40	12.33	Star Ruby	MxT	40	21.81
Marsh	X639	48	40.13	Star Ruby	MxT	48	36.48
Marsh	X639	64	40.00	Star Ruby	MxT	64	17.53
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Nelruby	C 35	27	0.39	Star Ruby	SC	27	9.61
Nelruby	C 35	32	1.16	Star Ruby	SC	32	6.96
Nelruby	C 35	36	3.48	Star Ruby	SC	36	18.27
Nelruby	C 35	40	11.83	Star Ruby	SC	40	20.47
Nelruby	C 35	48	39.98	Star Ruby	SC	48	30.93
Nelruby	C 35	64	43.17	Star Ruby	SC	64	13.76
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Nelruby	MxT	27	0.74	Star Ruby	X639	27	7.20
Nelruby	MxT	32	1.24	Star Ruby	X639	32	5.33
Nelruby	MxT	36	7.39	Star Ruby	X639	36	14.74
Nelruby	MxT	40	16.47	Star Ruby	X639	40	19.04
Nelruby	MxT	48	42.66	Star Ruby	X639	48	30.77
Nelruby	MxT	64	31.50	Star Ruby	X639	64	22.92

Table 5.5.11.4. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2013.

Cultivar	Rootstock	Kg/tree						6 Year Total	6 Year Mean
		(2008)	(2009)	(2010)	(2011)	(2012)	(2013)		
Marsh	C35	57.4	22.5	66.0	78.1	92.6	102.8	419.4	69.9
Marsh	MxT	64.4	44.3	34.5	62.4	37.9	46.4	289.9	48.3
Marsh	SC	79.3	95.4	140.3	157.8	126.5	95.4	694.7	115.8
Marsh	X639	66.7	27.3	44.7	95.9	81.7	68.0	384.3	64.1
Nelruby	C35	50.3	9.0	22.0	50.1	51.0	59.4	241.8	40.3
Nelruby	MxT	69	51.4	34.7	61.2	17.9	60.8	295	49.2
Nelruby	SC	95.2	35.3	18.4	115.8	59.6	104.2	428.5	71.4
Nelruby	X639	71.4	46.2	39.5	81.2	34.1	51.9	324.3	54.1
Star Ruby	C35	63.7	17.7	36.9	119.6	113.9	112.7	464.5	77.4
Star Ruby	MxT	74.2	60.9	100.3	150.6	106.6	120.4	613.0	102.2
Star Ruby	SC	60.2	63.7	44.8	119.8	72.0	108.2	468.7	78.1
Star Ruby	X639	31.4	31.3	41.2	102.0	84.7	109.8	400.4	66.7

5.5.12 **PROGRESS REPORT: Evaluation of various Navel selections on different rootstocks in the Burgersfort and Marble Hall area**

Project 590 A by J. Joubert (CRI)

Opsomming

Albei die vroeë nawel seleksies (Fukumoto en Newhall) het nie aan die uitvoer standaarde voldoen nie, agv lae suur vlakke onder die minimum standaard. Newhall het vertraagde eksterne kleur ontwikkeling op die vrugte getoon teenoor Fukumoto teen die selfde tyd met oes, maar intern was die sap en suurvlakke van Newhall laer gewees as Fukumoto. Vruggroote op albei seleksies het by telling 56 gepiek; ideale vruggrootte vir nawel produksie. Newhall se oes opbrengs per bostam: onderstam kombinasie was beter gewees as Fukumoto, alhoewel Fukumoto se totale opbrengs op die ses onderstam kombinasies hoër was.

Die twee laat nawel seleksies, waarvan Glen Ora Late op twee persele (Marble Hall en Burgersfort) aangeplant is teenoor California Lane Late slegs op die Marble Hall perseël, was vir die eerste keer tydens die 2013 season ge-evalueer. Lane Late was vroeër ryp gewees met laer suur vlakke as Glen Ora Late, maar soortgelyke sap en Brix vlakke by ŉ eksterne kleur ontwikkeling tussen T1 en T2. Glen Ora het in totaal op al die bostam: onderstam kombinasies meer vrugte geproduseer in vergelyking met Lane Late (512.5 kg teenoor 362.1 kg). Vruggrootte op albei seleksie het by telling 56 gepiek.

Summary

The two early navel varieties (Fukumoto and Newhall) did not comply with the export requirements due to low juice levels. The external colour development on Newhall was delayed compared to Fukumoto at the same time when evaluated, although internally the juice and acid levels were lower than Fukumoto. Fruit size peaked at count 56 on both selections; the optimum fruit size for navel production. Newhall produced a better crop per scion: rootstock combination, although the total crop on all six rootstocks with Fukumoto was higher.

The two late maturing navel selections, where Glen Ora Late was planted at two separate trial sites (Marble Hall and Burgersfort) and California Lane Late only at the Burgersfort site, were evaluated for the first time during the 2013 season. Lane Late matured first with lower acid levels compared to Glen Ora Late, but the juice and Brix levels were similar at external colour development of T1 to T2. Glen Ora produced the best crop in total on the scion: rootstock trees, followed by Lane Late (512.5 kg versus 362.1 kg). Fruit size peaked at count 56 on both selections.

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 Moosrivier in 2005, and the additional trees established at BBE Boerdery late in 2004. Trees were evaluated visually 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 also evaluated and analysed.

Table 5.5.12.1. List of cultivar and rootstock combinations in the Navel trial at Moosrivier in the Marble Hall area.

Selection	Rootstock	Qty Trees
Fukumoto	C35	4
Fukumoto	CC	5
Fukumoto	MxT	4
Fukumoto	SC	5
Fukumoto	Terrabella	3
Fukumoto	X639	5
Newhall	C35	5
Newhall	CC	3
Newhall	MxT	2
Newhall	SC	5
Newhall	Terrabella	2
Newhall	X639	5
Glen Ora Late	C35	5
Glen Ora Late	CC	3
Glen Ora Late	MxT	2
Glen Ora Late	SC	5
Glen Ora Late	Terrabella	4
Glen Ora Late	X639	5
Cal Lane Late	C35	5
Cal Lane Late	CC	5
Cal Lane Late	MxT	4

Cal Lane Late	SC	5
Cal Lane Late	Terrabella	4
Cal Lane Late	X639	5

Table 5.5.12.2. List of cultivar and rootstock combinations in the Navel trial at BBE Boerdery in the Burgersfort area.

Selection	Rootstock	Qty Trees
Glen Ora Late	C35	2
Glen Ora Late	CC	9
Glen Ora Late	KC	4
Glen Ora Late	MxT	11
Glen Ora Late	SC	14
Glen Ora Late	Terrabella	7
Glen Ora Late	X639	4

Results and discussion

This was the first evaluations for the trial site, due to severe cold damage in the past and poor crop set on the trees.

Fukumoto Navel
Fukumoto on MxT produced the best juice (47.3%), Brix in combination with C35 (10.4) and highest acid levels (0.85%) for this trial, followed by Terrabella with the highest Brix:acid ratio of 12.86. All the scion: rootstock combinations were below the minimum juice level for export of 48%. The external colour on all the combinations was between T1 and T4 (Table 5.5.11.2). Fruit size peaked from count 56 (MxT, CC and X639) to count 48 (C35, SC and Terrabella) on all rootstocks, followed by count 72 on all six (Table 5.5.12.3). Carrizo produced the best crop on the trees with 67.6 kg, followed by MxT with 65.3 kg per tree and X639 with 50.1 kg per tree. Swingle was the lowest with 21.6 kg per tree (Table 5.5.12.4).

Newhall Navel
Newhall on X639 developed the highest juice content of 47.2%, followed by Swingle (46%) and MxT (45.7%). The juice levels were too low to comply with requirements for export, similar to Fukumoto (both early navel selections) this season. The quality will improve when the trees mature, and the evaluations will have to be completed earlier in the season. MxT developed the best Brix (11.3) and Brix: acid ratio of 14.49:1, well over matured for a navel variety. The acid level ranged from 0.73 to 0.85%, complying with the minimum standards. Carrizo was well overmatured with a Brix: acid ratio of 13.1:1. The external colour on all the combinations was delayed from T1 to T5 (Table 5.5.12.2). Carrizo, Swingle and X639 peaked at count 56, followed by C35 and MxT at count 48 (Table 5.5.12.3). X639 was the best with 98 kg per tree, followed by Carrizo with 53.4 kg per tree and MxT with 20 kg per tree. The lowest crop was on Terrabella with no fruit on the trees. (Table 5.5.12.4).

Glen Ora Late Navel
Moosrivier:

Swingle in combination with Glen Ora peaked with juice levels of 54.8%, followed by Terrabella with Brix of 11.5 and acid level of 1.03%, developing a Brix: acid ratio of 11.17. The external colour on all the combinations was T1-T2. (Table 5.5.12.2). Glen Ora peaked on five of the six scion: rootstock combinations at count 56, except for MxT count 105/125. (Table 5.5.12.3). MxT produced the best crop on the Glen Ora Late trees and peaked at 147.5 kg per tree (small fruit size on MxT due to a heavy crop). Carrizo outperformed Swingle with 98.4 kg per tree. The remaining rootstocks peaked above 65 kg per tree (Table 5.5.12.4).

BBE Boerdery:

Glen Ora on Terrabella rootstock developed the best juice (52.6%) levels at the Burgersfort trial site. Koethen citrange was next with a high Brix of 12.9, excellent quality for this navel. X639 was below 1.0% acid, although Brix: acid ratio was excellent above 12.55. The external colour matured from T1 to T3 (Table 5.5.12.2). Fruit size peaked at count 56 on four of the six rootstocks, except for Koethen and X639 at count 72. Excellent fruit size for good quality export navel with medium to large fruit on the trees (Table 5.5.12.3).

At both trial sites MxT was the optimum rootstock choice for maximum crop production on the trees, bearing 101.4 kg fruit per tree. Koethen was second with 92.7 kg per tree, followed by Swingle and X639 with 91.2 and 91.4 kg per tree.

Lane Late Navel

The external colour on all four scion: rootstock combinations was uniform and peaked at T1 to T2. Lane Late performed well on MxT rootstock with the best juice (54%) and highest acid levels (0.9%) as well as good Brix: acid ratio of 11.67 (minimum standard 7.5:1). Terrabella had the lowest juice (48.9%), but highest Brix (10.8) and Brix: acid ratio of 13.5 when the trees were evaluated (Table 5.5.12.2). All six rootstocks peaked at a large fruit size (count 56, good navel export size) by the time of the final evaluation. The second highest peak was on count 72 (medium fruit size), except for Terrabella moving one size up to count 48 (Table 5.5.11.3). Minneola x Trifoliate produced the best yield on the trees and cropped 71.4 kg per tree. The second best combination was Lane Late on the dwarfing rootstock C35, cropping 63 kg per tree. Swingle was the lowest with 51.4 kg per tree. (Table 5.5.12.4).

Conclusions

Fukumoto on MxT performed well, considering that the trial was harvested for the first time in 2013. The internal quality was average with low juice levels. Fruit size was large to very large and the yield production peaked at 67.6 kg per tree. Newhall on X639 had high juice levels, but the second lowest acid content resulting in a Brix: acid ratio of 11.9:1. The fruit on the trees were over mature and Carrizo as well as MxT had Brix: acid ratios above 13:1. X639 produced the best crop on the trees, averaging 100 kg per tree.

The two late maturing navels were Glen Ora and Lane Late, where Glen Ora was evaluated at Marble Hall and Burgersfort trial sites. The internal quality was good and Terrabella performed well with high Brix, acid and Brix: acid ratios. Fruit size peaked at counts 57 and 72, excellent for navel production. Yield production on the trees at Marble Hall outperformed Burgersfort (147.5 kg per tree versus 101.4 kg per tree).

Lane Late in combination with MxT, Terrabella and X639 developed fruit with good internal quality and Brix: acid ratios above 11.5:1, indicating peak maturity for late navel selections. The external quality development ranged from T1 to T2 and crop production peaked at 71 kg per tree on MxT rootstock.

Table 5.5.12.2. Internal fruit quality data for Navels on different rootstocks at Moosrivier, Marble Hall during the 2013 season.

Selection	Root-stock	Date harvested	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Fukumoto	C35	02/05/2013	46.2	10.4	0.84	12.38	0.0	T1-3
Fukumoto	CC	02/05/2013	46.0	9.5	0.83	11.45	0.0	T2-3
Fukumoto	MxT	02/05/2013	47.3	10.4	0.85	12.24	0.0	T1-2
Fukumoto	SC	02/05/2013	46.7	8.4	0.72	11.67	0.0	T2-3
Fukumoto	TB	02/05/2013	45.5	9.9	0.77	12.86	0.0	T1-4
Fukumoto	X639	02/05/2013	45.5	10.2	0.71	14.37	0.0	T3-4
Newhall	C35	02/05/2013	44.9	10.2	0.85	12.00	0.0	T3-5
Newhall	CC	02/05/2013	43.5	9.5	0.73	13.01	0.0	T4-5
Newhall	MxT	02/05/2013	45.7	11.3	0.78	14.49	0.0	T3-5
Newhall	SC	02/05/2013	46.0	10.0	0.79	12.66	0.0	T2-4
Newhall	TB	02/05/2013	44.8	9.9	0.77	12.86	0.0	T1-3
Newhall	X639	02/05/2013	47.2	8.8	0.74	11.89	0.0	T2-5
Glen Ora Late	C35	16/07/2013	52.9	11.1	1.00	11.10	0.0	T1-2
Glen Ora Late	CC	16/07/2013	52.7	10.7	0.85	12.59	0.0	T1-2
Glen Ora Late	MxT	16/07/2013	52.9	10.0	1.11	9.01	0.0	T1-2
Glen Ora Late	SC	16/07/2013	54.8	11.3	1.01	11.19	0.0	T1-2
Glen Ora Late	TB	16/07/2013	50.2	11.5	1.03	11.17	0.0	T1-2
Glen Ora Late	X639	16/07/2013	53.2	10.6	0.93	11.40	0.0	T1-2
Lane Late	C35	16/07/2013	52.4	10.3	0.84	12.26	0.0	T1-2
Lane Late	CC	16/07/2013	51.6	9.6	0.80	12.00	0.0	T1-2

Lane Late	MxT	16/07/2013	54.0	10.5	0.90	11.67	0.0	T1-2
Lane Late	SC	16/07/2013	53.3	10.0	0.89	11.24	0.0	T1-2
Lane Late	TB	16/07/2013	48.9	10.8	0.80	13.50	0.0	T1-2
Lane Late	X639	16/07/2013	52.0	9.4	0.75	12.53	0.0	T1-2

Table 5.5.12.3. Internal fruit quality data for Navels on different rootstocks at BBE Boerdery, Burgersfort during the 2013 season.

Selection	Root-stock	Date harvested	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Glen Ora Late	C35	02/07/2013	49.2	12.6	1.02	12.35	0.0	T1-2
Glen Ora Late	CC	02/07/2013	50.9	12.8	1.21	10.58	0.0	T1-2
Glen Ora Late	KC	02/07/2013	52.1	12.9	1.07	12.06	0.0	T1-2
Glen Ora Late	MxT	02/07/2013	49.8	11.4	1.12	10.18	0.0	T1-3
Glen Ora Late	SC	02/07/2013	50.3	12.1	1.04	11.63	0.0	T1-3
Glen Ora Late	TB	02/07/2013	52.6	12.0	1.12	10.71	0.5	T1-3
Glen Ora Late	X639	02/07/2013	52.1	12.3	0.98	12.55	0.0	T1-2

Table 5.5.12.4. Fruit size distribution per rootstock at Moosrivier, Marble Hall during the 2013 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fukumoto	C35	48	37.58	Newhall	C35	48	40.06
Fukumoto	C35	56	32.01	Newhall	C35	56	35.02
Fukumoto	C35	72	16.72	Newhall	C35	72	14.20
Fukumoto	C35	88	7.96	Newhall	C35	88	7.26
Fukumoto	C35	105/125	5.25	Newhall	C35	105/125	3.15
Fukumoto	C35	144	0.48	Newhall	C35	144	0.32
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	MxT	48	16.30	Newhall	MxT	48	33.05
Fukumoto	MxT	56	31.15	Newhall	MxT	56	31.36
Fukumoto	MxT	72	25.96	Newhall	MxT	72	21.19
Fukumoto	MxT	88	14.75	Newhall	MxT	88	7.63
Fukumoto	MxT	105/125	10.75	Newhall	MxT	105/125	6.78
Fukumoto	MxT	144	1.09	Newhall	MxT	144	0.00
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	CC	48	29.19	Newhall	CC	48	17.63
Fukumoto	CC	56	34.34	Newhall	CC	56	37.29
Fukumoto	CC	72	18.73	Newhall	CC	72	27.27
Fukumoto	CC	88	8.79	Newhall	CC	88	11.13
Fukumoto	CC	105/125	7.51	Newhall	CC	105/125	5.19
Fukumoto	CC	144	1.44	Newhall	CC	144	1.48
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	SC	48	48.57	Newhall	SC	48	16.49
Fukumoto	SC	56	31.43	Newhall	SC	56	37.89
Fukumoto	SC	72	13.25	Newhall	SC	72	28.77
Fukumoto	SC	88	4.94	Newhall	SC	88	12.63
Fukumoto	SC	105/125	1.56	Newhall	SC	105/125	3.51
Fukumoto	SC	144	0.26	Newhall	SC	144	0.70
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	X639	48	22.71	Newhall	X639	48	13.28
Fukumoto	X639	56	32.74	Newhall	X639	56	29.59
Fukumoto	X639	72	21.44	Newhall	X639	72	27.22
Fukumoto	X639	88	12.29	Newhall	X639	88	16.60

Fukumoto	X639	105/125	9.14	Newhall	X639	105/125	11.90
Fukumoto	X639	144	1.67	Newhall	X639	144	1.42
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Fukumoto	TB	48	50.00	Newhall	TB	48	0.00
Fukumoto	TB	56	30.00	Newhall	TB	56	0.00
Fukumoto	TB	72	10.50	Newhall	TB	72	0.00
Fukumoto	TB	88	6.50	Newhall	TB	88	0.00
Fukumoto	TB	105/125	2.25	Newhall	TB	105/125	0.00
Fukumoto	TB	144	0.75	Newhall	TB	144	0.00
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	C35	48	14.57	Glen Ora Late	C35	48	41.78
Lane Late	C35	56	37.13	Glen Ora Late	C35	56	40.53
Lane Late	C35	72	28.22	Glen Ora Late	C35	72	13.68
Lane Late	C35	88	13.57	Glen Ora Late	C35	88	3.17
Lane Late	C35	105/125	6.20	Glen Ora Late	C35	105/125	0.50
Lane Late	C35	144	0.31	Glen Ora Late	C35	144	0.33
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	MxT	48	18.77	Glen Ora Late	MxT	48	3.85
Lane Late	MxT	56	38.25	Glen Ora Late	MxT	56	18.70
Lane Late	MxT	72	26.14	Glen Ora Late	MxT	72	19.89
Lane Late	MxT	88	11.93	Glen Ora Late	MxT	88	20.36
Lane Late	MxT	105/125	4.65	Glen Ora Late	MxT	105/125	26.99
Lane Late	MxT	144	0.26	Glen Ora Late	MxT	144	10.21
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	CC	48	17.24	Glen Ora Late	CC	48	19.11
Lane Late	CC	56	40.66	Glen Ora Late	CC	56	40.53
Lane Late	CC	72	24.62	Glen Ora Late	CC	72	23.89
Lane Late	CC	88	9.62	Glen Ora Late	CC	88	11.01
Lane Late	CC	105/125	5.29	Glen Ora Late	CC	105/125	5.12
Lane Late	CC	144	2.57	Glen Ora Late	CC	144	0.34
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	SC	48	15.21	Glen Ora Late	SC	48	18.97
Lane Late	SC	56	43.87	Glen Ora Late	SC	56	44.72
Lane Late	SC	72	28.26	Glen Ora Late	SC	72	22.77
Lane Late	SC	88	9.03	Glen Ora Late	SC	88	9.23
Lane Late	SC	105/125	3.43	Glen Ora Late	SC	105/125	4.02
Lane Late	SC	144	0.20	Glen Ora Late	SC	144	0.30
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	X639	48	14.51	Glen Ora Late	X639	48	26.27
Lane Late	X639	56	44.55	Glen Ora Late	X639	56	42.06
Lane Late	X639	72	24.75	Glen Ora Late	X639	72	22.56
Lane Late	X639	88	11.74	Glen Ora Late	X639	88	6.37
Lane Late	X639	105/125	4.03	Glen Ora Late	X639	105/125	1.93
Lane Late	X639	144	0.42	Glen Ora Late	X639	144	0.81
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Lane Late	TB	48	26.17	Glen Ora Late	TB	48	26.38
Lane Late	TB	56	42.62	Glen Ora Late	TB	56	28.86
Lane Late	TB	72	21.01	Glen Ora Late	TB	72	23.33
Lane Late	TB	88	7.68	Glen Ora Late	TB	88	12.48
Lane Late	TB	105/125	2.52	Glen Ora Late	TB	105/125	7.90

Lane Late	TB	144	0.00	Glen Ora Late	TB	144	1.05

Table 5.5.12.5. Fruit size distribution per rootstock at BBE Boerdery, Burgersfort during the 2013 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Glen Ora Late	C35	48	6.02	Glen Ora Late	SC	48	7.81
Glen Ora Late	C35	56	31.63	Glen Ora Late	SC	56	28.88
Glen Ora Late	C35	72	30.87	Glen Ora Late	SC	72	28.75
Glen Ora Late	C35	88	19.13	Glen Ora Late	SC	88	19.71
Glen Ora Late	C35	105/125	11.45	Glen Ora Late	SC	105/125	13.64
Glen Ora Late	C35	144	0.90	Glen Ora Late	SC	144	1.21
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Glen Ora Late	CC	48	7.18	Glen Ora Late	TB	48	17.06
Glen Ora Late	CC	56	30.89	Glen Ora Late	TB	56	41.33
Glen Ora Late	CC	72	29.07	Glen Ora Late	TB	72	25.22
Glen Ora Late	CC	88	18.08	Glen Ora Late	TB	88	11.44
Glen Ora Late	CC	105/125	13.15	Glen Ora Late	TB	105/125	4.08
Glen Ora Late	CC	144	1.63	Glen Ora Late	TB	144	0.87
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Glen Ora Late	KC	48	5.28	Glen Ora Late	X639	48	1.48
Glen Ora Late	KC	56	27.76	Glen Ora Late	X639	56	15.86
Glen Ora Late	KC	72	29.24	Glen Ora Late	X639	72	29.74
Glen Ora Late	KC	88	21.71	Glen Ora Late	X639	88	25.59
Glen Ora Late	KC	105/125	14.77	Glen Ora Late	X639	105/125	24.99
Glen Ora Late	KC	144	1.25	Glen Ora Late	X639	144	2.35
Cultivar	**Rootstock**	**Size**	**% Fruit**				
Glen Ora Late	MxT	48	8.31				
Glen Ora Late	MxT	56	30.85				
Glen Ora Late	MxT	72	30.36				
Glen Ora Late	MxT	88	18.22				
Glen Ora Late	MxT	105/125	11.32				
Glen Ora Late	MxT	144	0.94				

Table 5.5.12.6. Production per tree of Navel selections on different rootstocks at Moosrivier, Marble Hall during the 2013 season.

Cultivar	Rootstock	Kg/tree
		2013
Fukumoto	C35	41.8
Fukumoto	CC	67.6
Fukumoto	MxT	65.3
Fukumoto	SC	21.6
Fukumoto	TB	37.2
Fukumoto	X639	50.1
Newhall	C35	17.2
Newhall	CC	53.4
Newhall	MxT	20.5
Newhall	SC	14.2
Newhall	TB	0.0
Newhall	X639	98.0
Lane Late	C35	63.0
Lane Late	CC	61.9
Lane Late	MxT	71.4

Lane Late	SC	51.4
Lane Late	TB	54.8
Lane Late	X639	59.6
Glen Ora	C35	67.1
Glen Ora	CC	98.4
Glen Ora	MxT	147.5
Glen Ora	SC	68.7
Glen Ora	TB	65.5
Glen Ora	X639	65.3

Table 5.5.12.7. Production per tree of Navel selections on different rootstocks at BBE Boerdery, Burgersfort during the 2013 season.

Cultivar	Rootstock	Kg/tree
		2013
Glen Ora Late	C35	75.2
Glen Ora Late	CC	89.4
Glen Ora Late	KC	92.7
Glen Ora Late	MxT	101.4
Glen Ora Late	SC	91.2
Glen Ora Late	TB	49.1
Glen Ora Late	X639	91.4

5.5.13 **PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area**
Project 590 B by J. Joubert (CRI)

Opsomming

Delta op Koethen citrange het ŉ uitstekende 12.2 Brix : suur verhouding opgelewer, die hoogste vir die totale proef. Al die Delta onderstam kombinasies het nie aan die uitvoer standaarde voldoen. Delta se vruggrootte op al die onderstam kombinasies het by telling 105/125 gepiek, waarna telling 88 gevolg het. Die oes produksie op al die kombinasies het toegeneem behalwe by Koethen citrange, met Swingle die hoogste (140.4 kg/boom), ŉ 100% verbetering. Geen onverenigbaarheids tekens was by enige van die kombinasies sigbaar nie.

McClean SL het ook op al die onderstamme aan die uitvoer standaarde voldoen, die interne kwaliteit het heelwat verbeter op die vrugte. Swingle het die hoogste sap inhoud (60%) gelewer met Carrizo die hoogste Brix: suur verhouding van 9.74:1. Vruggrootte was uitstekend vir Valencias gewees, en die tellings het gepiek tussen 56, 72 en 88. McClean SL op al die bostam: onderstam kombinasie se produksie het toegeneem, met X639 die hoogste opbrens (182.2 kg/boom), gevolg deur Swingle met 170.8 kg per boom.

Midknight op Swingle en Koethen citrange het van die hoogste sap vlakke vir hierdie proef geproduseer (61.6%). Die hoogste suurvlak was op Swingle gewees, wat dan ook ŉ laer Brix: suur vlak van 9.01 tot gevolg gehad het. Hierdie bostam: onderstam kombinasie kan later in die seisoen geoes word, hou wel die eksterne kleur ontwikkeling goed dop. Midknight was die enigste seleksie met saad tellings in die vrugte (reeks van 0.2 tot 0.3 sade per vrug). Die vruggroottes het tussen telling 56 en 72 gepiek, behalwe vir X639 en Koethen citrange (telling 105/125). Oes produksie op vyf van die kombinasies was beter, met Swingle die hoogste, maar Carrizo en MxT het ongeveer ŉ derde van hulle oes gedaal. Warm temperature tydens blomset in hierdie produksie area het ŉ groot invloed op die prestasie.

Portsgate het hierdie seisoen uitstekend gevaar. Die interne kwaliteit van die vrugte op al die onderstam kombinasies het aan die uitvoer standaarde voldoen. Vruggroottes het gepiek by telling 72 (C35 en MxT) en telling 105/125 (CC, KC, SC, X639 en TB). Die hoogste oes obrengs in kombinasie met Portsgate was op X639 geproduseer (96.9 kg/boom), gevolg deur Swingle met 89.2 kg per boom. Slegs drie van die bostam: onderstam kombinasies het ŉ beter oes opgelewer (MxT, TB en X639). Al die vrugte op die bome was saadloos gewees.

Summary

Delta on Koethen citrange developed an excellent Brix: acid ratio of 12.2, the highest ratio for this trial. All the Delta rootstock combinations complied with the export requirements. Delta peaked on all the rootstock combinations with fruit size at count 105/125, followed by count 88. Yield production increased on all rootstock selections except for Koethen citrange, with Swingle the best (140.4 kg/tree), an increase of 100%. There were no incompatibility problems on the rootstock combinations visible.

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. Swingle developed the best juice level (60%) with Carrizo the highest Brix: acid ratio of 9.74:1. Fruit size for Valencias was excellent, fruit counts peaked between count 56, 72 and 88. McClean SL on all the scion: rootstock combinations increased their crop, with X639 the highest yield (182.2 kg/tree), followed by Swingle with 170.8 kg per tree.

Midknight on Swingle and Koethen citrange developed the best juice levels for this trial; above 61%. Midknight on Swingle produced the highest acid level, causing a low Brix: acid ratio of 9.01. Harvest this scion: rootstock combination later to improve ratio, but keep external colour development in mind. Midknight was the only selection to develop seeds in the fruit for this trial (ranging from 0.2 to 0.3 seeds per fruit). The fruit sizes peaked between count 56 and 72, except for X639 and Koethen citrange (count 105/125). Yield production on five of the combinations was better, with Swingle the best (95.9 kg per tree), although Carrizo and MxT dropped nearly a third in production. High temperature during the flower set period in this production area played a role.

Portsgate performed excellently this season. The internal quality of the fruit on all the rootstocks complied with the export requirements. Fruit size peaked at count 72 (C35 and MxT) and count 105/125 (CC, KC, SC, X639 and TB). The highest yield production in combination with Portsgate was produced on X639 (96.9 kg/tree), followed by Swingle (89.2 kg/tree). Only three of the scion: rootstock combinations produced a better crop this season (MxT, TB and X639). All the fruit on the trees were seedless.

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 5.5.13.1. 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 Koethen produced the best Brix (12.2) and lowest acid level (1.0), as well as Brix: acid ratio of 12.2:1, followed by X639 with the highest juice level (60.3%) and Carrizo the lowest Brix: acid ratio of 8.96. The external colour on all the selections was T1 (Table 5.5.13.2). Fruit size on all seven rootstocks peaked at count 105/125, followed by all seven at count 88 and five combinations at count 72, except for Carrizo and Koethen with count 144 (Table 5.5.13.3). Six of the seven scion: rootstock combinations increased crop production this season (except for Koethen which declined from 49.0 to 30.6 kg per tree), with Swingle more than 100% from 70.1 kg per tree to 140.4 kg per tree. The second highest production was in combination with X639 cropping 107.7 kg of fruit per tree (Table 5.5.13.4).

McClean SL Valencia

McClean SL on Swingle developed the highest juice content of 60%, in combination with 1.26% acid. The lowest acid levels for this trial were produced on Koethen and X639 (1.12%). C35 outperformed the other scion: rootstock combinations with the best Brix (11.9) and Carrizo with the Brix: acid ratio (9.74). The external colour on all the selections was T1 and McClean SL on all rootstocks was completely seedless (Table 5.5.13.2). Carrizo, Koethen, MxT and Terrabella peaked at count 72, followed by C35 and X639 at count 56 and Swingle at count 105/125. The small fruit size on the Swingle combination was due to the second highest crop (170.8 kg per tree) produced for this trial site (Table 5.5.12.3). All seven scion: rootstock combinations increased crop production this season. X639 was the best (from 49.2 kg per tree to 182.2 kg/tree), followed by Swingle from 74.0 kg per tree to 170.8 kg per tree (130% increase). The lowest crop was on Terrabella with 57.6 kg per tree (Table 5.5.13.4).

Midknight Valencia

Swingle and Koethen in combination with Midknight peaked with juice and Brix levels of 61.6% and 11.8, followed by MxT developing the best Brix: acid ratio of 10.6 and the lowest acid level of 1.1%. The external colour on all the selections was T1, except for C35 between T1 and T2 (Table 5.5.13.2). Carrizo, C35 and Terrabella peaked at count 72, followed by MxT and Swingle at count 56 and Koethen and X639 at count 105/125 (Table 5.5.13.3). Five of the seven scion: rootstock combinations increased crop production this season except for Carrizo (declined from 41.8 to 28.6 kg per tree) and MxT (30.3 kg per tree to 20.3 kg per tree). The highest 6 year total was on C35 with 337.2 kg and 56 .2 kg per tree 6 year mean (Table 5.5.13.4).

Portsgate Valencia

Portsgate on C35 and Swingle produced the best juice (59.8%) levels, followed by C35 with the best Brix level (11.5) and X639 the highest Brix: acid ratio of 10.61 as well as lowest acid level of 0.99%. The external colour on all the selections was T1 (Table 5.5.13.2). Five of the seven rootstocks peaked at count 105/125, except for MxT and C35 with count 72. The second highest peak was on count 88, except for C35 (count 56) and Carrizo (count 105/125) (Table 5.5.13.3). Three of the seven scion: rootstock combinations increased crop production this season. X639 outperformed the other combinations with 96.9 kg per tree. The second best was Swingle with 89.2 kg per tree, although 16.8 kg lower than in 2012. C35, Carrizo, Koethen and Swingle bore less fruit, with Carrizo the lowest (35 kg per tree) (Table 5.5.13.4).

Conclusions

Delta on all the rootstocks produced very good internal quality. The highest Brix: acid ratio was in combination with Koethen, peaking at 12.2:1 for this trial. The fruit size production for Delta peaked at count 105/125 on all the rootstock combinations, followed by 88. Production was even better this season, all the combinations increased their yield on the trees except for Koethen (30.6 kg per tree), with Swingle outperforming the rest at 140.4 kg per tree. X639 followed Swingle with 107.7 kg per tree.

McClean seedless on Swingle produced the highest juice levels (60.0%) in a row (2012 also best) for this trial. C35 outperformed the other rootstocks with the highest Brix level (11.9) for the second time this season. The best Brix: acid ratio average was 9.74 on Carrizo, very good for export quality and meets the requirements. Four of the rootstocks peaked at count 72, followed by the next two with count 56, and SC at count 88. McClean seedless produces on average a good fruit size for a Valencia selection. X639 bore a crop of 182.2 kg per tree, followed by Swingle with 170.8 kg per tree, excellent production for this trial. McClean seedless in combination with Terrabella performed poorly this season, producing a yield of only 57.6 kg per tree.

Midknight's performance was better this season and the internal quality improved. The juice and Brix levels increased; Koethen and Swingle with 61.8% juice and 11.8 Brix. Acids on C35 were high (1.29%), but still acceptable and below the export maximum; lowest was on MxT (1.1%). Midknight always tends to have larger fruit, but once again this season was an exception (similar to 2012), with only three rootstocks peaking at count 72 (C35, CC, TB). Swingle and MxT peaked at count 72, followed by Koethen citrange and X639 peaking at count 105/125. Crop set on the trees increased and five or the combinations were higher, with Swingle the best, producing 95.9 kg per tree (2012 season was 53 kg per tree).

Portsgate performed well internally with juice levels on six of the combinations above 59%, except for Koethen with 58.9%. The highest juice was on C35 and Swingle (59.8%), Brix on C35 (11.5) and Brix: acid ratio on X639 (10.61%), also developing the lowest acid (0.99%). Portsgate peaked at various counts from 105/125 (CC, KC, SC, TB and X639) up to 72 (C35 and MxT). The yield decreased for 2013 on four combinations (C35, CC, KC and SC), but improved on MxT, Terrabella and X639. X639 outperformed the rest, as well as producing the highest crop in total for this trial with 96.9 kg per tree.

Table 5.5.13.2. Internal fruit quality data for Valencias on different rootstocks at Golden Frontiers Citrus, Hectorspruit on 1 August 2013.

Selection	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Delta	C35	58.1	12.0	1.30	9.23	0.0	T1
Delta	CC	58.9	11.2	1.25	8.96	0.0	T1
Delta	KC	57.9	12.2	1.00	12.20	0.0	T1
Delta	MxT	57.5	10.9	1.17	9.32	0.0	T1
Delta	SC	58.7	10.8	1.09	9.91	0.0	T1
Delta	TB	59.4	10.8	1.05	10.29	0.0	T1
Delta	X639	60.3	11.0	1.14	9.65	0.0	T1
McClean SL	C35	57.9	11.9	1.36	8.75	0.0	T1
McClean SL	CC	56.8	11.1	1.14	9.74	0.0	T1
McClean SL	KC	58.5	10.7	1.12	9.55	0.0	T1
McClean SL	MxT	59.9	11.2	1.24	9.03	0.0	T1
McClean SL	SC	60.0	10.3	1.26	8.17	0.0	T1
McClean SL	TB	58.9	11.2	1.35	8.30	0.0	T1
McClean SL	X639	58.9	10.3	1.12	9.20	0.0	T1
Midknight	C35	59.6	11.4	1.29	8.84	0.0	T1-2
Midknight	CC	57.0	11.1	1.15	9.65	0.3	T1
Midknight	KC	61.6	11.8	1.17	10.09	0.0	T1
Midknight	MxT	59.8	11.6	1.1	10.55	0.3	T1
Midknight	SC	61.6	11.8	1.31	9.01	0.2	T1
Midknight	TB	60.2	11.0	1.17	9.40	0.3	T1
Midknight	X639	59.9	11.6	1.27	9.13	0.0	T1

Portsgate	C35	59.8	11.5	1.22	9.43	0.0	T1
Portsgate	CC	59.2	10.9	1.06	10.28	0.0	T1
Portsgate	KC	58.9	11.0	1.04	10.58	0.0	T1
Portsgate	MxT	59.7	10.1	1.12	9.02	0.0	T1
Portsgate	SC	59.8	10.8	1.17	9.23	0.0	T1
Portsgate	TB	59.4	10.7	1.16	9.22	0.0	T1
Portsgate	X639	59.7	10.5	0.99	10.61	0.0	T1

Table 5.5.13.3. Fruit size distribution per rootstock at Golden Frontiers Citrus, Hectorspruit during the 2013 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	0.44	Midknight	C35	48.0	1.61
Delta	C35	56	9.53	Midknight	C35	56.0	23.16
Delta	C35	72	25.06	Midknight	C35	72.0	35.82
Delta	C35	88	28.31	Midknight	C35	88.0	22.67
Delta	C35	105/125	31.04	Midknight	C35	105/125	15.01
Delta	C35	144	5.62	Midknight	C35	144.0	1.73
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	CC	48	0.17	Midknight	CC	48.0	4.48
Delta	CC	56	3.31	Midknight	CC	56.0	24.79
Delta	CC	72	12.96	Midknight	CC	72.0	34.27
Delta	CC	88	21.21	Midknight	CC	88.0	19.90
Delta	CC	105/125	47.64	Midknight	CC	105/125	14.58
Delta	CC	144	14.70	Midknight	CC	144.0	1.98
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	KC	48	0.20	Midknight	KC	48.0	4.44
Delta	KC	56	2.83	Midknight	KC	56.0	17.96
Delta	KC	72	10.80	Midknight	KC	72.0	18.84
Delta	KC	88	21.28	Midknight	KC	88.0	20.89
Delta	KC	105/125	50.86	Midknight	KC	105/125	29.78
Delta	KC	144	14.03	Midknight	KC	144.0	8.09
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	MxT	48	0.85	Midknight	MxT	48.0	6.61
Delta	MxT	56	8.47	Midknight	MxT	56.0	25.21
Delta	MxT	72	27.43	Midknight	MxT	72.0	20.80
Delta	MxT	88	28.59	Midknight	MxT	88.0	17.63
Delta	MxT	105/125	30.23	Midknight	MxT	105/125	21.49
Delta	MxT	144	4.43	Midknight	MxT	144.0	8.26
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	SC	48	0.58	Midknight	SC	48.0	22.56
Delta	SC	56	7.34	Midknight	SC	56.0	35.24
Delta	SC	72	24.74	Midknight	SC	72.0	21.43
Delta	SC	88	27.52	Midknight	SC	88.0	10.90
Delta	SC	105/125	35.12	Midknight	SC	105/125	9.02
Delta	SC	144	4.72	Midknight	SC	144.0	0.85
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Delta	TB	48	0.75	Midknight	TB	48.0	8.72
Delta	TB	56	9.63	Midknight	TB	56.0	27.40
Delta	TB	72	24.20	Midknight	TB	72.0	29.98
Delta	TB	88	27.64	Midknight	TB	88.0	18.92
Delta	TB	105/125	31.24	Midknight	TB	105/125	13.64
Delta	TB	144	6.53	Midknight	TB	144.0	1.35

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	0.10	Midknight	X639	48.0	1.53
Delta	X639	56	3.09	Midknight	X639	56.0	15.48
Delta	X639	72	14.86	Midknight	X639	72.0	18.13
Delta	X639	88	24.89	Midknight	X639	88.0	19.14
Delta	X639	105/125	44.96	Midknight	X639	105/125	32.38
Delta	X639	144	12.11	Midknight	X639	144.0	13.34
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	C35	48	6.04	Portsgate	C35	48.0	5.90
McClean SL	C35	56	37.12	Portsgate	C35	56.0	34.05
McClean SL	C35	72	30.48	Portsgate	C35	72.0	34.66
McClean SL	C35	88	16.10	Portsgate	C35	88.0	17.10
McClean SL	C35	105/125	9.36	Portsgate	C35	105/125	7.13
McClean SL	C35	144	0.91	Portsgate	C35	144.0	1.15
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	CC	48	1.40	Portsgate	CC	48.0	0.18
McClean SL	CC	56	14.23	Portsgate	CC	56.0	2.52
McClean SL	CC	72	30.95	Portsgate	CC	72.0	8.96
McClean SL	CC	88	27.07	Portsgate	CC	88.0	18.71
McClean SL	CC	105/125	24.13	Portsgate	CC	105/125	47.18
McClean SL	CC	144	2.21	Portsgate	CC	144.0	22.45
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	KC	48	1.01	Portsgate	KC	48.0	0.06
McClean SL	KC	56	14.31	Portsgate	KC	56.0	3.01
McClean SL	KC	72	28.79	Portsgate	KC	72.0	10.97
McClean SL	KC	88	27.95	Portsgate	KC	88.0	21.84
McClean SL	KC	105/125	23.57	Portsgate	KC	105/125	51.25
McClean SL	KC	144	4.38	Portsgate	KC	144.0	12.87
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	MxT	48	5.51	Portsgate	MxT	48.0	2.31
McClean SL	MxT	56	27.82	Portsgate	MxT	56.0	18.68
McClean SL	MxT	72	29.75	Portsgate	MxT	72.0	33.55
McClean SL	MxT	88	25.07	Portsgate	MxT	88.0	24.63
McClean SL	MxT	105/125	11.02	Portsgate	MxT	105/125	19.17
McClean SL	MxT	144	0.83	Portsgate	MxT	144.0	1.65
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	SC	48	1.57	Portsgate	SC	48.0	0.03
McClean SL	SC	56	9.89	Portsgate	SC	56.0	2.29
McClean SL	SC	72	22.57	Portsgate	SC	72.0	7.78
McClean SL	SC	88	29.11	Portsgate	SC	88.0	20.32
McClean SL	SC	105/125	32.15	Portsgate	SC	105/125	54.96
McClean SL	SC	144	4.72	Portsgate	SC	144.0	14.62
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	TB	48	2.52	Portsgate	TB	48.0	0.19
McClean SL	TB	56	25.35	Portsgate	TB	56.0	3.31
McClean SL	TB	72	36.77	Portsgate	TB	72.0	13.90
McClean SL	TB	88	22.83	Portsgate	TB	88.0	28.44
McClean SL	TB	105/125	10.94	Portsgate	TB	105/125	46.81
McClean SL	TB	144	1.57	Portsgate	TB	144.0	7.35
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	X639	48	11.70	Portsgate	X639	48.0	0.23
McClean SL	X639	56	30.47	Portsgate	X639	56.0	5.74

McClean SL	X639	72	28.30	Portsgate	X639	72.0	19.13
McClean SL	X639	88	18.02	Portsgate	X639	88.0	30.50
McClean SL	X639	105/125	9.34	Portsgate	X639	105/125	40.24
McClean SL	X639	144	2.17	Portsgate	X639	144.0	4.15

Table 5.5.13.4. Production per tree of Valencia selections on different rootstocks at Golden Frontiers Citrus, Hectorspruit during the 2013 season.

Cultivar	Rootstock	Kg/tree						6 Year Total	6 Year Mean
		2008	2009	2010	2011	2012	2013		
Delta	C35	60.1	45.6	51.7	37.6	50.5	53.8	299.3	49.9
Delta	CC	33.4	25.6	21.5	38.4	58.3	60.2	237.4	39.6
Delta	KC	38.5	26.2	10.4	32.6	49.0	30.6	187.3	31.2
Delta	MxT	39.4	40.0	6.4	13.8	48.4	73.8	221.8	37.0
Delta	SC	73.9	76.8	65.7	41.7	70.1	140.4	468.6	78.1
Delta	TB	34.9	30.5	32.3	31.0	44.4	87.8	260.9	43.5
Delta	X639	35.1	51.8	44.7	51.1	69.1	107.7	359.5	59.9
McClean SL	C35	81.0	64.3	32.5	46.3	46.1	69.0	339.2	56.5
McClean SL	CC	29.2	54.6	22.6	27.5	32.4	83.7	250.0	41.7
McClean SL	KC	24.7	58.3	21.6	38.8	46.7	90.3	280.4	46.7
McClean SL	MxT	19.4	26.6	10.1	4.5	16.1	71.8	148.5	24.8
McClean SL	SC	35.7	98.1	49.7	47.5	74.0	170.8	475.8	79.3
McClean SL	TB	46.9	39.4	49.6	37.2	55.5	57.6	286.2	47.7
McClean SL	X639	29.3	96.2	49.9	80.6	49.2	182.2	487.4	81.2
Midknight	C35	33.9	83.4	54.8	39.0	49.7	76.4	337.2	56.2
Midknight	CC	20.9	32.1	3.6	12.3	41.8	28.6	139.3	23.2
Midknight	KC	11.3	29.7	15.2	16.9	44.0	81.4	198.5	33.1
Midknight	MxT	8.2	27.0	1.2	8.6	30.3	20.3	95.6	15.9
Midknight	SC	13.8	57.7	8.1	30.7	53.0	95.9	259.2	43.2
Midknight	TB	19.6	53.7	14.3	15.5	36.6	56.0	195.7	32.6
Midknight	X639	5.2	17.9	3.3	13.0	36.3	68.7	144.4	24.1
Portsgate	C35	33.6	53.5	22.3	26.2	68.4	60.4	264.4	44.1
Portsgate	CC	26.6	31.3	9.6	4.2	50.9	35.0	157.6	26.3
Portsgate	KC	19.0	31.3	4.9	11.8	58.2	47.4	172.6	28.8
Portsgate	MxT	30.5	19.4	3.3	7.5	25.1	76.0	161.8	27.0
Portsgate	SC	55.2	44.0	19.9	15.3	106.0	89.2	329.6	54.9
Portsgate	TB	48.0	40.8	30.4	31.8	55.8	77.7	284.5	47.4
Portsgate	X639	35.6	73.0	37.1	73.1	91.7	96.9	407.4	67.9

5.5.14 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)**
Project 57D by S.Meeding (CRI)

Opsomming

Die data van die 2013 seisoen verskil van die 2012 seisoen. Ohtsu seleksie was die eerste seleksie om ryp te word gevolg deur Dobashi-Beni. Ueno volg op Dobashi-Beni met goeie opbrengs en vruggrootte. Na die Ueno seleksie volg Aoshima op Carrizo en dan Aoshima op Swingle. Aoshima op Swingle behou die suurvlakke langer as op Carrizo. Die seisoen word dan afgesluit met Owari. Al vyf hierdie seleksies het goeie opbrengs en vruggrootte getoon. Die laatste Satsuma seleksie bly die Imamura wat geen vrugte gedra het gedurende die 2013 seisoen nie, al was bome geringeleer. Die Imamura is groeikragtige bome wat opmerkbaar groter is as die ander Satsuma seleksies. Pluk periodes vir Satsumas sal strek tussen 2-3 weke aangesien vrugte se sure vinnig daal en die skil powwerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

Summary

The data of the 2013 season changed from that of the 2012 season. The Ohtsu selection was the first to mature followed by Dobashi-Beni. Ueno was next to mature with good internal quality and fruit size. After Ueno the Aoshima selection on Carrizo matured with Aoshima on Swingle next. Aoshima on Swingle tends to have higher acids and a delayed colour development. The season ended with Owari. All five selections showed good yield and fruit size. The last Satsuma selection to mature is still the Imamura. The Imamura selection bore no fruit for the 2013 season even after being girdled. Imamura is a very vigorous tree and is bigger than the rest of the Satsumas. 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 5.5.14.1. List of Satsuma selections evaluated at Lustigaan (Paarl) during 2013.

Selection	Rootstock	Planted
Aoshima	Carrizo	2006
Aoshima	Swingle	2006
Dobashi Beni	Carrizo	2006
Imamura	Carrizo	2006
Ohtsu	Carrizo	2006
Owari	Carrizo	2006
Ueno	Carrizo	2006

Results and discussion

These results are from a six-year-old trial block in the area.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which 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.

The results from the 2013 season (Table 5.5.14.2) show that Ohtsu was the earliest to mature. This was followed by Dobashi-Beni, Ueno, Aoshima on Carrizo, Aoshima on Swingle and the season was ended with Owari. Imamura being the latest selection bore no fruit on the trees. Aoshima on Swingle has a delayed colour development and the acid is higher than Aoshima on Carrizo. Fruit size in all the Satsuma selections increased by 1-2 counts.

Table 5.5.14.2. Internal fruit quality data for Satsuma selections in the Paarl region (Lustigaan) of the Western Cape during the 2013 season.

Date harvested:	Selection	Rootstock	Colour	Count	Juice (%)	Brix °	Acid (%)	Avg. Seeds	Ratio
12/04/13	Aoshima	CC	T6-7	1	54.9	8.5	0.83	0.5	10.20
03/05/13	Aoshima	CC	T5	1X	43.0	10.8	0.80	0.1	13.50
22/05/13	Aoshima	CC	T2-3	1XX	48.0	11.1	0.85	0.8	13.04
12/04/13	Aoshima	SC	T7	1XX	55.8	8.9	1.09	0	8.20
03/05/13	Aoshima	SC	T6	1XX	49.0	10.9	0.93	0.3	11.70
22/05/13	Aoshima	SC	T4-5	1XXX	52.0	10.8	0.91	0.5	11.88
12/04/13	Dobashi-Beni	CC	T6	1XX	59.8	9.3	0.85	0	10.90
03/05/13	Dobashi-Beni	CC	T5	1X	45.0	11.4	0.36	0	31.24
22/05/13	Dobashi-Beni	CC	T1-2	1XX	46.0	12.1	0.94	0	12.81
12/04/13	Ohtsu	CC	T6	1XX	58.5	9.4	0.87	0.1	10.80
03/05/13	Ohtsu	CC	T4	1XX	41.0	11.9	0.88	0.6	13.57
22/05/13	Ohtsu	CC	T1-2	1XX	46.0	12.0	0.87	0.4	13.83
12/04/13	Owari	CC	T6	1	59.6	8.3	1.04	0	8.00
03/05/13	Owari	CC	T5	1X	43.0	10.9	0.93	0.1	11.74
22/05/13	Owari	CC	T2	1X	47.0	11.4	0.95	0.1	12.03
12/04/13	Ueno	CC	T6	1XX	66.5	9.1	0.86	0	10.60
03/05/13	Ueno	CC	T5	1XX	44.0	11.2	0.81	0.2	13.88
22/05/13	Ueno	CC	T2-3	1XXX	45.0	11.4	0.76	0.2	14.96

Conclusion

Ohtsu was the earliest selection with good yields and highest juice percentage. This was followed by Dobashi-Beni, Ueno and Aoshima on Carrizo producing good internal quality and acceptable external colour. Next to mature was Aoshima on Swingle followed by Owari. Imamura remains the latest maturing Satsuma for this trial site and had very poor yields in previous seasons and not one fruit in the 2013 season, although colour development and internal quality were good. At this trial Imamura was the selection with the smoothest skin.

5.5.15 **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 begin met Nules wat goeie vruggrootte, opbrengs en interne kwaliteit toon. Nules word gevolg deur Clemenpons en dan Bonanules wat ‘n eksperimentele seleksie is. Die seisoen word afgesluit deur Marisol. Marisol moes volgens die rypwordings tabelle voor Nules wees. Bonanules het ‘n kenmerkende platter voorkoms as Marisol en Nules. Die skil is ook gladder. Al vier seleksies het ‘n goeie vruggrootte van 1X. Bonanules en Clemenpons se sap persentasie het albei met net meer as 10% geval van 2012 se seisoen af weens granulasie in beide seleksies. Al vier seleksies het ‘n vertraging in eksterne kleur gehad. Dit was in 2012 se seisoen ook sigbaar.

Summary

The season begins with Nules with good fruit size, yield and internal quality. Nules is followed by Clemenpons and then Bonanules which is an experimental selection. The season is ended by Marisol. According to the maturity tables Marisol is supposed to mature before the Nules selection. The Bonanules selection has a flatter shape than Marisol and Nules and the skin is smoother as well. All four selections

have a good fruit size with a count of 1X. Bonanules and Clemenpons juice percentage dropped by over 10% from the 2012 season due to granulation in both selections. All four selections have delayed colour development; this was also observed in the 2012 season.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Wellington region of the Western Cape; the planting age is unknown. The following varieties were evaluated: Bonanules Clemenpons, Marisol and Nules.

Table 5.5.15.1. List of Clementine selections evaluated at Bonathaba (Wellington) during 2013.

Selection	Rootstock	Planted
Bonanules	Troyer	Unknown
Clemenpons	Carrizo	Unknown
Marisol	Troyer	Unknown
Nules	Troyer	Unknown

Results and discussion

These results are from commercial orchards in the area. This project is ongoing and additional varieties are planned for inclusion in the future.

According to the results shown in Table 5.5.15.2, Nules is the earliest maturing selection followed by Clemenpons for this season. Bonanules follows next in line but it is safe to say the Bonanules and Clemenpons matured in the same window this season. The latest maturing selection for this site was Marisol. The fruit size increased for Bonanules, Clemenpons and Nules this season and peaked at count 1X. The highest Brix: acid ratio was Nules with (10.2). The lowest was Marisol with 8.3. The external colour development was delayed this season compared to 2012 for the Bonanules and Clemenpons but this may be because both selections are planted beside the windbreak. Bonanules has the flattest fruit shape. There were no seeds in any of the evaluated fruit this season.

Conclusion

Nules was the earliest to mature followed by Clemenpons, Bonanules and then Marisol. Fruit size increased for all 4 selections. There was a delay in external colour as found in the 2012 season. All four selections were seedless when evaluated. The skin of the Bonanules selection was a lot smoother than the other three selections.

Table 5.5.15.2. Internal fruit quality data for Clementine selections in the Wellington region (Bonathaba) of the Western Cape during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
12/04/13	Bonanules	TC	1x	45.9	9.2	0.92	10.0	0	T5
12/04/13	Clemenpons	CC	1x	43.3	9.5	0.95	10.0	0	T6
12/04/13	Marisol	TC	1x	53.5	9.1	1.09	8.3	0	T6
12/04/13	Nules	TC	1x	53.9	10.3	1.01	10.2	0	T5

5.5.16 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)**
Project 997A by S. Meeding (CRI)

Opsomming

Volgens 2013 se data het Tahoe Gold eerste ryp geword gevolg deur Nadorcott. Tahoe Gold het ŉ goeie vruggrootte met ŉ telling van 1X en 1XX. Na Nadorcott word Gold Nugget en Tango ryp. Gold Nugget het die hoër Brix. Die seisoen word afgesluit in die volgende volgorde Shasta Gold, Yosemite Gold en dan Winola. Nadorcott met 1.2 en Tahoe Gold met 1.3 per vrug het gemiddeld die meeste saad bevat. Tango het geen saad bevat. Nadorcott, Tango, Winola en Gold Nugget het die kleinste vrugte gedra met telling 1-3. Winola het saad in wat verskil met 2012 se data toe daar geen saad gevind is nie. Shasta Gold en Yosemite Gold het die grootste vrugte met tellings van 1XXX. In die jong bome neig Shasta Gold na groot growwe vrugte met ribbing. Tahoe Gold en Shasta Gold kry ook meer sonbrand as die ander seleksies. Yosemite Gold sukkel met drag en dra nie so baie vrugte nie.

Summary

The results of 2013 show Tahoe Gold is first to mature and is followed by Nadorcott. Tahoe Gold has a good fruit size with a count of 1X and 1XX. After Nadorcott, Gold Nugget and Tango mature. Gold Nugget had the higher Brix. The season ends in this order: Shasta Gold, Yosemite Gold and Winola. Nadorcott with 1.2 and Tahoe Gold with 1.3 seeds per fruit had the highest seed count of all seven selections. Tango had no seed and that was the case in the 2012 season as well. Tango, Winola and Gold Nugget had the smallest fruit with counts between 1 and 3. Shasta Gold and Yosemite Gold had the largest fruit with counts of 1XXX. In the young trees Shasta Gold tends to have large fruit with a rough skin and also ribbing. Tahoe Gold and Shasta Gold do get more sunburn than the other selections. Yosemite Gold struggles with fruit set.

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 2013 season. The following varieties were evaluated: Gold Nugget, Nadorcott, Shasta Gold, Tahoe Gold, Tango, Winnola and Yosemite Gold.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new 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 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.

Table 5.5.16.1. List of Mandarin Hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2013 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Winnola	CC	2010
Yosemite Gold	CC	2010

Table 5.5.16.2. List of Mandarin Hybrid selections in the Fort Beaufort (Riverside) region of the East Cape Midlands during the 2013 season.

Selection	Rootstock	Topwork
Gold Nugget	TC	2010
Shasta Gold	TC	2010
Tahoe Gold	TC	2010
Tango	TC	2010
Yosemite Gold	TC	2010

Results and discussion

These results are from semi-commercial orchards in the area. This project has been boosted by the addition of several new Mandarin Hybrid selections.

According to the results shown in Tables 5.5.16.3 and 5.5.16.4, Tahoe Gold matured first, followed by Nadorcott, Gold Nugget and Tango. These were followed by Shasta Gold and Yosemite Gold. The latest selection to mature at the two trial sites was Winnola, ending off the Mandarin Hybrid season. These are still young trees and some of them had their first crop this year. Shasta Gold, Tahoe Gold and Gold Nugget had the largest fruit with counts of 1X-1XXX. Winnola ended the season with Brix of 12.2 but still had high acid of 1.88. Colour was very good for all the selections with no degreening required as all selections were fully coloured when harvested (T1). Tango had no seeds and Gold Nugget 0.1 seeds per fruit: this was the lowest seed count. The highest were Nadorcott with 1.2 and Tahoe Gold with 1.3 seeds per fruit.

Conclusion

Shasta Gold tends to have a light crop when still a young tree so fruit is very large with a rough skin and ribbing. Tahoe Gold has the smallest tree of all the TDE selections so if there is a heavy crop on the tree the branches tend to break if not supported. The colour development on all the selections was good.

Table 5.5.16.3. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
22/04/13	Nadorcott	CC	3	54.2	6.6	2.24	2.9	1.2	T8
14/05/13	Nadorcott	CC	2	58.5	6.9	1.72	4.0	0.7	T7
18/06/13	Nadorcott	CC	1	56.9	8.1	1.27	6.4	0.3	T5-6
01/07/13	Nadorcott	CC	2	55.4	9.0	1.23	7.3	1.2	T2-3
19/07/13	Nadorcott	CC	1	59.9	9.7	1.06	9.2	0.1	T1-2
19/08/13	Nadorcott	CC	1x	51.5	11.2	1.08	10.4	0.8	T1
22/04/13	Gold Nugget	CC	2	51.6	7.5	2.83	2.7	0	T8
14/05/13	Gold Nugget	CC	3	52.3	7.5	2.33	3.2	0	T8
18/06/13	Gold Nugget	CC	1	55.0	9.4	1.51	6.2	0.1	T5-6
01/07/13	Gold Nugget	CC	1x	51.8	10.3	1.44	7.2	0	T3-4
19/07/13	Gold Nugget	CC	1	55.5	11.3	1.37	8.2	0.1	T1-2
19/08/13	Gold Nugget	CC	1xx	56.0	12.7	1.16	10.9	0	T1
29/08/13	Gold Nugget	CC	1xxx	57.2	11.9	1.13	10.5	0.3	T1
22/04/13	Shasta Gold	CC	1xx	39.4	7.1	3.36	2.1	0	T8
01/07/13	Shasta Gold	CC	1xx	56.9	10	1.48	6.8	0.8	T2-3
19/07/13	Shasta Gold	CC	1xx	59.3	11.2	1.39	8.1	0.9	T1
19/08/13	Shasta Gold	CC	1xxx	56.4	12.4	1.18	10.5	0.3	T1
29/08/13	Shasta Gold	CC	1xxx	56.0	14.4	1.12	12.9	0.7	T1
22/04/13	Tahoe Gold	CC	2	56.1	7.4	3.16	2.3	0.3	T8
14/05/13	Tahoe Gold	CC	1	60.6	7.8	1.83	4.3	1.3	T8

18/06/13	Tahoe Gold	CC	1	62.5	8.8	1.76	4.8	0.7	T6-7
01/07/13	Tahoe Gold	CC	1xx	55.0	9.7	1.01	9.6	1.3	T5-6
19/07/13	Tahoe Gold	CC	1x	54.3	10.2	0.81	12.6	1.1	T1
19/08/13	Tahoe Gold	CC	1	60.8	10.6	1.22	8.7	0.6	T1
29/08/13	Tahoe Gold	CC	1xxx	56.3	13.9	1.08	12.9	1.1	T1
22/04/13	Tango	CC	3	58.7	6.8	2.28	3.0	0	T8
14/05/13	Tango	CC	2	55.9	6.6	1.58	4.2	0	T7
18/06/13	Tango	CC	1	56.8	7.9	1.34	5.9	0	T5-6
01/07/13	Tango	CC	2	51.4	9.3	1.24	7.5	0	T1-2
19/07/13	Tango	CC	1	61.4	9.6	1.07	9	0	T1-2
19/08/13	Tango	CC	1x	56.5	11.5	1.14	10.1	0	T1
29/08/13	Tango	CC	2	54.0	13.3	1.12	11.9	0	T1
18/06/13	Winola	CC	3	54.2	9.9	2.17	4.6	0.5	T5
19/07/13	Winola	CC	1	63.1	11.6	1.94	6	0.3	T1
19/08/13	Winola	CC	3	60.3	12.2	1.88	6.5	0	T1
22/04/13	Yosemite Gold	CC	3	46.0	6.7	2.98	2.2	0.3	T8
14/05/13	Yosemite Gold	CC	1x	52.8	7.5	2.09	3.6	0.1	T8
18/06/13	Yosemite Gold	CC	1xx	54.7	7.1	1.86	3.8	0.4	T7-8
01/07/13	Yosemite Gold	CC	1xxx	56.8	8.6	1.34	6.4	0.5	T5-6
19/07/13	Yosemite Gold	CC	1xxx	57.3	8.5	1.24	6.9	0.3	T4-5
19/08/13	Yosemite Gold	CC	1xxx	56.2	10.4	1.21	8.6	0.4	T1
29/08/13	Yosemite Gold	CC	1xxx	57.2	11.9	1.13	10.5	0.3	T1

Table 5.5.16.4. Internal fruit quality data for Mandarin hybrid selections from the Fort Beaufort (Riverside) region of the East Cape Midlands during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg Seed	Colour
01/07/13	Gold Nugget	**CC**	1xxx	48.8	10.2	1.18	8.6	0	T3
19/07/13	Gold Nugget	**CC**	1xx	50.0	10.6	1.13	9.4	0	T1-2
01/07/13	Shasta Gold	**CC**	1XXX	55.5	10.1	1.6	6.3	0	T1-2
19/07/13	Shasta Gold	**CC**	1xx	56.8	10.0	1.46	7.3	0.3	T1-2
01/07/13	Tango	**CC**	1	51.4	9.5	1.01	9.4	0	T1
19/07/13	Tango	**CC**	1x	49.5	10.2	0.90	11.3	0	T1
29/08/13	Tango	**CC**	2	60.9	14	1.21	11.6	0.3	T1
01/07/13	Yosemite Gold	**CC**	1xxx	49.6	8.7	1.26	6.9	0.6	T4-5
19/07/13	Yosemite Gold	**CC**	1xxx	49.8	9.8	1.13	8.7	0.7	T1-3

5.5.17 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)**
Project 997B by S. Meeding (CRI)

Opsomming

Die uitslae van 2013 toon Clemcott het die vroegste ryp geword. Vruggrootte was goed met tellings van 1XX en 1XXX. Interne kwaliteit was baie goed met sure wat nie vinnig gedaal het nie. Vrugte het ook goeie kleur ontwikkeling getoon. Na Clemcott het Gold Nugget en Tango ryp geword. Tahoe Gold het na Tango ryp geword en die seisoen is afgesluit met Shasta Gold en Yosemite Gold. Hierdie was die UC 5 se eerste oes. Gold Nugget, Tahoe Gold, Shasta Gold en Yosemite Gold het die beste vruggrootte gehad met tellings van 1X-1XXX. Tango het kleinste vrugte met tellings van 3 en ook 1. Tango en Gold Nugget was saadloos. Clemcott het die meeste sade bevat met 8-9 sade per vrug. Daar word aanbeveel dat die oes periode nie langer as 3 tot 4 weke neem nie, om goeie interne kwaliteit te behou en na-oes skil probleme te vermy.

Summary

The results of the 2013 season show Clemcott was the first to mature. Fruit size was good with counts of 1XX and 1XXX. Internal quality was also good with acid still present when the brix reached 12.2. The Clemcott selections had good colour development with a colour of T1 when harvested. After Clemcott, Gold Nugget and Tango matured followed by Tahoe Gold. The season was ended with Shasta Gold and then Yosemite Gold. This was the first crop for the UC 5 selections. Gold Nugget, Tahoe Gold, Shasta Gold and Yosemite Gold had the best fruit size with counts of 1X-1XXX. Tango has the smallest fruit with counts of 3 and 1. Both Tango and Gold Nugget were seedless. Clemcott had the most seed with 8-9 seeds per fruit. It is suggested that the picking period should not exceed more than 3-4 weeks to ensure good internal quality and also to prevent post harvest rind problems.

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 andwere bearing fruit in the 2013 season. The following varieties were evaluated: Valley Gold, African Sunset, Clemcott, Nova, HE Mandarin and Gold Nugget.

Table 5.5.17.1. List of Mandarin hybrid selections evaluated in the Sundays River Valley region during the 2013 season.

Selection	Rootstock	Topwork
Clemcott	Carrizo	2004
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Yosemite Gold	Carrizo	2011
Tango	Carrizo	2011

Results and discussion

The Clemcott that was evaluated was from a commercial orchard. The UC 5 was bearing its first crop.

Clemcott had the largest fruit with counts of 1XX and 1XXX. Tango had the smallest fruit with counts of 1 and 3. The acid level was the highest in Shasta Gold (1.42%) and Yosemite Gold (1.21%) when fully coloured up. All 6 selections showed good colour development with a colour of T1 at harvest. Gold Nugget had the highest brix with 14.1 while the acid was still as high as 1.19%.

Conclusion

Clemcott was the first to mature followed by Gold Nugget and then Tango. Tahoe Gold followed Tango and the season ended with Shasta Gold and Yosemite Gold. Tango and Gold Nugget were seedless. Clemcott had the most seed with 8-9 seeds per fruit. All the Mandarin selections had good colour development with a colour of T1 at harvest.

Table 5.5.17.2. Internal fruit quality data for Mandarin hybrid selections from various regions of the Sundays River Valley during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
14/04/13	Clemcott	CC	1xx	51.5	8.8	1.78	4.9	9.4	T8
17/05/13	Clemcott	CC	1xx	60.2	11.4	1.38	8.3	11	T6
02/07/13	Clemcott	CC	1xxx	59.4	12.2	1.13	10.8	8.2	T1
02/07/13	Gold Nugget	CC	1x	55.6	9.8	1.2	8.2	0	T6
25/07/13	Gold Nugget	CC	1x	54.3	11.4	1.13	10.1	0	T1-3
07/08/13	Gold Nugget	CC	1xxx	50.2	12.0	1.07	11.2	0	T1
04/09/13	Gold Nugget	CC	1	55.0	14.1	1.19	11.8	0	T1
02/07/13	Shasta Gold	CC	1xxx	58.3	10.2	1.69	6	0.3	T3
25/07/13	Shasta Gold	CC	1xxx	58.8	11.3	1.58	7.2	0	T2-3
07/08/13	Shasta Gold	CC	1xxx	56.7	11.8	1.42	8.3	0	T1
02/07/13	Tahoe Gold	CC	1xxx	61.5	10.1	1.17	8.6	0.2	T6
25/07/13	Tahoe Gold	CC	1xx	58.3	11.3	1.34	8.4	0.5	T3-4
07/08/13	Tahoe Gold	CC	1xx	58.4	11.3	0.96	11.8	0	T1
04/09/13	Tahoe Gold	CC	1xxx	55.8	12.7	0.89	14.3	0.2	T1
02/07/13	Tango	CC	3	57.9	10.3	1.17	8.8	0	T2-3
25/07/13	Tango	CC	1x	53.7	9.7	1.05	9.2	0	T1
07/08/13	Tango	CC	1	56.9	10.9	1.06	10.3	0	T1
04/09/13	Tango	CC	3	61.0	14.3	1.08	13.2	0	T1
25/07/13	Yosemite Gold	CC	1xxx	55.2	10	1.32	7.6	0.2	T4-5
07/08/13	Yosemite Gold	CC	1xxx	54.3	9.5	1.21	7.9	0.5	T1

5.5.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)

Project 997C by S. Meeding (CRI)

Opsomming

Die 2013 seisoen was die eerste drag vir die UC 5. Nadorcott was eerste om ryp te word. Nadorcott het ook die kleinste vruggrootte met tellings van 2 en 1. Tango was na Nadorcott ryp en Tango was gevolg deur Tahoe Gold. Na Tahoe Gold het Gold Nugget ryp geword. Die seisoen is geeïndig deur Yosemite Gold en Shasta Gold na die Yosemite Gold. Tango het op sy piek tyd einde Julie nog ŉ brix van 9.6 gehad waar Nadorcott ‘n brix van 10.7 gehad. Tahoe Gold en Tango het saam ryp geword. Gold Nugget het voor Shasta Gold en Yosemite Gold ryp geword, waar dit volgens die rypwordings tabelle na albei eers moet ryp word.

Summary

The 2013 season was the first harvest for the UC 5 selections. Nadorcott matured first and also had the smallest fruit with counts of 2 and 1. Nadorcott was followed by Tango, then Tahoe Gold and after Tahoe Gold, Gold Nugget matured. The season was ended with Yosemite Gold followed by Shasta Gold. Tango had a brix of only 9.6 on its peak at the end of July whereas Nadorcott had a brix of 10.7. Tahoe Gold and Tango matured in the same time frame. Gold Nugget matured before Shasta Gold and Yosemite Gold whereas the maturity tables suggest Gold Nugget should mature after both of them.

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 (Hankey). A range of new Mandarin Hybrids had been added to this area and were bearing fruit in the 2013 season. The following varieties were evaluated: Shasta, Tahoe and Yosemite Gold, as well as Gold Nugget and Tango.

Table 5.5.18.1. List of experimental Mandarin hybrid selections evaluated in the Patensie region of the Gamtoos River Valley during the 2013 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

The 2013 season was the first year these UC 5 trees bore fruit. Visual impressions were good with all the trees growing strongly and filling the space well. Tahoe Gold has the smallest tree of the UC 5 selections and some branches did break with a heavy crop. Yosemite Gold had the largest tree but had a problem bearing fruit. Fruit size was good with counts of 1XXX. Shasta Gold also had a light crop with large fruit with counts of 1XX and 1XXX. The fruit had a very rough skin with ribbing. Tango had the smoothest skin and was seedless. Gold Nugget had a rough skin and tends to get puffy if left on the tree for too long. All the UC 5 selections were seedless with Nadorcott having the most seeds with 0.3 seeds per fruit.

Conclusion

Colour development for all 6 selections was good and they were fully coloured when it was time to harvest. Nadorcott was the only selection that had seed. This trial site was the only one in the Western Cape and Eastern Cape where Shasta Gold, Tahoe Gold, Gold Nugget and Yosemite Gold were completely seedless. It is suggested that the picking period should not exceed more than 3-4 weeks to ensure good internal quality and also to prevent postharvest rind problems.

Table 5.5.18.2. Internal fruit quality data for experimental Mandarin hybrid selections from the Hankey region of the Gamtoos River Valley region during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
06/05/13	Nadorcott	CC	1	55.1	7.7	1.17	6.6	0	T7
15/05/13	Nadorcott	CC	1	56.4	8.7	1.44	6.0	0	T7
28/05/13	Nadorcott	CC	1	54.3	7.6	1.16	6.6	0	T6
24/06/13	Nadorcott	CC	1	56.7	9.7	1.14	8.5	0	T1-3
03/07/13	Nadorcott	CC	2	54.9	9.9	1.14	8.7	0	T1-2
24/07/13	Nadorcott	CC	1x	50.2	10.7	1.03	10.1	0.3	T1
15/05/13	Gold Nugget	CC	1	56.8	8.3	1.38	6.0	0	T7
28/05/13	Gold Nugget	CC	1x	54.4	8.4	1.31	6.4	0	T7
24/06/13	Gold Nugget	CC	1xxx	53.7	9.7	1.11	8.7	0	T3-4
03/07/13	Gold Nugget	CC	1xx	53.5	10.8	1.18	9.2	0	T1-2
24/07/13	Gold Nugget	CC	1xx	50.5	11.6	1.04	11.2	0	T1-2
05/08/13	Gold Nugget	CC	1xxx	48.5	11.9	0.98	12.1	0	T1
05/08/13	Gold Nugget	CC	1xx	55.0	11.9	0.89	13.4	0	T1
14/08/13	Gold Nugget	CC	1xx	54.3	13	0.98	13.3	0	T1
06/05/13	Shasta Gold	CC	1x	58.0	7.4	1.96	3.8	0	T7-8
15/05/13	Shasta Gold	CC	1x	58.9	7.7	1.81	4.3	0	T6

28/05/13	Shasta Gold	CC	1x	58.3	8.4	1.6	5.3	0	T6
24/06/13	Shasta Gold	CC	1xxx	58.5	9.1	1.33	6.8	0	T1-2
03/07/13	Shasta Gold	CC	1xx	58.5	9.9	1.34	7.4	0	T1-2
24/07/13	Shasta Gold	CC	1xx	50.1	10.9	1.25	8.7	0	T1
05/08/13	Shasta Gold	CC	1xx	56.1	11.3	1.19	9.5	0	T1
14/08/13	Shasta Gold	CC	1xxx	55.1	11.4	1.1	10.4	0	T1
26/08/13	Shasta Gold	CC	1xxx	53.6	12.9	1.05	12.3	0	T1
06/05/13	Tahoe Gold	CC	1xx	62.8	6.9	1.25	5.5	0	T7
15/05/13	Tahoe Gold	CC	1x	63.1	7.7	1.01	7.6	0	T6
28/05/13	Tahoe Gold	CC	1x	61.4	7.8	1.05	7.4	0	T5
24/06/13	Tahoe Gold	CC	1xx	60.4	8.6	0.9	9.6	0	T5
03/07/13	Tahoe Gold	CC	1x	59.6	8.5	0.81	10.5	0	T1-2
24/07/13	Tahoe Gold	CC	1xx	54.4	8.2	0.68	12.1	0	T1-2
06/05/13	Tango	CC	1	57.8	7.5	1.21	6.2	0	T6
15/05/13	Tango	CC	1	57.4	7.9	1.19	6.6	0	T6
28/05/13	Tango	CC	1	54.6	8.1	1.05	7.7	0	T6
24/06/13	Tango	CC	1	55.5	8.8	1.04	8.5	0	T1-3
03/07/13	Tango	CC	2	55.9	9.5	1.02	9.3	0	T1
24/07/13	Tango	CC	1x	48.8	9.6	0.96	10	0	T1
05/08/13	Tango	CC	1xx	45.5	9.9	0.85	11.6	0	T1
14/08/13	Tango	CC	1x	48.2	10	0.92	10.9	0	T1
26/08/13	Tango	CC	1	54.5	11.7	0.87	13.4	0	T1
06/05/13	Yosemite Gold	CC	1xx	52.5	6.6	1.39	4.7	0	T8
15/05/13	Yosemite Gold	CC	1xx	56.4	6.9	1.44	4.8	0	T7
24/06/13	Yosemite Gold	CC	1xxx	55.2	7.8	1.05	7.4	0	T3-4
03/07/13	Yosemite Gold	CC	1xxx	55.1	8.4	1.03	8.2	0	T1-2
24/07/13	Yosemite Gold	CC	1xxx	53.6	9	0.94	9.6	0	T1
05/08/13	Yosemite Gold	CC	1xxx	54.9	10	0.91	11	0	T1
14/08/13	Yosemite Gold	CC	1xxx	52.8	10	0.82	12.2	0	T1
26/08/13	Yosemite Gold	CC	1xxx	54.4	11.5	0.87	13.2	0	T1

5.5.19 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Western Cape)**
Project 997D by S. Meeding (CRI)

Opsomming

Vir die Porterville en Piketberg proef was dit die eerste jaar van drag. Piketberg se bome het ŉ swaarder drag op gehad moontlik weens die growweskil onderstam wat gebruik is. Al 5 seleksies se bome is ook groter weens die groeikragtigheid van die growweskil onderstam. Weens die swaar drag was daar nie tekens van kleiner vrugte nie behalwe vir die Tango seleksie.

Summary

For the Porterville and Piketberg site it was the first season that the trees bore fruit. The trees at the Piketberg site had a heavier crop than the trees at Porterville because of the Rough lemon rootstock that had been used. All 5 selections had a heavier crop than the Porterville trees due to the vigorous growth of the Rough lemon rootstock. None of the selections except for Tango showed signs of smaller fruit due to the heavy crop.

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 a Mandarin Hybrid selection from the Clanwilliam, Citrusdal, Piketberg, Porterville and Paarl region of the Western Cape.

Table 5.5.19.1. List of experimental Mandarin hybrid selections evaluated in the Paarl region of the Western Cape during the 2013 season.

Selection	Rootstock	Planted
Tahoe Gold	CC	2008
Shasta Gold	CC	2008
Yosemite Gold	CC	2008
Gold Nugget	CC	2008

Table 5.5.19.2. List of experimental Mandarin hybrid selections evaluated in the Piketberg region of the Western Cape during the 2013 season.

Selection	Rootstock	Topwork
Tahoe Gold	RL	2010
Shasta Gold	RL	2010
Yosemite Gold	RL	2010
Gold Nugget	RL	2010
Tango	RL	2010

Table5.5.19.3. List of experimental Mandarin hybrid selections evaluated in the Porterville region of the Western Cape during the 2013 season.

Selection	Rootstock	Topwork
Tahoe Gold	CC	2010
Shasta Gold	CC	2010
Yosemite Gold	CC	2010
Gold Nugget	CC	2010
Tango	CC	2010

Results and discussion

Tahoe Gold was first to mature followed by Gold Nugget. After Gold Nugget Tango reached its peak. The season was ended by Yosemite Gold and then Shasta Gold. Gold Nugget tends to mature at the end of July where in the maturing table it has to mature at the end of August. The colour development was good with a colour of T1 at harvest. Tango was seedless except for the 0.6 seeds found per fruit at the Porterville trial site. Gold Nugget also had a low seed count of 0.1 seeds per fruit. Tahoe Gold, Shasta Gold and Yosemite Gold had the most seeds with 0.5 – 0.9 seeds per fruit. If fruit is left on the trees for too long the birds damage and eat them. On the rough lemon rootstock Tango had a heavier crop with smaller fruit. Tahoe gold and Shasta Gold get the most sunburn of all the selections and more on the bottom of the fruit.

Conclusion

Colour development is good in all 5 selections with a colour of T1 at harvest. Gold Nugget, if kept too long on the tree becomes puffy. The Tahoe Gold trees have to be supported to prevent any branches breaking due to their heavy crop. It is suggested that the picking period should not exceed more than 3-4 weeks to ensure good internal quality and also to prevent post harvest rind problems.

Table 5.5.19.4. Internal fruit quality data for experimental Mandarin hybrid selections from the Paarl region of the Western Cape during the 2013 season.

Date harvested	Cultivar	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
13/06/2013	Gold Nugget	CC	1X	44	10.4	1.39	7.47	0	4 - 5
26/06/2013	Gold Nugget	CC	1XX	42	11.6	1.24	9.39	0	2 - 3
17/07/2013	Gold Nugget	CC	1X	44	12.2	1.15	10.59	0	2
29/07/2013	Gold Nugget	CC	1X	46	12.3	0.98	12.60	0.1	2
20/08/2013	Gold Nugget	CC	1X	43	13.6	0.82	16.66	0	1
13/06/2013	Shasta Gold	CC	1XX	42	8.8	2.14	4.12	0.3	4
26/06/2013	Shasta Gold	CC	1XXX	43	10.1	1.92	5.26	0	2 - 3
17/07/2013	Shasta Gold	CC	1XX	41	11.2	1.79	6.27	0.3	1
29/07/2013	Shasta Gold	CC	1XX	41	10.4	1.60	6.50	0.1	1 - 2
13/06/2013	Tahoe Gold	CC	1XX	39	9.2	1.18	7.79	0.9	4 - 5
26/06/2013	Tahoe Gold	CC	1XXX	38	9.5	1.00	9.54	0.3	3 - 4
17/07/2013	Tahoe Gold	CC	1X	38	9.9	0.91	10.93	0.3	1
13/06/2013	Yosemite Gold	CC	1XX	44	9.4	1.95	4.82	1.1	4
26/06/2013	Yosemite Gold	CC	1XX	43	10	1.73	5.79	0.1	2 - 3
17/07/2013	Yosemite Gold	CC	1X	45	11.2	1.60	7.00	0.3	1
29/07/2013	Yosemite Gold	CC	1XX	46	11.5	1.44	7.98	0.5	1
20/08/2013	Yosemite Gold	CC	1XX	44	12.7	1.22	10.44	0.5	1

Table 5.5.19.5. Internal fruit quality data for experimental Mandarin hybrid selections from the Piketberg region of the Western Cape during the 2013 season.

Date harvested	Cultivar	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
13/06/2013	Gold Nugget	RL	1XX	49	9.4	1.26	7.47	0	5
26/06/2013	Gold Nugget	RL	1XX	49	9.9	1.02	9.73	0	5 – 6
16/07/2013	Gold Nugget	RL	1XX	47	10.9	0.98	11.13	0	2
13/06/2013	Shasta Gold	RL	1XXX	44	8.3	1.54	5.38	0.3	4
26/06/2013	Shasta Gold	RL	1XXX	43	9.3	1.38	6.73	0.1	2 – 3
16/07/2013	Shasta Gold	RL	1XXX	45	9.4	1.12	8.41	0.1	1
13/06/2013	Tahoe Gold	RL	1XXX	40	8.5	0.88	9.62	0.3	4 – 5
26/06/2013	Tahoe Gold	RL	1XX	40	8.5	0.95	8.94	0.3	3 – 4
16/07/2013	Tahoe Gold	RL	1XXX	45	9.5	0.79	11.97	0.6	1
13/06/2013	Tango	RL	1X	44	9.0	1.06	8.52	0	4
26/06/2013	Tango	RL	1	44	9.6	0.97	9.90	0	2
16/07/2013	Tango	RL	1	43	10.2	0.99	10.35	0	1
13/06/2013	Yosemite Gold	RL	1XX	46	8.7	1.29	6.74	0.5	4
26/06/2013	Yosemite Gold	RL	1XXX	45	8.9	1.29	6.88	0.3	3 – 4
16/07/2013	Yosemite Gold	RL	1XX	46	10.1	1.13	8.94	0	1

Table 5.5.19.6. Internal fruit quality data for experimental Mandarin hybrid selections from the Porterville region of the Western Cape during the 2013 season.

Date harvest	Cultivar	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Colour
26/06/2013	Gold Nugget	CC	1XX	45	12.5	1.42	8.80	0	3 - 5
16/07/2013	Gold Nugget	CC	1X	42	14.3	1.31	10.95	0	2
29/07/2013	Gold Nugget	CC	1XX	46	13.7	0.96	14.27	0	2
26/06/2013	Shasta Gold	CC	1XXX	42	11.0	1.83	6.02	0.6	3
16/07/2013	Shasta Gold	CC	1XX	42	12.0	1.60	7.51	0.3	1
29/07/2013	Shasta Gold	CC	1XXX	42	12.5	1.45	8.64	0.3	1
26/06/2013	Tahoe Gold	CC	1X	38	13.2	1.74	7.57	0.3	2 - 3
16/07/2013	Tahoe Gold	CC	1	39	14.3	1.58	9.04	0.5	1
26/06/2013	Tango	CC	1	42	11.9	1.54	7.71	0.6	3 - 5
16/07/2013	Tango	CC	2	43	12.3	1.45	8.46	0	1
29/07/2013	Tango	CC	1	44	11.8	1.09	10.78	0	1
26/06/2013	Yosemite	CC	1XXX	44	10.7	1.63	6.55	0.1	5 - 6
29/07/2013	Yosemite	CC	1XXX	48	11.0	1.15	9.55	0.5	1

5.5.20 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Navel oranges in a cold production region (Sundays River Valley)

Project 998B by S. Meeding (CRI)

Opsomming

Die eerste seleksie was Lina (TC) met goeie interne kwaliteit. Fukumoto (TC) was na Lina ryp met goeie kleur ontwikkeling. Palmer (SC) het die jaar voor Tulegold (TC) en Washington (SC) ryp geword waar dit in die 2012 seisoen na die twee seleksies ryp geword het. Na Palmer het die Tulegold seleksie ryp geword met Washington wat die seisoen geeïndig het.

Vir die laat seleksies by Dunbrody het Cambria eerste ryp geword met swak eksterne kleur asook lae suur en brix. Witkrans het op Cambria gevolg met hoër brix en suur. Autumn Gold was na Witkrans ryp met goeie kleur ontwikkeling. Na Autumn Gold was dit Summer Gold, Glen Ora Late, Lane laat en die seisoen is geeïndig met Powell Summer. In die 2012 seisoen het Glen Ora die seisoen geeïndig maar in die die 2013 seisoen het Glen Ora laat voor Lane laat en Powell Summer rypgeword.

Summary

The first selection to mature was Lina (TC) with good internal quality. Fukumoto (TC) was mature after Lina with good colour development. Palmer (SC) matured earlier in the 2013 season, maturing before Tulegold (TC) and Washinton (SC) whereas in the 2012 season Palmer matured after both of them. Tulegold followed Palmer and Washington ended the season.

For the late selections at Dunbrody Estate, Cambria was first to mature with poor colour as well as low brix and acid. Cambria was followed by Witkrans with higher brix and acid. Autumn Gold matured after Witkrans with good colour development. After Autumn Gold, Summer Gold, Glen Ora late, Lane late matured and the season was ended with Powell Summer. In the 2012 season Glen Ora late ended the season but in the 2013 season Glen Ora late matured before Lane late and Powell Summer.

Objectives

- To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (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 and Washington. The following late maturing selections were evaluated: Autumn Gold, Cambria, Glen Ora Late, Lane Late, Powell Summer, Summer Gold and Witkrans.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it 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 5.5.20.1. List of Navel selections evaluated at Sundays River Valley (Penhill) during 2013.

Selection	Rootstock	Planted
Fukumoto	Troyer	2007
Lina	Troyer	2007
Palmer	Swingle	2007
Tulegold	Troyer	2007
Washington	Swingle	2007

Table 5.5.20.2. List of Navel selections evaluated at Sundays River Valley (Dunbrody) during 2013.

Selection	Rootstock	Planted
Autumn Gold	Carrizo	2004
Cambria	Rough Lemon	2004
Glen Ora Late	Rough Lemon	2004
Lane Late	Carrizo	2004
Powell Summer	Carrizo	2004
Summer Gold	Carrizo	2004
Witkrans	Carrizo	2004

Results and discussion

These results are from commercial orchards in the area on various rootstocks.
At the Penhill site Lina (TC) was the earliest selection to mature with good internal quality. This was followed by Fukumoto (TC) with good colour development and good fruit size. Palmer (SC) was next to mature with a delayed colour development. After Palmer, Tulegold (TC) matured with Washington (SC) ending the season. The delayed colour development of Washington (SC) could be due to the Swingle rootstock that is 1-2 weeks later than Troyer and Carrizo. Tulegold had good colour development and also had the smallest fruit with a count of 72. Fukumoto and Palmer had the largest fruit with counts of 56. All the selections were seedless.

At Dunbrody Estate, Cambria was the first to mature, followed by Witkrans, Autumn Gold, Summer Gold, Glen Ora late and Lane Late. Powell Summer was the latest selection. All of the selections were of similar size (count 56). The selections with the poorest colour development were Cambria and Summer Gold, both with a colour of T3 at peak maturity. All the selections were seedless. Autumn Gold (11.0) and Lane late (11.6) had the highest brix. Cambria had the lowest acid with 0.85.

Conclusion

Of the early to mid maturing navels, Lina (TC) was first to mature followed by Fukumoto (TC), Palmer (TC), Tulegold (TC) and then Washington (SC). For the late Navels Cambria matures first, followed by Witkrans, Autumn Gold, Summer Gold, Glen Ora late, Lane late and Powell Summer ends the season. Some of the early Navels had better colour development in the 2013 season as well as greater yields.

Table 5.5.20.3. Internal fruit quality data for early and mid Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
18/04/13	Lina	TC	64	53.3	9.3	1.33	6.99	0	T6
07/05/13	Lina	TC	64	52.6	9.7	1.02	9.51	0	T4
22/05/13	Lina	TC	64	53.8	10.2	1.09	9.36	0	T1

18/04/13	Fukumoto	TC	64	48.9	9.9	1.53	6.47	0	T4
07/05/13	Fukumoto	TC	64	50.4	10.9	1.33	8.20	0	T1
22/05/13	Fukumoto	TC	56	52.2	11.5	1.17	9.83	0	T1
18/04/13	Palmer	SC	64	51.4	9.0	1.10	8.18	0	T6
07/05/13	Palmer	SC	56	50.7	10.1	1.09	9.27	0	T6
22/05/13	Palmer	SC	56	50.7	9.4	0.94	10.00	0	T4
18/04/13	Tulegold	TC	64	49.2	9.1	1.25	7.28	0	T4
07/05/13	Tulegold	TC	64	52.5	9.0	1.09	8.26	0	T3
22/05/13	Tulegold	TC	72	52.5	9.9	1.12	8.84	0	T1
18/04/13	Washington	SC	64	51.4	9.7	1.43	6.78	0	T7
07/05/13	Washington	SC	64	51.5	10.3	1.26	8.17	0	T6
22/05/13	Washington	SC	64	53.4	9.6	1.15	8.35	0	T4

Table 5.5.20.4. Internal fruit quality data for late Navel selections from the Kirkwood (Dunbrody) region of the Sundays River Valley during the 2013 season.

Date harvested	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg Seed	Colour
02/07/13	Autumm Gold	CC	56	52.4	11.0	1.16	9.5	0	T1-2
02/07/13	Cambria	RL	56	54.6	8.5	0.85	10.0	0	T3-4
13/07/13	Cambria	RL	56	54.1	9.5	0.74	12.8	0	T1-2
07/08/13	Cambria	RL	56	53.6	9.7	0.74	13.1	0	T1
02/07/13	Glen Ora Late	RL	56	52.8	9.9	1.04	9.5	0	T1
25/07/13	Glen Ora Late	RL	56	52.8	9.7	0.93	10.4	0	T1
07/08/13	Glen Ora Late	RL	56	53.6	10.3	0.95	10.8	0	T1
15/08/13	Glen Ora Late	RL	56	51.6	12.4	0.88	14.1	0	T1
02/07/13	Lane Late	CC	56	53.6	11.6	1.28	9.1	0	T1
25/07/13	Lane Late	CC	56	53.0	10.8	0.98	11.0	0	T1
02/07/13	Powell Summer	CC	56	54.7	10.9	1.20	9.1	0	T1
25/07/13	Powell Summer	CC	56	53.4	10.6	1.01	10.5	0	T1
02/07/13	Summer gold	CC	56	53.9	10.8	1.08	10.0	0	T3
25/07/13	Summer gold	CC	56	52.9	10.4	0.91	11.4	0	T2-3
02/07/13	Witkrans	CC	56	56.9	10.1	0.93	10.7	0	T1

5.5.21 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental Navel oranges in a cold production region (Sundays River Valley)**
Project 1001A by S. Meeding (CRI)

Opsomming

Die 99 Nawel was eerste om ryp te word en kleur ontwikkeling was goed. Die 99 Nawel ‘n ook die kleinste vrugte met ‘n telling van 64. Die HE laat Nawel het die seisoen geeïndig met goeie interne kwaliteit en groter vrugte met ‘n telling van 56. Kleur ontwikkeling was goed. Vrugte was al opgekleur toe is die brix:acid nog 8.3:1. Vrugte sal nog ‘n tyd kan hang.

Summary

The 99 Navel was the first to mature with good colour development. The 99 Navel had the smallest fruit size with a count of 64. The HE late Navel ended the season with good internal quality and large fruit with a count of 56. Colour development was good. The fruit had a colour of T1 when the brix:acid was 8.3:1. The fruit can be kept on the tree for a while longer.

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 Sundays River Valley. The following selections were evaluated: HE Late and 99 Navel.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it 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 5.5.21.1. List of Navel selections evaluatedat various sites in the Eastern Cape during the 2013 season.

Selection	Rootstock	Planted
99 Navel	Rough Lemon	Unknown
HE Late	Carrizo	Unknown

Results and discussion

The 99 Navel had a poor yield, perhaps due to alternate bearing. The 99 Navel is a large tree and in the off-year fruit usely bears higher up in the tree. HE late Navel had the best internal quality with a brix of 10.9 while the acid was still at 1.31 (Table 5.5.21.2). Both selections had good colour development and were fully coloured before their peak. Both selections are seedless.

Conclusion

The 99 Navel was first to mature but this selection seems to have alternative bearing problems with poor yield in the 2013 season. HE late Navel ended the season with good internal quality and good colour development. Both selections had a colour of T1 before their peak.

Table 5.5.21.2. Internal fruit quality data for Experimental Navel selections from the Sundays River Valley region of the Eastern Cape during the 2013 season.

Date harvested	Grower	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
18/04/13	Invercloy	99 Navel	RL	64	43.2	7.1	1.29	5.5	0	T7
08/05/13	Invercloy	99 Navel	RL	64	47.1	7.6	1.09	7.0	0	T5
22/05/13	Invercloy	99 Navel	RL	64	51.4	7.4	1.01	7.3	0	T4
25/07/13	H Ehlers	HE Late	CC	56	51.1	10.9	1.31	8.3	0	T1-2

5.5.22 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental Navel oranges in a cold production region (Gamtoos River Valley)**
Project 1001B by S. Meeding (CRI)

Opsomming

Die vroegste nawel seleksie in Suid Afrika vir die afgelope vyf jaar is Patensie Early. EDP1 (De Wet 1) was volgende, met uitstekende kleur ontwikkelling, goeie opbrengs en goeie vruggrootte. Fischer nawel het na De Wet 1 ryp geword. Na Fischer het Cambia en Lazy Boy ryp geword. Beide Lazy Boy en Cambria se kleur was laat met ŉ kleur van T3 op piek tyd. Suitangi volg op Lazy Boy en die seisoen word dan afgesluit deur die KS Nawel. Die oes periode moet nie langer as 3 tot 4 weke wees nie om goeie interne kwaliteit te verseker sonder skil probleme.

Summary

Patensie Early is the earliest maturing navel selection in South Africa and has maintained its earliness for five years. EDP 1 (De Wet 1) was next to mature with very good colour development, good yields and good fruit size. Fischer Navel matured after De Wet 1. After Fischer, Cambria and Lazy Boy matured. Both the latter selections had a slow colour development with a colour of T3 when both selections were on their peak. Suitangi matured after Lazy Boy with KS Navel ending the season. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

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: Patensie Early, Fischer, EDP 1&2, KS navel, Suitangi, Lazy Boy with Cambria as a control.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it 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 5.5.22.1. List of Mandarin hybrid selections evaluated at various sites in the Eastern Cape area during the 2013 season.

Selection	Rootstock	Planted
Patensie Early	Rough Lemon	Old
Fischer	Rough Lemon	Old
EDP1	Rough Lemon	Young
Cambria	Rough Lemon	Old
KS Navel	Rough Lemon	Young
Suitangi	Rough Lemon	Old
Lazy Boy	Rough Lemon	Young

Results and discussion

Cambria had the smallest fruit with counts of 88 and 72. Selections that had good fruit size as well were De Wet 1 (72), KS Navel (72) and Patensie early (72). The selections with the biggest fruit were Suitangi (56), Lazy Boy (56) and Fischer (56). All the selections were seedless. Lazy Boy has a round and bushy shape and is bearing most of the fruit on the outside of the tree. All of the selections had a delay in external colour development with fruit still at colour plate T3 when at peak maturity.

Conclusion

Patensie Early is the earliest navel selection. This is followed by EDP1 (De Wet 1), Fischer, Cambria and Lazy Boy and Suitangi. KS Navel is the latest navel selection. Yields were best in KS Navel, Cambria and Fischer. Cambria has the smallest fruit size with Suitangi, Lazy Boy and Fischer the biggest.

Table 5.5.22.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2013 season.

Date harvested	Grower	Selection	Rootstock	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. Seed	Colour
15/05/13	K Scheepers	Cambria	RL	88	48.1	8.6	1.15	7.5	0	T6
28/05/13	K Scheepers	Cambria	RL	64	51.6	7.5	1.03	7.3	0	T6
24/07/13	K Scheepers	Cambria	RL	64	57.8	10.2	0.79	12.9	0	T-3
05/08/13	K Scheepers	Cambria	RL	64	49	10.2	0.68	15	0	T1
14/08/13	K Scheepers	Cambria	RL	72	49.4	10.1	0.7	14.4	0	T1
06/05/13	E Du Preez	De Wet	RL	72	50.9	8.4	0.95	8.8	0	T6
15/05/13	E Du Preez	De Wet	RL	64	49.1	7.4	0.86	8.6	0	T5
28/05/13	E Du Preez	De Wet	RL	64	50.9	7.5	0.79	9.5	0	T5
06/05/13	C Rautanberg	Fischer	RL	56	51.0	9.7	1.12	8.7	0	T5
15/05/13	C Rautanberg	Fischer	RL	56	51.0	10.9	1.19	9.2	0	T2-3
28/05/13	K Scheepers	KS	RL	64	51.1	8.9	1.03	8.6	0	T6
24/07/13	K Scheepers	KS	RL	64	51.7	10.7	0.76	14.1	0	T1-2
05/08/13	K Scheepers	KS	RL	64	48.0	11	0.78	14.1	0	T1
14/08/13	K Scheepers	KS	RL	72	50.4	11.5	0.73	15.8	0	T1
03/08/13	Pieter Streso	Lazy Daughter	RL	56	51.4	10.9	0.97	11.2	0	T3-4
24/08/13	Pieter Streso	Lazy Daughter	RL	56	48.8	11.6	0.8	14.5	0	T2-3
05/08/13	Pieter Streso	Lazy Daughter	RL	56	49.2	10.9	0.74	14.7	0	t1-3
14/08/13	Pieter Streso	Lazy Daughter	RL	56	51.0	11.9	0.79	15.1	0	T1
03/07/13	Pieter Streso	Lazyboy	RL	56	46.3	12.2	1.12	10.9	0	T3
24/07/13	Pieter Streso	Lazyboy	RL	56	49.8	12.6	0.95	13.2	0	T1-2
05/08/13	Pieter Streso	Lazyboy	RL	56	48.5	13.7	1	13.7	0	t1
14/08/13	Pieter Streso	Lazyboy	RL	56	47.5	14	1	14	0	T1
06/05/13	C Malan	Patensie early	RL	72	49.0	10.7	0.86	12.4	0	T1
24/06/13	I Ferreira	Suitangi	RL	56	55.8	10.5	1.13	9.3	0	T3
24/07/13	I Ferreira	Suitangi	RL	56	54.1	14.2	0.96	14.8	0	T1-2

5.5.23 PROGRESS REPORT: Establishment of a molecular citrus genotype reference database for citrus cultivar verification within the Citrus Improvement Scheme

Project TS 514022 (2012/3 – 2014/5) by Anita Severn-Ellis (ARC-ITSC)

Summary

Citrus (*Citrus reticulata*) accessions entering the Virus Free Nucleus Block at the Institute for Tropical and Subtropical Crops have been characterised based on important morphological or agronomical features. These defining morphological or agricultural characteristics are not continuously expressed within the potted greenhouse environment. It is therefore not always possible to check or verify the trueness-to-type of an accession which may prevent the detection of misidentifications or duplicates. Thus, to ensure correct cultivar identity, a procedure for accurate identification using molecular data is urgently needed. Twenty three of the previously selected 26 microsatellite (SSR) markers were finally included and used to differentiate between all citrus accessions included in the Citrus Improvement Scheme. PCR amplification, visualisation and documentation of DNA fragments generated for the Mandarin hybrid-, Clementine-, Satsuma-, lemon-, lime-, grapefruit-, pumello-, rootstock-, sweet orange and diverse citrus cultivars as per CIS list has been completed. PCR results were verified and the SSR-PCR results were captured on an Excel spreadsheet which will form part of the genotype reference database.

Although genetic differences were detected between most of the cultivars using the selected SSR markers, limited genetic variation was detected between the sweet orange cultivars. Additional marker systems such as sequence related amplified polymorphism or SRAP markers may provide the supplementary genetic information required to distinguish between these closely related cultivars. The use of SRAP markers to

distinguish between the sweet orange cultivars in addition to the current set of SSR markers will be investigated.

Opsomming

Sitrus (*Citrus reticulata*) genotipes wat opgeneem word in die Virus vrye Kern Blok by die Instituut vir Tropiese en Subtropiese Gewaase word gekarakteriseer deur belangrike morfologiese of lanboukundige eienskappe. Hierdie eienskappe kom nie altyd tot uiting in die glashuis omgewing waar die plante in potte groei. Dit is dus nie altyd moontlik om die tipe-egtheid van ŉ genotipe to bepaal of verifieer nie, wat kan lei tot duplikasies of die insluiting van verkeerde materiaal in die skema. Om hierdie rede is dit belangrik dat ŉ akkurate indentifikasie metode ontwikkel word deur die gebruik van molekulere data. Drie en twintig uit ŉ vorige geselekteerde groep van 26 mikro-sateliet (SSR) merkers is gebruik om te onderskei tussen al die sitrus genotipes vervat in die Sitrusverbeteringsskema (SVS).

Polimerase kettingreaksie (PCR) versterking, visualisering en dokumentasie van DNA-fragmente gegenereer vir die Mandaryn basters, Clementine-, Satsuma-, suurlemoen-, lemmetjie-, pomelo-, onderstamme-, soetlemoen en diverse sitrus kultivars soos per SVS lys is voltooi. PCR resultate is geverifieer en die SSR-PCR resultate is vasgelê op ŉ Excel spreistaat wat deel van die genotipe verwysing databasis sal vorm.

Alhoewel genetiese verskille tussen die meeste van die kultivars bepaal is met behulp van die geselekteerde SSR merkers, is beperkte genetiese variasie egter bespeur tussen die soetlemoen kultivars. Addisionele merker stelsels soos ‘volgorde verwante versterkte polimorfisme’ of SRAP merkers kan die aanvullende genetiese inligting wat nodig is om te onderskei tussen hierdie nou verwante kultivars verskaf. Die gebruik van SRAP merkers om te onderskei tussen die soetlemoen kultivars in vergelyking met die huidige stel SSR merkers sal ondersoek word.

5.6 **Climatic Regions of Southern Africa and cultivars being evaluated**

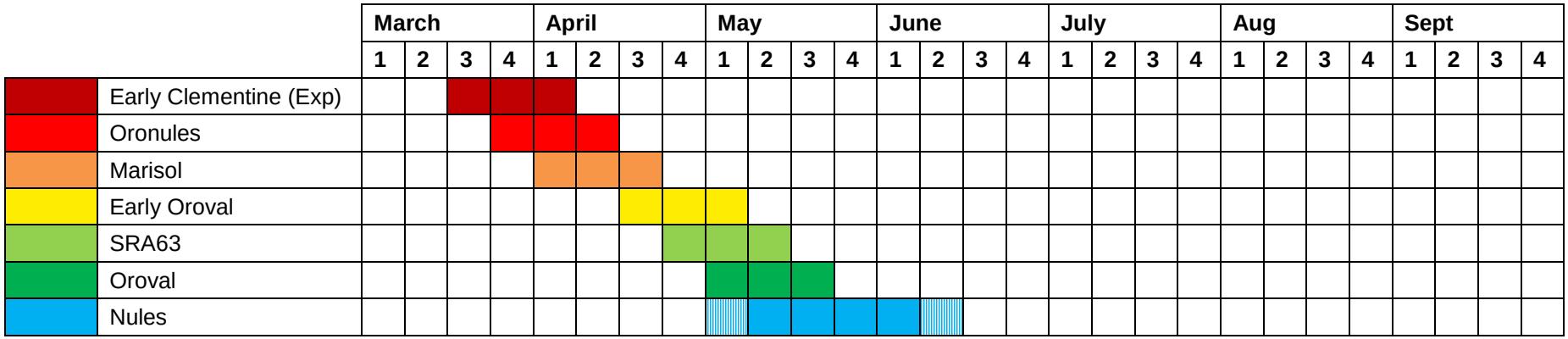
CLIMATIC REGION	AREA	PLACE	CULTIVARS
Hot-Dry	Limpopo	Tshipise	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Musina	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Letsitele	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
		Hoedspruit	Grapefruit
			Valencias
			Mandarin Hybrids (Late)
Hot-Humid	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

Approximate Clementine 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

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																												

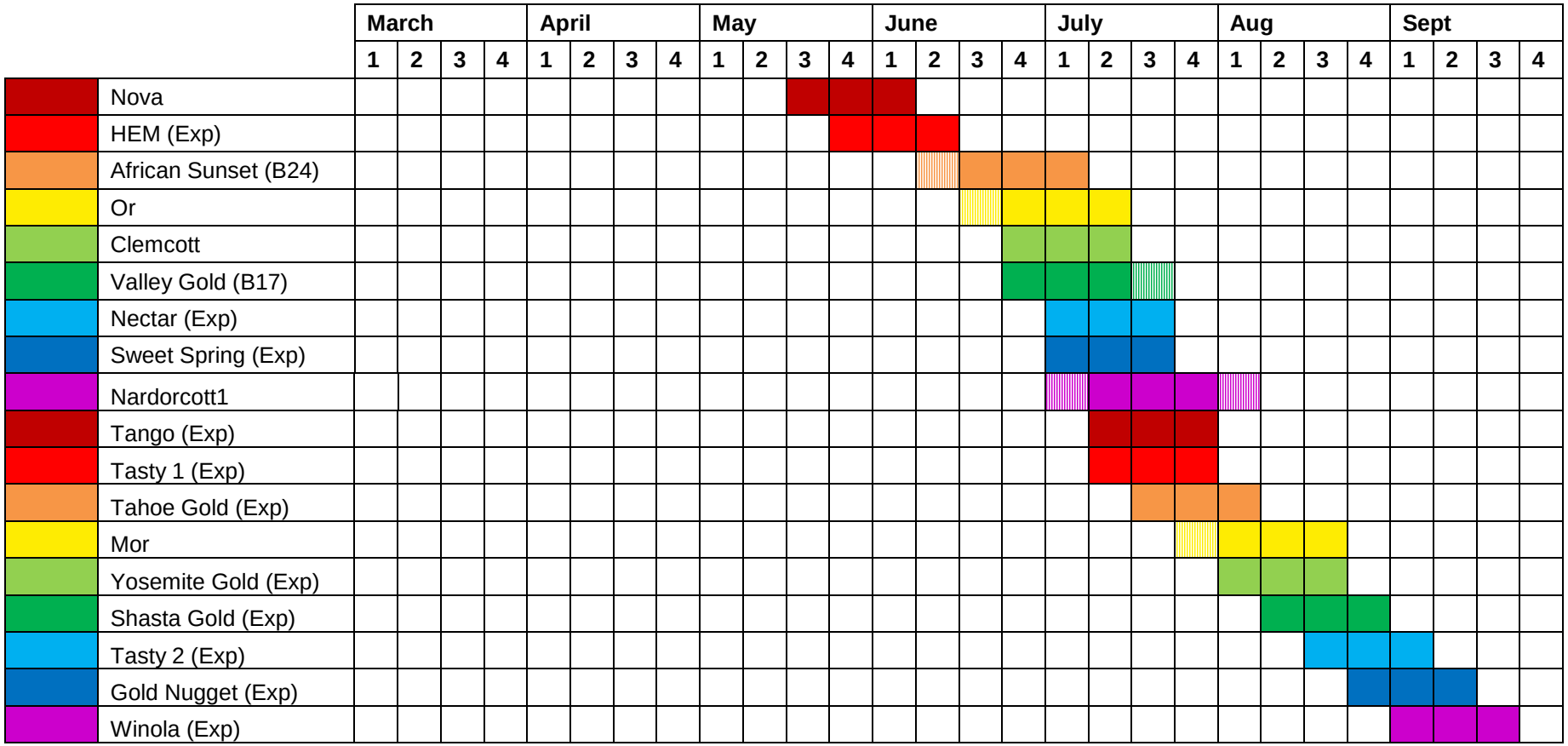
Exp = Experimental Cultivar

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

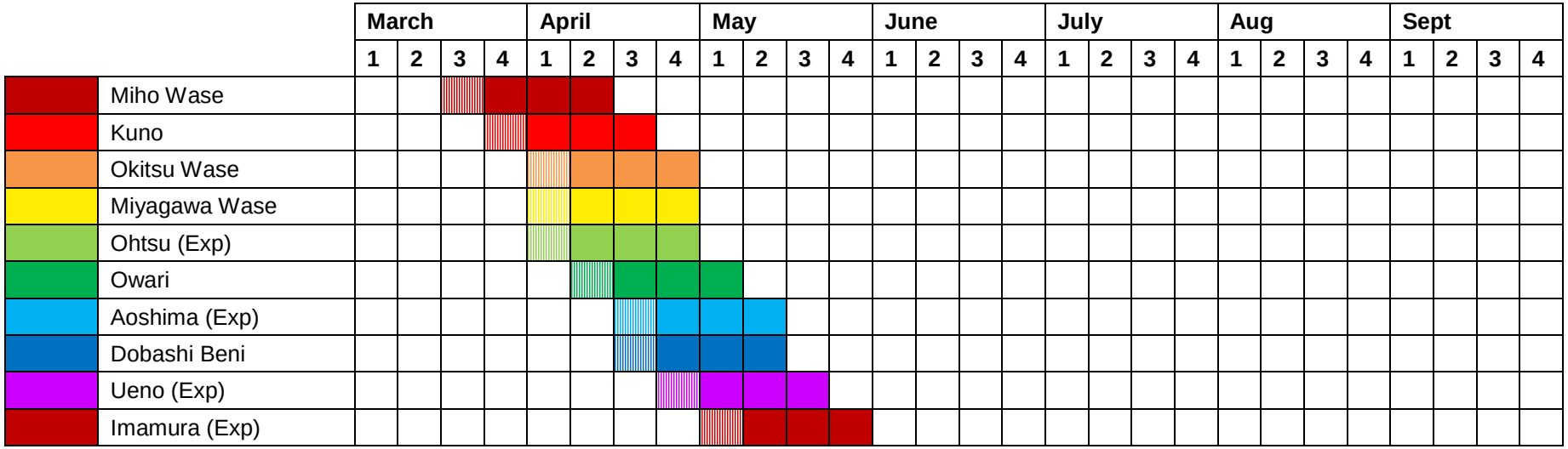
Approximate Mandarin Hybrid Maturity Periods in the Cape region of South Africa



Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

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

Approximate Valencia Maturity Periods in the Northern region of South Africa

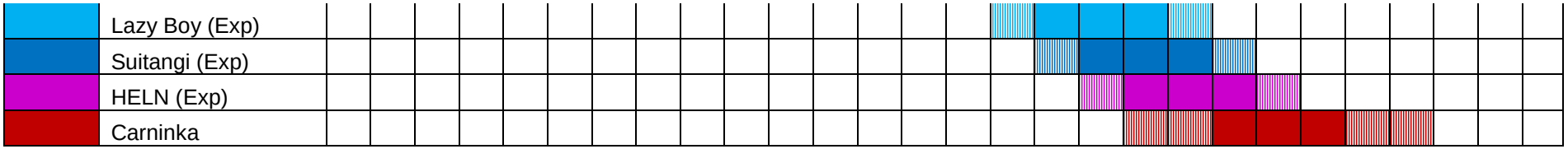
	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Turkey																												
Mouton Early (Exp)																												
Portsgate																												
Benny 2																												
Bend 8A1 (Exp)																												
Benny 1																												
Bend 8A2 (Exp)																												
Alpha																												
Midknight																												
Jassie (Exp)																												
Henrietta (Exp)																												
Delta																												
Du Roi																												
McClean SL																												
Louisa (Exp)																												
Ruby (Exp)																												
Skilderkrans (Exp)																												
Lavalle																												
Moosrivier Late 1 (Exp)																												

Exp = Experimental Cultivar

	Solid blocks indicate average maturity periods for the area overall
	Striped blocks indicate variation due to microclimates

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																												



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)

By P.H. Fourie, M.M. N. du Toit, M. le Roux, L. Olivier, S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

6.1 Budwood

This report summarises the seasonal supply of budwood from May 2013 to April 2014. A total of 3,513,599 buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries. This is 9.6% more buds than in the same period of 2013 and 11% more buds than in the same period of 2012. During this period 7,565 buds were exported to neighbouring countries. Increased demand was mostly from the Western Cape and Eastern Cape nurseries. Mandarin (42.18%) was the most popular citrus type, followed by lemon (20.31%), Valencia (18.57%) and navels (10.71%); in 2012 this proportion was 28.7%, 22.5%, 20.9% and 18.1%, respectively, which indicates a marked shift in cultivar demand (Tables 6.1.1 and 6.1.2). The Top 30 varieties comprised 91.2% of total number of buds supplied. Nadorcott 1 mandarin, the 2nd most popular cultivar in 2012 and the 7th most popular cultivar in 2013; has surpassed Eureka Lemon and is now the most popular cultivar (Table 6.1.3). The need for authorised cutting in nurseries has decreased by 9.7% from 40.6% in 2012/13 to 30.9% in 2013/14 (Figure 6.1.1).

6.2 Seed

During May to April 2014, 2788 litres of seed were supplied locally and 173 litres of seed were exported. Carrizo Citrange remains the most popular rootstock (40.23%), followed by C35 citrange (19.23%), Rough Lemon (11.86%) and Swingle Citrumelo (10.17%). Uncharacteristically low and variable germination rates were experienced. Exports were stopped early in the season to address local demand. During this period 1065 litres were replaced locally and 17 litres were replaced for SADC countries. Carrizo citrange seed was imported from Australia and Carrizo citrange, Swingle citrumelo and Rough Lemon were imported from the United States of America to address the shortages.

6.3 Production

With multiplication trees in production, the CFB presently carries a potential budwood stock of 6.5 million buds of approximately 300 varieties per year. As the top 30 varieties comprise 92% of demand, multiplication tree stocks are being managed in order for CFB to be timeously able to supply demand of the sought-after varieties. Heating and lighting were installed in two tunnels. In the one tunnel seedlings were transplanted in 2-L plastic bags and in 230 ml plastic tubes and 9,898 rootstocks were budded with high demand cultivars. This option allowed for a much higher concentration of increase trees. Thus far this initiative seems to be successful as active growth was observed into winter and budwood could already be harvested in the summer on the small increase trees. In the second tunnel the normal 10 L plastic bags were used and 1,898 rootstocks budded with high demand cultivars for rapid multiplication under controlled environment. A new 5-bay (1,904 m²) extension was added to the existing Greenhouse 4 A&B to increase the capacity with 12,348 increase trees in 10-L planting bags.

6.4 Tree Certification

There were 423,075 trees certified during April 2013 to March 2014. This is 566,019 less trees than in the same period of 2013 and 1,488,303 less than in 2012. The implementation of an extensive database to verify the pathogen status of nursery trees as a certification criterion has resulted in a number of pending tree certification requests. Michelle le Roux has also been on maternity leave in the last quarter, which necessitated Louise Olivier to focus much of her time on training and performance of other duties. The backlogged certification requests will be concluded in autumn and winter 2014.

6.5 Nursery Registration

Twenty-five nurseries were visited during the May 2013 audits. Twenty nurseries retained their certification status, while two new nurseries and two existing nurseries were provisionally certified. Upon completion of the outstanding requirements, these four nurseries could be certified. One nursery has lost its certification status. Twenty-three nurseries were visited during the November 2013 audits. Twenty-one nurseries retained their certification status, while two new nurseries were provisionally certified. Upon completion of the outstanding requirements, these two nurseries could be 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.

6.6 Statutory Improvement Scheme

The statutory CIS proposal was received back from the Registrar and updates were made in line with the new Plant Improvement Act as latest changes to the scheme's Procedural Guide. A draft discussion document stating the benefits and detriments of a voluntary or compulsory statutory improvement scheme was compiled and sent to all stakeholders and growers for inputs. The statutory scheme initiative was also discussed in meetings with all participating nurseries, a domestic nursery, cultivar management companies and growers at the regional CRI production workshops. A status document summarising feedback and inputs was compiled and submitted to CRI's Board for further guidance. A public workshop facilitated by the NAMC was held on 9 April 2014.

The workshop was attended by most stakeholders, including growers, nurserymen, cultivar managers and DAFF representatives. The workshop debated matters arising from the consultation process on which more clarity or consensus was required. The meeting's minutes were circulated, and are briefly summarised in this report.

New changes to the Plant Improvement Act make it obligatory that the Scheme in the near future be promulgated as a statutory scheme. The meeting agreed that the CGA can be proposed as designated authority with CRI as its CIS management agent, provided that the CIS Advisory Committee (CISAC) represents all stakeholders. This proposal was supported by arguments that the CGA can operate the CIS at own cost and has ability to financially underwrite the CIS, as it has been doing since it was established; the CGA is the representative body of the majority stakeholder grouping, the citrus growers of South Africa, who are also the clients of all CIS stakeholders with commercial interests; and CRI is the current operator of the CIS and is an independent, not-for-profit, research and technical service company. Regarding representation on CISAC, it was agreed that CISAC is an adequate forum for all interested parties to make inputs. However, CISAC will be restructured into an advisory committee with two sub-committees. Participation and inputs of technical and operational personnel will largely be on sub-committee level, and the sub-committee chairperson will then report to a more streamlined advisory committee with less members. CISAC's decision-making process will also be formulated, but it will remain an advisory and consensus forum. Other matters discussed were addressed to satisfaction of the meeting, and/or will form part of ongoing discussions within CISAC. A statutory CIS can either be proposed as a voluntary scheme or a compulsory scheme. It was agreed that the best option for the South African Citrus industry was a compulsory participation scheme; however, this option was not necessarily supported by all the role-players e.g. certain cultivar management companies, who indicated that they support the voluntary statutory scheme option. The CGA tabled a letter requesting that the CIS should be compulsory statutory scheme. The meeting was closed by Mr Ramabulana from NAMC, who concluded that a compulsory scheme offered the most advantages as well as protection from biosecurity risks for the citrus industry in South Africa. However, the needs of all role players including those not supportive of a compulsory scheme should be considered.

6.7 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 and feedback were received that some residents have already started to remove their citrus trees. DAFF has made follow-up visits to owners refusing to remove trees, and will again follow up after they sought legal advice how to address the few problem situations where owners refuse to remove their trees.

6.8 Establish and maintain a virus-free gene source at CRI

Project 790 by J.H.J. Breytenbach S.P. van Vuuren & G. Cook (CRI)

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 seven new selections were submitted for STG and a further 49 submissions from previous years are at various stages in the process before release. Eight of the latter are being indexed to ensure virus-free status. Virus-free material is pre-immunised with a suitable *Citrus tristeza virus* source before it is supplied to the Citrus Foundation Block (CFB). A virus-free gene source of citrus cultivars is maintained in an insect-free tunnel at CRI. Seven new cultivars and selections were supplied to the CFB in this report period and added to the CRI gene source, which now comprises 277 cultivars and selections. Erection of a new tunnel for housing the nucleus block is complete. This facility was funded by SACNA and CRI.

6.9 Diagnostic services for graft transmissible diseases

Project 796 by J.H.J. Breytenbach, S.P. van Vuuren & G. Cook (CRI)

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 CFB as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents are present or have been inadvertently introduced. These ongoing diagnostic activities of the CIS 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 177 mother trees was completed and indexing of the remaining 176 mother trees have been initiated and await evaluation. Viroid indexing of 194 mother trees was initiated and analysis is pending. Current test protocols are updated and improved on an ongoing basis. Our STG process was evaluated and relevant adjustments were made to the existing processes following the inputs of visiting experts during the hosting of the IOCV in South Africa in August 2013. General diagnostics and investigations into *ad hoc* industry problems and concerns relating to graft transmissible diseases are also reported within this project.

6.10 Citrus Biosecurity activities

Feedback from the biosecurity activities that are funded / coordinated from CIS in support of DAFF are briefly discussed here.

African Citrus Greening surveys

Western Cape

- **Knysna magisterial district:** As a follow up to previous years, orchards on the farm Candlewood were surveyed and six samples were collected. At the farm Portland Manor, neighboring farm five samples were collected from a neglected orchard. The results were negative for both sites; however, the Candlewood grower was removing the old Clementine orchards.
- **Malmesbury magisterial district:** Five positive trees were found in a farm in the Malmesbury district; destruction orders were issued and trees removed. Delimiting surveys will be conduicted in 2014.
- **Piketberg magisterial district:** In the Piketberg area five farms and seven home gardens were surveyed. No psylla was found but six samples were taken from trees with discoloured leaves on farms; these tested negative.

Eastern Cape

- **Fort Beaufort magisterial district:** A survey was conducted; all samples collected tested negative.
- Except for two magisterial districts in Transkei, the Eastern Cape is still Greening-free. To ensure its freedom the regulated Control Measures (R.110) under the Agricultural Pest Act was amended to include buffer zones protecting the Eastern Cape from the Western Cape and KZN where African Greening does occur. These buffer zones include the greening-free Knysna and Uniondale districts, and the Alfred Nzo and Oliver Tambo districts south of the KZN border, where greening is under eradication. These buffer zones will be regarded as greening-free and more intensive surveys will be conducted to ensure its sustained pest freedom.
- An provincial working group will be established between DAFF, the Eastern Cape department of agriculture, CGA and CRI to drive the required awareness campaigns, delimiting surveys and eradication in Transkei.

Asiatic Citrus Greening (HLB) and Asian Citrus Psyllid (ACP) surveys

A national HLB/ACP action plan was developed by CRI and approved by DAFF. Important actions are contingency surveys and awareness programmes. These are coordinated between DAFF, CGA and CRI at bi-annual Greening Stakeholder meetings. Given the seriousness of this risk to the southern African citrus growers, it has to be acknowledged that not enough resources are being allocated to these actions.

KwaZulu-Natal

- **Durban:** The survey in KZN focussed on Asiatic Citrus Greening (HLB) and its vector, *Diaphorina citri*. In order to improve awareness and increase the scope and effectiveness of the survey, schools were targeted and learners requested to brings samples from home. 106 samples were collected; all tested negative for HLB, except 26 positive for African Greening.

Yellow sticky traps were also placed in the different areas around Durban and in the botanical garden to determine the possible presence of *Diaphorina*; only the African triozid, *Trioza erytreae* was caught on the traps.

Citrus biosecurity in Africa

Angola

In the recent past, CRI became aware that citrus nursery trees were being imported from Brazil. Forunately, these trees originated from excellent nurseries in Sao Paulo state, which has a mandatory citrus improvement scheme. This reduced the risk of inadvertent import of exotic diseases; the practice is nonetheless risky, especially considering *Citrus leprosis virus* that is vectored by the *Brevipalpus* mite. New evidence has emerged that Brazilian trees were imported into Angola from Minas Gerais province. As this Brazilian state does not have a formal citrus improvement scheme, these imports are extremely risky as many of the feared citrus diseases that occur in Brazil, and are exotic to Africa, could have been exported with these consignments.

CRI invited a high-level Angolan ministry of agriculture (MINADERP) delegation to SA-CIS and CRI facilities. The delegation consisted out of Dr Adelino Rodrigues (Director: Plants and Livestock), Julio José Nascimento (Head of Department: Plant Protection) and Sidonio Mateus (Head of Department: Agriculture). They visited CFB, a certified nursery, packhouse, CRI facilities in Nelspruit and orchards. A meeting was held with SA-DAFF [Alice Baxter, Mashudu Silimela, Salomina (Inspection Services), Kgabo Matlala (National contact point) and Mike Holtzhausen]. The outcomes are briefly summarized below:

- MINADERP acknowledged the risk of propagation material import and indicated that they will immediately cease to authorize imports from Brazil
- MINADERP was encouraged to rather buy trees from CIS certified nurseries in South Africa. Feedback from nurseries indicates trade in citrus trees with Angola and other African countries.
- MINADERP will liase with DAFF to get information to get their import conditions in line with those of RSA
- This matter will also be raised on a workshop specifically aimed at actualizing a RSA-Angola bilateral protocol that has been signed years ago.
    - From this protocol, funding should become available to support training, translation, research, surveys, and services.
- On invitation from MINADERP, Hennie le Roux attended an Agricultural Trade Fair and presented a talk on Citrus Biosecurity (end-November 2013). Hennie's excellent presentation at Trade show was also televised. A report sent to MINADERP proposing follow-up surveys to be conducted. No feedback was received from Angola.

The initiatives in Angola will be continued in 2014.

Mozambique

Evidence of citrus development in Mozambique with Brazilian links raised concern that they might use Brazilian-made trees. MC Pretorius and Hennie le Roux arranged a meeting with Mozambique officials to discuss the citrus biosecurity risks and to promote the use of CIS nurseries. The meeting was postponed and will be held in 2014.

Challenges: Resource, time and personnel constraints. Given the capacity constraints within DAFF, a lot of these biosecurity actions are handled by CRI personnel. More resources are urgently required for the HLB/ACP surveys and African Biosecurity actions.

SITRUSVERBETERINGSKEMA (SVS)

Deur M.M.N. du Toit, P.H. Fourie, M. le Roux, L. Olivier, S.P. van Vuuren, J.H.J. Breytenbach en G. Cook (CRI)

6.1 **Okuleerhout**

Hierdie verslag gee ŉ opsomming van die seisoenale verskaffing van okuleerhout vanaf Mei 2013 tot April 2014. ŉ Totaal van 3,513,599 ogies is verskaf deur die Sitrus Grondvesblok (SGB) en gemagtig vir die sny in gesertifiseerde kwekery. Dit is 9.6% meer ogies as in dieselfde tydperk van 2013 en 11% meer ogies as in dieselfde tydperk van 2012. 7,565 ogies is gedurende hierdie tydperk uitgevoer na buurlande. Die verhoogde aanvraag was meestal van die kwekerye in die Oos-Kaap en Wes-Kaap. Mandaryne (42.18%) was die gewildste sitrus tipe, gevolg deur suurlemoene (20.31%), Valencias (18.57%) en nawels (10.71%); in 2012 was hierdie verhouding onderskeidelik 28.7%, 22.5%, 20.9% en 18.1% wat op ŉ merkbare verskuiwing in kultivar aanvraag dui. Die Top 30 kultivars behels 91.2% van die totale aantal ogies verskaf. Nadorcott 1

mandaryn (2de gewildste kultivar in 2012 en 7de gewildste kultivar in 2013) oortref Eureka suurlemoen in 2013/14 en is nou die gewildste kultivar. Die behoefte vir gemagtigde sny in kwekerye het afgeneem met 9.7% vanaf 40.6% in 2012/13 tot 30.9% in 2013/14.

6.2 Saad

Gedurende Mei tot April 2014 is 2,788 liter saad plaaslik voorsien en 173 liter saad is uitgevoer. Carrizo Citrange is steeds die gewildste onderstam (40.23%), gevolg deur C35 Citrange (19.23%), Growweskil Suurlemoen (11.86%) en Swingle Citrumelo (10.17%). Ongekenmerkde lae en wisselvallige ontkieming is ondervind. Uitvoere is vroeg in die seisoen gestaak om sodoende die plaaslike aanvraag te voorsien. Gedurende hierdie tydperk is 1,065 liter plaaslik vervang en 17 liter is aan SADC-lande vervang. Carrizo Citrange saad is vanaf Australië, en Carrizo Citrange, Swingle Citrumelo en Growweskil Suurlemoen is vanaf die Verenigde State van Amerika ingevoer om die tekorte aan te spreek.

6.3 Produksie

Met vermeerderingsbome in die produksie, dra die SGB tans ŉ potensiële okuleerhout voorraad van 6.5 miljoen ogies van ongeveer 300 kultivars per jaar. Omdat die top 30 kultivars uit 92% van die aanvraag bestaan, word vermeerderingsboom voorraad so bestuur dat die SGB instaat is om betyds aan die aanvraag na gesogte kultivars te voldoen. Verhitting en beligting is in twee tonnels geïnstalleer. In die een tonnel is saailinge oorgeplant in 2-L plastieksakke en 230-ml plastiese buise en 9,898 onderstamme is geokuleer met hoë-aanvraag kultivars. Hierdie opsie maak voorsiening vir ŉ heelwat hoër konsentrasie van vermeerderingsbome. Tot dusver lyk dit of hierdie inisiatief suksesvol kan wees omdat aktiewe groei gedurende die winter waargeneem is en okuleerhout kon reeds vroeg in die somer van hierdie klein vermeerderingsbome geoes word. In die tweede tonnel is die normale 10-L plastieksakke gebruik en 1,898 onderstamme is geokuleer met hoë-aanvraag kultivars vir die vinnige vermeerdering in ŉ gekontroleerde omgewing. Die bestaande Kweekhuis 4 A & B is met ŉ nuwe 5-koepel (1,904 m²) uitbreiding vergroot wat die kapasiteit met 12,348 vermeerderingsbome in 10-L plant sakke, verhoog.

6.4 Boomsertifisering

Daar was 423,075 bome gedurende April 2013 tot Maart 2014 gesertifiseer. Dit is 566,019 minder bome as in dieselfde tydperk van 2013 en 1,488,303 minder as in 2012. Die implementering van ŉ uitgebreide databasis om die patogeen-status van kwekery bome as ŉ sertifisering kriterium te verifieer, het gelei tot ŉ groot aantal agterstallige boomsertifiseringsaansoeke. Michelle le Roux is ook op kraamverlof in die laaste kwartaal, wat Louise Olivier genoodsaak het om baie van haar tyd aan opleiding en die uitvoering van ander pligte te fokus. Die agterstallige sertifiseringsaansoeke sal gedurende die herfs en winter 2014 afgehandel word.

6.5 Kwekery-sertifisering

Vyf-en-twintig kwekerye is gedurende die Mei 2013 oudits besoek. Twintig kwekerye behou hul sertifiseringstatus, terwyl twee nuwe kwekerye en twee bestaande kwekerye voorlopig gesertifiseer is. Na voltooiing van die uitstaande vereistes, kan hierdie vier kwekerye gesertifiseer word. Een kwekery het sy sertifiseringstatus verloor. Drie-en-twintig kwekerye is gedurende die November 2013 oudits besoek. Een-en-twingtig kwekerye behou hul sertifiseringstatus, terwyl twee nuwe kwekerye voorlopig gesertifiseer is. Na voltooiing van die uitstaande vereistes, kan hierdie twee kwekerye gesertifiseer word. Die toename in *Phytophthora* besmetting in sekere kwekerye is rede tot kommer en bykomende ondersteuning word aan hierdie kwekerye gebied om te help om die probleem reg te stel.

6.6 Statutêre Verbeteringskema

Die voorgestelde skema dokument is terug ontvang van die Registrateur. Die nuutste veranderinge aan die Prosedureële Riglyn is gemaak om sodoende in ooreenstemming te wees met die nuut-voorgestelde Plantverbeteringswet. ŉ Besprekingsdokument, wat die voordele en nadele van ŉ vrywillige of verpligte statutêre verbeteringskema opsom, is opgestel en aan alle belanghebbendes en produsente vir hul insette gestuur. Die statutêre skema inisiatief is ook met alle deelnemende sitrus kwekerye, een ornamentele kwekery, kultivar agente tydens individuele vergaderings bespreek, asook met produsente tydens die plaaslike CRI produksie werkswinkels. ŉ Statusdokument wat die terugvoering en insette opsom, is opgestel en aan die CRI Raad voorgelê vir verdere leiding. ŉ Openbare werkswinkel, gefasiliteer deur die NAMC, is op 9 April 2014 gehou. Die werkswinkel is deur die meeste belanghebbendes bygewoon, insluitend produsente, kwekerye, kultivar agente en DAFF verteenwoordigers. Die werkswinkel het aangeleenthede

wat na die konsultasieproses meer duidelikheid of konsensus vereis, bespreek. Die vergadering se notule is versprei en word kortliks in hierdie verslag saamgevat.

6.7 Beskermende sone rondom die Sitrus Grondvesblok

Die wetgewing wat die area binne ŉ 5-km radius rondom die SGB as ŉ sitrus-vrye area verklaar, is op 21 Januarie 2011 in die Skaatskoerant gepubliseer. Lasgewings om alle sitrus in hierdie sone te verwyder, is deur DAFF uitgereik en terugvoering is ontvang dat sommige inwoners hul bome verwyder het, terwyl ander geweier het. DAFF het opvolgbesoeke by die inwoners wat weier om hul bome te verwyder afgelê en is tans besig om regsadvies in te win om te verseker dat die oorblywende bome ook verwyder sal word.

6.8 Vestiging en instandhouding van ŉ virus-vrye genebron by CRI

Groeipuntenting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is sewe nuwe seleksies ingedien vir GPE en ŉ verdere 49 seleksies van vorige introduksies is in verskeie fases voor vrystelling. Agt van laasgenoemde word reeds geïndekseer om virusvrye status te bevestig. Virusvrye materiaal word met ŉ toepaslike *"Citrus tristeza virus"* bron gepreïmuniseer voordat dit aan die Sitrus Grondvesblok (GVB) vrygestel word. Virusvrye boompies van verskillende cultivars en seleksies word as ŉ genebron in ŉ insekvrye tonnel by CRI bewaar. Sewe nuwe seleksies van 2013 is aan die GVB voorsien en die is by die CRI genebron gevoeg, wat tans uit 277 kultivars en seleksies bestaan. Oprigting van ŉ nuwe tonnel om die genebron te huisves is voltooi. Hierdie fasiliteit is deur SACNA en CRI befonds.

6.9 Diagnostiese dienste vir ent-oordraagbare siektes

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 verpreiding van gesonde voortplantingsmateriaal. Biologiese en molekulêre indeksering word gedoen op nuwe toevoegings tot die SVS voordat die material aan die Grondvesblok verskaf word, asook her-indeksering van moederbome wat by die Grondvesblok gehuisves word. Daar word hier verslag gelewer op die voortdurende diagnostiese aktiwiteite van die SVS. Die moederbome by die Grondvesblok word op ŉ rotasie basis elke tweede jaar ge-herindekseer om te bepaal of enige strawwe CTV rasse of enige sitrus viroïede in die moedermateriaal voorkom. Die biologiese evaluasie van die CTV virulensie in 177 moederbome is gedurende die jaar voltooi waarna ŉ verdere 176 geïndekseer word waarvan die resultate nog afwagtend is. Viroied indeksering van 194 moederbome is ook begin waarvan die resultate ook nog afwagtend is. Huidige prosedures word voortdurend aangepas en verbeter. Die groeipunt-entingsproses is ge-evalueer en die nodige aanpassings gedoen na ŉ besoek van wêreldkundiges tydens die IOCV kongres wat Suid Afrika in Augustus 2013 aangebied het. 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.

6.10 Sitrus Biosekuriteit aktiwiteite

Gegewe die kapasiteitsbeperkinge binne DAFF, word baie van hierdie biosekuriteit aksies deur CRI personeel hanteer. Meer hulpbronne word dringend benodig vir die HLB/ACP-opnames en biosekuriteit aksies in Afrika. Terugvoer van die biosekuriteit aktiwiteite wat befonds of gekoördineer word deur die SVS ter ondersteuning van DAFF, word kortliks in hierdie verslag bespreek.

6.10.1 <u>Afrika Sitrus Vergroening opnames</u>

Terugvoering van die Afrika Sitrus Vergroening opnames wat in die volgende areas uitgevoer is, word kortliks bespreek in hierdie verslag:

a) Wes-Kaap: Knysna; Malmesbury; Piketberg
b) Oos-Kaap: Fort Beaufort; Transkei

6.10.2 <u>Asiatiese Sitrus Vergroening (HLB) en Asiatiese Sitrus Psyllid (ACP) opnames</u>

ŉ Nasionale HLB/ACP aksieplan is deur CRI ontwikkel en is deur DAFF goedgekeur. Belangrike aksies sluit in: gebeurlikheidsopnames en bewusmakingsprogramme. Dit word deur DAFF, CGA en CRI by halfjaarlikse vergaderings tussen die verskillende rolspelers gekoördineer. Gegewe die erns van hierdie risiko vir die Suider-Afrikaanse sitrus produsente, moet daar erken word dat daar nie genoeg hulpbronne aan hierdie aksies toegeken word nie.

6.10.3 Sitrus biosekuriteit in Afrika

a) Angola:

CRI het onlangs bewus gemaak dat sitrus kwekerybome vanuit Brasilië na Angola ingevoer is. Hierdie invoere is uiters riskant omdat baie van die gevreesde sitrus siektes wat in Brasilië voorkom, en eksotiese aan Afrika is, moontlik saam met hierdie besendings ingevoer kon word. Op uitnodiging van CRI het ŉ afvaardiging van die Angolese Ministerie van Landbou die Skema, kwekerye en DAFF besoek. Hulle het hierdie risiko erken en het aangedui dat hulle dadelik die magtiging van invoere uit Brasilië sal staak.

b) Mosambiek:

Bewyse van ŉ sitrus-ontwikkeling in Mosambiek is kommerwekkend weens hul Brasiliaanse skakels en die risiko betrokke indien hulle van Brasiliaanse kwekerybome gebruik sou maak. MC Pretorius en Hennie le Roux het ŉ vergadering met Mosambiekse amptenare gereël om sitrus biosekuriteit risiko's te bespreek en om die gebruik van SVS gesertifiseerde kwekerye, te bevorder. Die vergadering is uitgestel en sal in 2014 gehou word.

Table 6.1.1. Buds supplied during the period: May to April 2012-2014.

Area	2011/12	2012/13	2013/14	Total
Local	3 138 552	3 198 295	3 506 034	9 756 884
Exports	27 050	8 250	7 565	42 865
Total	3 165 602	3 206 545	3 513 599	9 799 749

Area	2011/12	2012/13	2013/14	Total
Eastern Cape	611 325	638 623	771 140	2 009 521
Gauteng	100			100
KwaZulu-Natal	34 300	41 800	24 400	100 500
Limpopo	1 259 732	1 268 530	1 141 113	3 612 475
Mpumalanga	260 583	225 485	271 847	740 385
North-West Province	246 194	121 000	46 500	413 694
Northern Cape	141 465	149 669	188 600	479 734
Western Cape	584 853	753 188	1 062 434	2 400 475
	3 138 552	3 198 295	3 506 034	9 756 884

Area	2011/12	2012/13	2013/14	Total
Namibia	14 150			14 150
Other African States		8 100	7 565	15 665
Zimbabwe	12 900	150		13 050
	27 050	8 250	7 565	42 865

Variety	2011/12	%	2012/13	%	2013/14	%	Total
Mandarin Hybrid	828 989	26.19%	920 847	28.72%	1 482 118	42.18%	3 205 683
Lemon	681 002	21.51%	724 850	22.61%	713 535	20.31%	2 103 287
Valencia	647 766	20.46%	671 837	20.95%	652 604	18.57%	1 934 907
Navel	643 722	20.33%	573 701	17.89%	376 276	10.71%	1 588 373
Grapefruit	24 385	0.77%	88 670	2.77%	125 715	3.58%	238 770
Clementine	116 247	3.67%	128 700	4.01%	85 460	2.43%	330 407
Satsuma	159 696	5.04%	36 270	1.13%	40 436	1.15%	236 402
Kumquat	10 550	0.33%	7 710	0.24%	12 100	0.34%	30 360
Lime	47 600	1.50%	37 170	1.16%	11 955	0.34%	95 725
Midseason	735	0.02%	9 340	0.29%	6 590	0.19%	16 665
Diverse	2 500	0.08%	4 800	0.15%	4 515	0.13%	11 815
Ellendale	1 540	0.05%	1 800	0.06%	1 550	0.04%	4 890
Pummelo	850	0.03%	850	0.03%	665	0.02%	2 365
Rootstock	20	0.00%		0.00%	80	0.00%	100
	3 165 602	100.00%	3 206 545	100.00%	3 513 599	100.00%	9 799 749

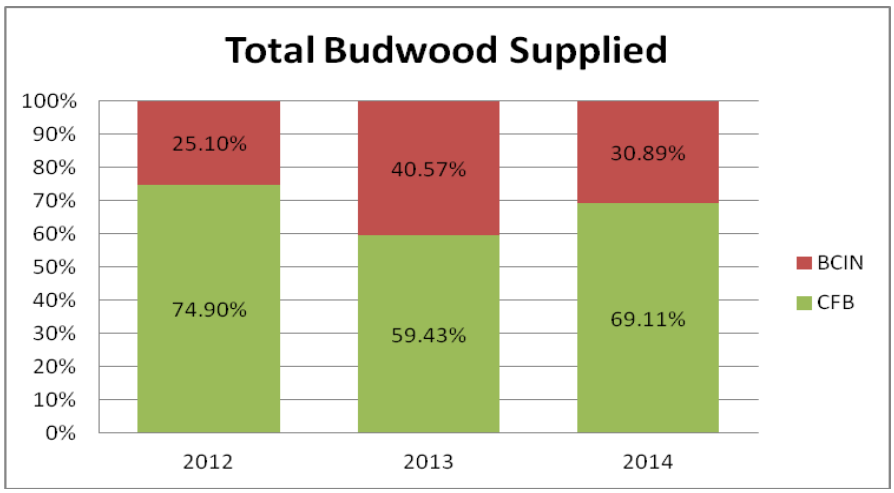


Figure 6.1.1. Budwood supplied by the CFB and cut in nurseries (BCIN) during the period: May to April 2012-2014.

Table 6.1.2. Buds supplied per variety per area during the period: May to April 2012-2014.

Variety	Year	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpuma-langa	Namibia	North-West Province	Northern Cape	Other African States	Western Cape	Zimbabwe	Total
Clementine	2011/12	4600			64710	1068		3200	4300		38369		116247
	2012/13	2600		500	10000	600			7000		108000		128700
	2013/14	19952			7500	85			5500	50	52373		85460
Diverse	2011/12								1000		1500		2500
	2012/13				3100			400			1150	150	4800
	2013/14	455		1750	1900	40			200		170		4515
Ellendale	2011/12					20			920		600		1540
	2012/13								300		1500		1800
	2013/14							1200			350		1550
Grapefruit	2011/12	800		1000	8150	10130	500	1300	20		1585	900	24385
	2012/13	1100		2000	54660	28120				1000	1790		88670
	2013/14			1400	32870	41600			49550	25	270		125715
Kumquat	2011/12	150		600	1800	2000		3000	500		2500		10550
	2012/13	500		3000	200	200		500	10		3300		7710
	2013/14	150		2000	3400	1400		3100	1000		1050		12100
Lemon	2011/12	202882	100	8000	191050	57740	100	106900	500		113730		681002
	2012/13	194460		7000	325780	18305		47700	41500	150	89955		724850
	2013/14	264768		3750	267800	31545		5200	28300	25	112147		713535
Lime	2011/12	2050			10700	5100	50	19500			10200		47600
	2012/13	5560		500	10200	2860		9000	550		8500		37170
	2013/14	20			2300	3460		0		25	6150		11955
Mandarin Hybrid	2011/12	155014		6000	270100	29796	7000	33600	63600		263879		828989
	2012/13	200752		500	344860	39395		26100	40700	1150	267390		920847
	2013/14	301217		7500	322663	53050		23900	83200	130	690458		1482118
Midseason	2011/12							350			385		735
	2012/13										9340		9340
	2013/14	315				40		700			5535		6590
Navel	2011/12	156259		9700	248000	80919	4500	55044	26075		63225		643722
	2012/13	171928		11500	95240	55660		18200	28540	2800	189833		573701

	2013/14	140764		1000	67600	15385		7100	13450	3100	127877		376276
Pummelo	2011/12					650					200		850
	2012/13				600				50		200		850
	2013/14				650					15			665
Rootstock	2011/12					20							20
	2012/13					80							80
Satsuma	2011/12	37968		6000	56000	4933		2000	2000		50795		159696
	2012/13	4700		12000	3500	1660					14410		36270
	2013/14	5635		5000	6400	195		900		1000	21306		40436
Valencia	2011/12	51602		3000	409222	68207	2000	21300	42550		37885	12000	647766
	2012/13	57023		4800	420390	78685		19100	31019	3000	57820		671837
	2013/14	37864		2000	428030	124967		4400	7400	3195	44748		652604
		2021088	100	100500	3669375	757915	14150	413694	479734	15665	2400475	13050	9885746

Totals:

2011/12	**3 165 602**
2012/13	**3 206 545**
2013/14	**3 513 599**

Table 6.1.3. Top 30 budwood cultivars supplied during the period: May to April 2012-2014.

Rank	2011/12					2012/13					2013/14				
	Cultivar	**BCIN**	**CFB**	**Total**	**%**	**Cultivar**	**BCIN**	**CFB**	**Total**	**%**	**Cultivar**	**BCIN**	**CFB**	**Total**	**%**
1	Eureka Lem.	238329	230800	469129	14.8%	Eureka Lem.	263185	287705	550890	17.2%	Nadorcott 1	405088	185909	590997	16.8%
2	Nadorcott 1 Man.	120618	145423	266041	8.4%	Or 4 Man.	194950	50800	245750	7.7%	Eureka	60750	372560	433310	12.3%
3	Midknight Val.		179801	179801	5.7%	Midknight Val.	35025	189672	224697	7.0%	Tango	305240	112468	417708	11.9%
4	Nova Man.		177628	177628	5.6%	Late Val.	71000	124790	195790	6.1%	Late	44000	167720	211720	6.0%
5	Chislett M7 Nav.	112849	58000	170849	5.4%	Chislett M7 Nav.	121404	45530	166934	5.2%	Midknight	0	170605	170605	4.9%
6	Late Val.	10000	144098	154098	4.9%	ARCCIT1614 (B17) (Valley Gold) Man.	116750	37000	153750	4.8%	ARCCIT1614 (B17) (Valley Gold)	65456	92607	158063	4.5%
7	Bahianinha Nav.		131020	131020	4.1%	Nadorcott 1 Man.	55932	95001	150933	4.7%	Limoneira 8A	53800	75466	129266	3.7%
8	Or 4 Man.	79120	43765	122885	3.9%	Nova Man.	2000	120959	122959	3.8%	Witkrans 3	39798	69740	109538	3.1%
9	Lisbon Lem.	17500	88418	105918	3.3%	Tango Man.	76359	45451	121810	3.8%	Star Ruby		107280	107280	3.1%
10	Benny 2 Val.		100772	100772	3.2%	Carninka Late Nav.	71605	25502	97107	3.0%	Lisbon	9000	93566	102566	2.9%
11	Sonet Sat.	39922	54148	94070	3.0%	Witkrans 3 Nav.	54610	41290	95900	3.0%	Nova		80157	80157	2.3%
12	Limoneira 8A Lem.	42000	50170	92170	2.9%	Lisbon Lem.	21000	70330	91330	2.8%	Or 4	32300	39515	71815	2.0%
13	Delta Val.		66935	66935	2.1%	Andes 1 - Clemenluz Clem.	79300	6250	85550	2.7%	Cambria 3	21800	38574	60374	1.7%
14	Washington Nav.		66356	66356	2.1%	Star Ruby Grapefruit		79760	79760	2.5%	Gusocora (G5)	12700	42565	55265	1.6%
15	Palmer Nav.		60720	60720	1.9%	Benny 2 Val.	8000	57571	65571	2.0%	Benny 2		50495	50495	1.4%
16	Nules Clem.	19450	39600	59050	1.9%	Gusocora (G5) Val.	31500	29282	60782	1.9%	Mor 26		48857	48857	1.4%
17	Miho Wase Sat.		55178	55178	1.7%	Cambria 3 Nav.	23558	32871	56429	1.8%	Delta		47589	47589	1.4%
18	Mor 26 Man.		54378	54378	1.7%	Cambria 4 (K-tak) Nav.	35263	17018	52281	1.6%	Alpha		47006	47006	1.3%
19	Cambria 3 Nav.	11510	39615	51125	1.6%	Limoneira 8A Lem.		44540	44540	1.4%	Nules		34984	34984	1.0%
20	Bearss Lime	3000	43950	46950	1.5%	Delta Val.		43042	43042	1.3%	Chislett M7		33881	33881	1.0%
21	Empress Man.	1000	45500	46500	1.5%	Genoa Lem.		37340	37340	1.2%	Empress		33320	33320	0.9%
22	ARCCIT1614 (B17) (Valley Gold) Man.	21950	23485	45435	1.4%	Bearss Lime		36770	36770	1.1%	Lane Late (Cal.)		30003	30003	0.9%
23	Gusocora (G5) Val.		39775	39775	1.3%	Mor 26 Man.		33870	33870	1.1%	Glenora Late		26303	26303	0.7%
24	Cambria 4 (K-tak) Nav.	13000	22700	35700	1.1%	Empress Man.	5500	26410	31910	1.0%	Carninka Late		25266	25266	0.7%
25	Witkrans 3 Nav.	23794	9475	33269	1.1%	Lavalle 2 Val.		22000	22000	0.7%	Nules (2)		24670	24670	0.7%
26	Tango Man.		23060	23060	0.7%	Nules Clem.		21500	21500	0.7%	2PH Eureka SL		23951	23951	0.7%
27	Midknight 1 (I15) Val.		19450	19450	0.6%	Bahianinha Nav.		20940	20940	0.7%	Or 4 (2)		23435	23435	0.7%
28	Star Ruby Grapefruit		19210	19210	0.6%	Miho Wase Sat.	1000	17770	18770	0.6%	Bahianinha		20375	20375	0.6%
29	Lavalle 2 Val.		18865	18865	0.6%	Washington Nav.	3000	15407	18407	0.6%	Genoa		20211	20211	0.6%
30	Carninka Late Nav.	8500	9215	17715	0.6%	Palmer Nav.		16270	16270	0.5%	Washington		16627	16627	0.5%
	Top 30	762542	2061510	2824052	89.2%	Top 30	1270941	1692641	2963582	92.4%	Top 30	1049932	2155705	3205637	91.2%
		27.00%	73.00%	100%			42.89%	57.11%	100%			32.75%	67.25%	100%	
	Total	794492	2371110	3165602	100.0%	Total	1300931	1905614	3206545	100.0%	Total	1085411	2428188	3513599	100.0%
		25.10%	74.90%	100%			40.57%	59.43%	100%			30.89%	69.11%	100%	

Table 6.2.1. Seed supplied during the period: May to April 2012-2014.

Area	2011/12	2012/13	2013/14		
			Order	Replacement	Total
South Africa	3069	2993	2861.50	1065	3853
Exported	575	372	173.00	17	190
Total	3644	3365	3034.50	1082	4043

Area	2011/12	2012/13	2013/14		
			Order	Replacement	Total
Eastern Cape	459	366.5	607	244	804
KwaZulu-Natal	14	14	10		10
Limpopo	1986	1703	1296	525	1793
Mpumalanga	45	11	71	6	77
North-West Province	52	118	16.50	12.75	28.9
Northern Cape	58	75	132	67	199
Western Cape	455	705.5	729	212	941
	3069	2993	2861.50	1065	3853

Area	2011/12	2012/13	2013/14		
			Order	Replacement	Total
Australia/NZ	135	72	33		33
Caribbean	8	0			0
Europe	72	100	80		80
Mozambique	3				0
Namibia	8				0
Other African States	148	176	19	17	36
South America	201	21	41		41
Zimbabwe		2.5			0
	575	372	173	17	190

Table 6.2.2. Most popular rootstocks in 2013-2014.

Cultivar	2011/12	%	2012/13	%	2013/14					%
					Ordered	Supplied	Out of Stock	Replace-ment	Credit or Returned	
C35	531	14.6%	412	12.2%	613.25	613.25		366		19.2%
CC	1507	41.4%	1554	46.2%	1283.25	1173.25		346	-16	40.2%
CCexAus		0.0%		0.0%		110.00		106		0.00%
CM	25	0.7%	5	0.2%	6.25	6.00		6		0.2%
FD	208	5.7%	227	6.8%	120.25	120.25		0		3.8%
MXT	81	2.2%	74	2.2%	62.25	57.25	5	41		2.0%
RLC	243	6.7%	363	10.8%	378.25	332.25		109	-2	11.9%
RLS	60	1.7%	50	1.5%		46.00		4		0.0%
RLVN	14	0.4%		0.0%		0.00		2		0.0%
SC	516	14.2%	435	12.9%	324.25	324.25		69	-10	10.2%
TC	146	4.0%	75	2.2%	40.25	40.25		4		1.3%
VA	64	1.8%	57	1.7%	63.25	63.25		12		2.0%
X639	234	6.4%	78	2.3%	279.25	129.25	150	0	-47	8.8%
YC	15	0.4%	36	1.1%	19.25	19.25		17		0.6%
Total	3644	100%	3365	100%	3189.75	3034.50	155	1082	-75	100%

Table 6.4.1. Trees certified during the period: April to March 2012-2014.

Variety	Eastern Cape	KwaZulu Natal	Limpopo	Mozambique	Mpumalanga	North West Province	Northern Cape	Swaziland	Western Cape	Total
Clementine			1800		2200				22047	26047
Grapefruit		1082			13744				500	15326
Lemon	20362		8245	2500	500	2000			16500	50107
Lime									500	500
Mandarin Hybrid	19423	2000	13450		32327	8200			112413	187813
Navel	57663		100		25005		5847	6100	3827	98542
Satsuma			53				400		3560	4013
Valencia	889	1820	11288		13030	3500			10200	40727
Total	98337	4902	34936	2500	86806	13700	6247	6100	169547	423075

2011/12	**1 911 378**	
2012/13	**989 094**	
2013/14	**423 075**	1 633 939
	3 323 547	

6.8 **Establish and maintain a virus-free gene source at CRI**
Project 790 by J.H.J. Breytenbach S.P. van Vuuren and G. Cook (CRI)

Opsomming

Groeipuntenting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is sewe nuwe seleksies ingedien vir GPE en ʼn verdere 49 seleksies van vorige introduksies is in verskeie fases voor vrystelling. Agt van laasgenoemde word reeds geïndekseer om virusvrye status te bevestig. Virusvrye materiaal word met ʼn toepaslike *"Citrus tristeza virus"* bron gepreïmuniseer voordat dit aan die Sitrus Grondvesblok (GVB) vrygestel word. Virusvrye boompies van verskillende cultivars en seleksies word as ʻn genebron in ʼn insekvrye tonnel by CRI bewaar. Sewe nuwe seleksies van 2013 is aan die GVB voorsien en die is by die CRI genebron gevoeg, wat tans uit 277 kultivars en seleksies bestaan. Oprigting van ʻn nuwe tonnel om die genebron te huisves is voltooi. Hierdie fasiliteit is deur SACNA en CRI befonds.

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 seven new selections were submitted for STG and a further 49 submissions from previous years are at various stages in the process before release. Eight of the latter are being indexed to ensure virus-free status. Virus-free material is pre-immunised with a suitable *Citrus tristeza virus* source before it is supplied to the Citrus Foundation Block (CFB). A virus-free gene source of citrus cultivars is maintained in an insect-free tunnel at CRI. Seven new cultivars and selections were supplied to the CFB in this report period and added to the CRI gene source, which now comprises 277 cultivars and selections. Erection of a new tunnel for housing the nucleus block is complete. This facility was funded by SACNA and CRI.

Introduction

The overall objective of the southern African Citrus Improvement Scheme (CIS) is to enhance the productivity of the industry by ensuring supply of the highest quality propagation material. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees and are responsible for stunting, decline, small fruit and a range of other harmful effects. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro *et al*., 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). Some cultivars and selections of the virus-free gene source maintained at the ARC-ITSC have been

duplicated in part at CRI Nelspruit as a back-up source. STG facilities at CRI are used to introduce new virus-free cultivars and selections which are added to the gene source after STG and indexing. Cross-protection for severe CTV infection is a function of the CIS and specific pre-immunising CTV sources are applied to all citrus varieties before supply to the CFB.

Objectives

Receive and introduce new cultivar selections.
Do STG of new editions and index for GTD to ensure that they are virus-free.
Maintain the virus-free gene source in an insect-free tunnel.
Pre-immunise selections with a suitable cross-protecting *Citrus tristeza virus* (CTV) source before budwood supply to the Citrus Foundation Block (CFB) at Uitenhage.

Materials and methods

In vitro cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige & Skoog, 1962). Germination takes place at a constant temperature of 28ºC in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4ºC in darkness.

Scion preparation: Method 1; buds of the source plant are budded on a standard rootstock in the glasshouse. After bud grown and maturation (approximately 3–4 months), the source plant is defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile distilled water. Method 2; bud sticks from the source plant are cut in 50 mm lengths and surface sterilised by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile distilled water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32ºC and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG: The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth tip, containing the leaf primordia, is placed on the horizontal cut of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28ºC exposed to 16 h light/day.

STG plant increase. The shoot tip starts growing 3 to 4 weeks after STG. The growing shoot tip is micro-grafted with the seedling rootstock onto a vigorous-growing virus-free rootstock in the glasshouse. After micro-grafting, the graft is closed by a plastic bag for 8 days. Once the graft has sufficiently grown, buds for indexing are taken from this material.
Virus indexing. Elimination of graft transmissible pathogens is established by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter confirmed with molecular diagnostic techniques. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) is used to detect viroids, CPsV and ASGV. PCR is used to detect the bacterial pathogen causing citrus greening. Virus-free plants are maintained in an insect-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage.

Results and discussion

Objective / Milestone	Achievement
• Receive and maintain new selections/cultivars.	Ongoing: 49 brought forward from previous year; 7 new selections received in current year.
• Do shoot tip grafting (STG) of new selections/cultivars and index for graft transmissible diseases, to ensure they are virus-free.	Ongoing: 254 STGs, 64 successful micro-grafts.
• Maintain the virus-free nucleus block in an insect-free tunnel.	Ongoing: currently 277 cultivars and selections.

• Establish a pre-immunised source of the new selection/cultivar with a suitable CTV cross-protection source and supply budwood to the CFB.	Ongoing: 7 additions supplied to CFB.
• Re-index the virus-free selection every three years.	Ongoing: Partly PCR'd for CVd, CPsV and Greening

STG:

The STG procedure was initiated at CRI in 2004 and the existing facilities completed in 2005. The introductions for STG and subsequent releases to the CFB from 2008 to date are summarised in Table 6.8.1. Seven new selections of three cultivar groups were submitted for STG in the current year and 49 brought forward from the previous year. During this report period a total of 254 STGs were done on these introductions. Of these, 64 were successfully micro-grafted.

Eight of the successful STGs have been indexed biologically while the remainder are duplicate ex-plants or micro-grafts which are still too small for indexing. Six of the eight successful STGs indexed negative for CTV, ASGV and CVd by biological indexing, and two tested positive for CVd (Table 6.8.2). Thirteen STG's were biologically indexed for CPsV and CID of which the results are still awaiting (Table 6.8.3). On average it takes 24 to 30 months to obtain a virus-free STG followed by the scheduled indexing to confirm the virus-free status of the cultivar. However, delays can occur with elimination of some pathogens. The reason for these "difficult to remove" cases is unknown.

To facilitate a faster turn-around with the STG process, new introductions are tested directly with PCR prior to STG to determine the viroid and CTV status as well as post STG, once enough material is available for testing. These additional steps help to identify the pathogens to be eliminated and allow quicker detection of pathogens not eliminated by an STG step. This shortens the time before the STG can be repeated if positive. This is quicker than the biological indexing used previously. This process does not replace the biological indexing and PCR done as a confirmation of pathogen free status prior to final release of the accession. These additional tests are routinely done and the number of tests conducted is not reported here.

Confirmation of biological indexing by PCR on a number of STG submissions prior to final release is reflected in Table 6.8.4. Seven STG submissions free of CTV, CVd, and ASGV were pre-immunised successfully and bud wood was supplied to the CFB.

Table 6.8.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Cultivar Variety Group[2]	STG introductions and releases 2008 to 2012[1]															
	2009			2010			2011			2012			2013			
	Bf from 2006	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Balance
C	2	0	2	0	1	0	1	0	0	1	5	1	5	1	0	6
G	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
L	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mi	5	0	4	1	0	2	1	0	0	1	0	0	1	0	0	1
Ma	1	1	0	2	3	2	3	0	2	1	0	1	0	4	0	4
N	20**	6	7	19	6	3	22	11	4	29	10	5	34	2	4	32
R	1***	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
V	7***	2	1	6	0	0	6	2	2	6	2	2	6	0	2	4
Or	2	0	0	2	0	1	1	0	0	1	0	0	1	0	0	1
Rs	1	0	0	1	0	1	0	1	0	1	1	0	2	0	1	1
Total	**41**	**9**	**15**	**32**	**10**	**10**	**34**	**14**	**8**	**40**	**18**	**9**	**49**	**7**	**7**	**49**

[1] Bf = Brought forward from previous year; Balance = Balance for the current reporting year.
[2] Cultivar/variety group: C = Clementine; G = Grapefruit; L = Lemon; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.
* Seven navel selections withdrawn by owner.
** Release of 1 navel selection withdrawn by client.
*** One Reticulata, 1 navel and 2 Valencia selections withdrawn by owners.

Table 6.8.2. STG submissions indexed biologically for CTV, ASGV and CVd.

Variety Group	Number of plants	Negative	Positive
Navel	4	3	1
Midseason	1	1	-
Mandarin	-	-	-
Valencia	2	1	1
Clementine	-	-	-
Grapefruit	-	-	-
Lemon	-	-	-
Ornamental citrus	-	-	-
Rootstock	1	1	-
Total	**8**	**6**	**2**

Table 6.8.3. STG submissions indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive
Navel	9	awaiting results	
Midseason	-	awaiting results	
Valencia	1	awaiting results	
Reticulata	-	awaiting results	
Mandarin	-	awaiting results	
Grapefruit	-	awaiting results	
Clementine	-	awaiting results	
Lemon	-	awaiting results	
Ornamental	1	awaiting results	
Rootstock	2	awaiting results	
Total	**13**		

Table 6.8.4. STG plants indexed by PCR for CVd, ASGV and CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	23	5	-	11
Midseason	2	-	-	-
Valencia	4	-	-	1
Reticulata	-	-	-	-
Mandarin	1	-	-	1
Grapefruit	-	-	-	-
Clementine	4	-	-	1
Lemon	1	-	-	-
Ornamental	-	-	-	1
Rootstock	2	2	-	2
Total	**37**	**7**	**0**	**17**

Maintaining the virus-free gene source:
The number of selections maintained at CRI is listed per cultivar/variety group in Table 6.8.5. Seven new additions were made to the gene source this reporting year (Table 6.8.1). Two trees of each selection are maintained in the gene source and trees have to be re-budded to new rootstocks every five years as part of the routine maintenance. Tree in the nucleus block are allowed to fruit to support identification and trueness-to-type evaluation.

Erection of a new tunnel for housing the nucleus block was completed. This facility was funded by SACNA and CRI. The transfer of the gene source to the new tunnel was finalised by end June 2013.

Table 6.8.5. The number of accessions per cultivar/variety group maintained at the CRI nucleus block.

Variety Group	No. of selections maintained at CRI
Clementine	24
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	18

Kumquat	1
Lemon	20
Lime	4
Mandarin	5
Midseason	27
Navel	59
Ornamnetal	4
Pummelo	7
Reticulata	33
Rootstock	22
Satsuma	8
Valencia	47
Total	**277**

Conclusion

- Successful elimination of GTDs from new selections was achieved. On average it takes 30 months for the entire process from STG to final release (the quickest being 21 months) although some selections proved to be problematic and still remain infected despite repeated STG.
- Seven new selections were added to the gene source and also released to the CFB.
- Seven new selections were received this year for elimination of GTD and are in the STG process.

Technology transfer

None.

Further objectives and work plan

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2014 and Jan-Mar 2015

- Receive material
- Bud to virus-free rootstocks and maintain at high temperature
- Prepare liquid and solid Murashige & Skoog culture mediums
- Prepare and plant seed in culture tubes with solid medium
- Germinate seed in darkness
- Store rootstocks at 4°C
- Prepare rootstocks under stereo microscope under aseptic conditions
- Collect new shoots from source maintained at high temperature
- Prepare etiolated rootstock from culture tube
- Under the stereo microscope, cut and place shoot tip on rootstock
- Put the rootstock with shoot tip into a culture tube with liquid medium
- Keep tubes in growth room (do weekly trimmings of rootstock suckers)
- Graft shoot tip with rootstock on virus-free rootstocks in the glasshouse
- Let shoot tip grow for indexing
- Index for graft transmissible agents
- Pre-immunise rootstock with suitable cross protector
- Bud virus-free shoot tip grafted material to pre-immunised rootstock
- Do ELISA to confirm pre-immunisation
- Multiply pre-immunised budwood on virus-free rootstocks
- Supply budwood to Citrus Foundation block
- Maintain virus-free material in nucleus block

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6.9 Diagnostic services for graft transmissible diseases: October to December 2013

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 verpreiding van gesonde voortplantingsmateriaal. Biologiese en molekulêre indeksering word gedoen op nuwe toevoegings tot die SVS voordat die material aan die Grondvesblok verskaf word, asook her-indeksering van moederbome wat by die Grondvesblok gehuisves word. Daar word hier verslag gelewer op die voortdurende diagnostiese aktiwiteite van die SVS. Die moederbome by die Grondvesblok word op ‘n rotasie basis elke tweede jaar ge-herindekseer om te bepaal of enige strawwe CTV rasse of enige sitrus viroïede in die moedermateriaal voorkom. Die biologiese evaluasie van die CTV virulensie in 177 moederbome is gedurende die jaar voltooi waarna ‘n verdere 176 geïndekseer word waarvan die resultate nog afwagtend is. Viroied indeksering van 194 moederbome is ook begin waarvan die resultate ook nog afwagtend is. Huidige prosedures word voortdurend aangepas en verbeter. Die groeipunt-entingsproses is ge-evalueer en die nodige aanpassings gedoen na ‘n besoek van wêreldkundiges tydens die IOCV kongres wat Suid Afrika in Augustus 2013 aangebied het.
Algemene diagnostiese dienste en ondersoeke na probleme t.o.v ent-oordraagbare siektes in die industrie word op ‘n *ad hoc* basis gedoen en verslag word ook in hierdie projek gelewer.

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 CFB as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents are present or have been inadvertently introduced. These ongoing diagnostic activities of the CIS 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 177 mother trees was completed and indexing of the remaining 176 mother trees have been initiated and await evaluation. Viroid indexing of 194 mother trees was initiated and analysis is pending. Current test protocols are updated and improved on an ongoing basis. Our STG process was evaluated and relevant adjustments were made to the existing processes following the inputs of visiting experts during the hosting of the IOCV in South Africa in August 2013. General diagnostics and investigations into *ad hoc* industry problems and concerns relating to graft transmissible diseases are also reported within this project.

Introduction

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and, in some cases, unidentified pathogens. The GTDs affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and is used in South Africa since 1977 (de Lange *et al*., 1981).

Indexing, or establishing whether GTD disease agents are present in plant material, is done mostly by means of biological indicator plants. A range of virus-free plants are propagated in the glasshouse, each of which is used for detection of a specific graft transmissible pathogen. Previously only biological indexing was used for the detection of GTDs following STG, but this is now supplemented with molecular based

techniques, which target regions of the pathogen's nucleic acid and are used to specifically identify the pathogen. These techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR), PCR and dot-blot have an enhanced sensitivity compared to symptom expression on indicators.

Since *Citrus tristeza virus* (CTV) and its vector, *Toxoptera citricida*, is endemic in South Africa, virus-free material should be protected by pre-immunisation with a suitable cross-protection source (Müller & Costa, 1987). Currently three CTV sources are used for cross-protection in the southern African Citrus Improvement Scheme (CIS) depending on the scion material to be protected (von Broembsen & Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000). ELISA is used to confirm pre-immunisation with CTV (Roistacher, 1991). The STG and pre-immunisation procedures have been improved to suite South African conditions (Fourie & van Vuuren, 1993). Re-indexing of the mother trees, maintained at the Citrus Foundation Block (CFB), is done to ensure these trees remain free of graft transmissible pathogens and that the pre-immunising CTV remains mild within these cultivars. CTV severity indexing is done on an annual basis, indexing for Citrus viroids (CVd) is done biennially and other GTD are indexed every 10 years.

Indexing for GTD is also done to support growers where field problems are experienced and is necessary to ensure appropriate recommendations. Bud wood sent in by growers or collected during field visits, are budded to indicator plants and kept in the glasshouse at optimum temperatures according to the requirements for disease detection.

Objectives

1. Biological and molecular indexing of material that went through STG.
2. Biological and molecular re-indexing of mother trees at the CFB.
3. Requests from growers and institutions to index suspected material for graft GTD.
4. *Ad*-hoc indexing as required

Materials and methods

Specific virus-free indicator plants are propagated from seed or clonally from virus-free material for the detection of the various GTDs. These are maintained in an insect-free glasshouse and kept in stock until needed. When budwood for indexing is received, two buds are budded on each of three indicator seedlings for each disease. For CPsV indexing 4 buds are used to inoculate each of 3 indicator plants. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Known positive and negative control samples are included. A minimum indexing time of 6 months is required for CTV, CVd, *Apple stem grooving virus* (tatter leaf) (ASGV) and greening, while 12 months are required for *Citrus psorosis virus* (CPsV) and Citrus Impietratura Disease (CID) indexing.

Field material is usually not suitable for the serological or molecular techniques (PCR, s-PAGE, DOT blots etc.), since the organisms are usually present in low concentrations or are poorly distributed, and therefore false negative results may be obtained. Field material is inoculated on suitable indicator plants, maintained at optimal temperatures in the glasshouse and then tested at least 3 months after inoculation for specific pathogens. Molecular results are regarded as a confirmation of the biological result.

Results and discussion

Objective / Milestone	Achievement
• Biological and molecular indexing of STG plants for CTV, CVd, ASGV, CPsV and CID.	Achieved and ongoing.
• Annual biological and molecular indexing of the CFB mother trees (every year for CTV severity; every third year for the presence of CVd; every 10 years for the presence of CPsV and ASGV).	Achieved and ongoing.
• Indexing samples send in by growers and institutions using ELISA, PCR and biological indicators.	General samples were received for indexing for possible ASGV, CPsV, CVd and CTV infection. Analyses communicated to clients

1. STG material

After the STG process, citrus cultivars undergo initial biological indexing for CTV, ASGV and CVd (Table 6.9.1). Once indexed negative for CTV, ASGV and CVd, a process which takes 6 months, the source is pre-immunised with a suitable CTV cross-protection source. After confirmation of positive pre-immunisation, budwood is supplied to the CFB to establish mother trees. The cultivar is then also introduced into the nucleus block (Table 6.9.4). Following interim release to the CFB, plants are further biologically indexed for CPsV and CID (Table 6.9.3). The presence of greening is continuously monitored since all the cultivars and selections, except the trifoliate types, are self-indexing with symptoms clearly visible if the pathogen is present, but PCR confirmation is done prior to final release to the CFB. Thirty-seven cultivars, further in the release process, were tested by PCR for the presence of CVd and seven for the presence of ASGV and 17 for Greening. All indexed negative for these pathogens (Table 6.9.2).

Table 6.9.1. Status of STG plants indexed biologically for CTV, ASGV and CVd[1].

Cultivar	Number of plants	CTV			ASGV			CVDs		
		+	-	±	+	-	±	+	-	±
Navel	10	-	4	6	-	4	6	1	-	-6
Midseason	1	-	1	-	-	1	-	-	1	-
Mandarin	-	-			-		-	-	-	-
Valencia	4	-	2	2	-	2	2	1	1	2
Clementine	1	-	-	1	-	-	1	-	-	1
Lemon	-	-	-	-	-	-	-	-	-	-
Grapefruit	-	-	-	-	-	-	-	-	-	-
Ornamental citrus	-	-	-	-	-	-	-	-	-	-
Rootstock	2	-	1	1	-	1	1	-	1	1

[1] + = positive; ± = awaiting final results; - = negative.

Table 6.9.2. STG plants tested by PCR for CVd, ASGV, CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	23	5	-	11
Midseason	2	-	-	-
Valencia	4	-	-	1
Reticulata	-	-	-	-
Mandarin	1	-	-	1
Grapefruit	-	-	-	-
Clementine	4	-	-	1
Lemon	1	-	-	-
Ornamental	-	-	-	1
Rootstock	2	2	-	2
Total	**37**	**7**	**0**	**17**

Table 6.9.3. STG plants indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive
Navel	9	awaiting results	-
Midseason	-	awaiting results	-
Valencia	1	awaiting results	-
Reticulata	-	awaiting results	-
Mandarin	-	awaiting results	-
Grapefruit	-	awaiting results	-
Clementine	-	awaiting results	-
Lemon	-	awaiting results	-
Ornamental	1	awaiting results	-
Rootstock	2	awaiting results	-
Total	**13**		

Table 6.9.4. Pre-immunisation status and new cultivar additions to the gene source and Foundation Block.

Cultivars	Number of plants pre-immunised	Pre-immunisation to be confirmed	Pre-immunisation confirmed by ELISA	Supplied to the CFB and additions to Nucleus Block
Navel	9	5	4	4
Midseason	-	-	-	-
Valencia	4	2	2	2
Reticulata	-	-	-	-
Mandarin	-	-	-	-
Grapefruit	-	-	-	-
Clementine	1	1	-	-
Lemon	-	-	-	-
Ornamental	-	-	-	-
Rootstock	-	-	-	1
Total	**14**	**8**	**6**	**7**

2. Re-indexing of mother trees at the CFB

The CTV severity status of 177 mother trees was indexed as part of the re-indexing program. Mother trees indexed are presented in Table 6.9.5. The Mexican lime indicators were inspected for growth, the presence of mild to severe stem pitting and vein clearing six months after inoculation. Thirty-nine mother trees tested negative for CTV after inspection and CTV absence was confirmed by ELISA. The presence of severe strains of CTV was indicated for 2 mother trees. A further 176 mother trees are being indexed for CTV severity and await final analysis (Table 6.9.6). Additionally, viroid indexing of 194 mother trees was initiated (Table 6.9.7).

Table 6.9.5. Mother trees at the CFB indexed for CTV severity.

Cultivar	Number of mother trees	Number of trees with severe SP*	Number of trees with mild CTV	Number of trees negative for CTV
Nadorcott 1	6	0	6	0
Mor 26	6	0	4	2
2 PH LS Murcott	6	0	0	6
Cami	2	2	0	0
Haddas	3	0	3	0
Irradiated I22	3	0	0	3
Murcott x Clem	3	0	0	3
Nova	3	0	3	0
Valley Gold	6	0	0	6
Shasta Gold	2	0	0	2
Yosemite Gold	2	0	0	2
Tahoe Gold	2	0	0	2
African Sunset	3	0	3	0
Ortanique Tangor	3	0	3	0
B17 (I-12)	3	0	2	1
Or 4	1	0	0	1
Total Mandarins	**54**	**2**	**24**	**28**
None	0	0	0	0
Total Midseasons	**0**	**0**	**0**	**0**
Kirkwood Red	3	0	3	0
Newhall	3	0	3	0
Chislett M7	6	0	3	3
Cambria 3	4	0	3	1
Carninka Late	5	0	1	4
Bahianinha	6	0	6	0
Navelina	6	0	6	0
Palmer	7	0	7	0
Autumn Gold	3	0	3	0
Barnfield Summer	3	0	3	0
Chislett Summer	3	0	3	0
Powell Summer	3	0	3	0

Summer Gold	3	0	3	0
Dream	3	0	3	0
Cambria 4	3	0	3	1
Washington	3	0	3	0
Witkrans 3	6	0	6	0
Total Navels	**68**	**0**	**59**	**9**
Turkey	6	0	6	0
Lavelle 2	3	0	3	0
McClean SL	5	0	5	0
Total Valencias	**14**	**0**	**14**	**0**
Sonet	6	0	4	2
Kuno	4	0	4	0
Total Satsumas	**10**	**0**	**8**	**2**
Mandared C1739	3	0	3	0
Esbal	3	0	3	0
Total Clementines	**6**	**0**	**6**	**0**
Flamingo (H17)	5	0	5	0
Star Ruby	4	0	4	0
Nelruby	3	0	3	0
Marsh	5	0	5	0
Rose	3	0	3	0
Total Grapefruit	**20**	**0**	**20**	**0**
Eureka	2	0	2	0
Total Lemons	**2**	**0**	**2**	**0**
	0	0	2	0
Total Limes	**0**	**0**	**0**	**0**
Nagami	3	0	3	0
Total Kumquats	**3**	**0**	**3**	**0**
Grand Total	**177**	**2**	**136**	**39**

Table 6.9.6. Mother trees at the CFB indexed for CTV severity.

Cultivar	Number of mother trees	Number negative	Number positive
Nadorcott SL	3		Analysis pending
Empress	3		Analysis pending
Mandalate	3		Analysis pending
Nova mutant	3		Analysis pending
Tango	4		Analysis pending
Shani SL	3		Analysis pending
Sweet Spring	3		Analysis pending
Clara	3		Analysis pending
Gold Nugget	3		Analysis pending
Tacle	3		Analysis pending
Total Mandarins	**31**		
Clarke	3		Analysis pending
Lane Late California	6		Analysis pending
Fukumoto	6		Analysis pending
Glen Ora Late	3		Analysis pending
Lina	6		Analysis pending
Fischer	3		Analysis pending
Cara Cara	6		Analysis pending
Gerhard (Letaba Early)	4		Analysis pending
Witkrans 3	6		Analysis pending
Total Navels	**43**		
Delta	4		Analysis pending
Late	4		Analysis pending
McClean	4		Analysis pending
Midknight	4		Analysis pending
G5	4		Analysis pending
Bennie 2	6		Analysis pending
Alpha	5		Analysis pending

Midknight 1	1		Analysis pending
Ruby	3		Analysis pending
Total Valencias	**35**		
Miho Wase	6		Analysis pending
Owari	3		Analysis pending
Bela	5		Analysis pending
Total Satsumas	**14**		
Basol	3		Analysis pending
Nules	6		Analysis pending
SRA 63	3		Analysis pending
Total Clementines	**12**		
Nartia	6		Analysis pending
Total Grapefruit	**6**		
2PH Eureka	3		Analysis pending
Genoa	3		Analysis pending
Eureka SL	6		Analysis pending
Limoneira	6		Analysis pending
Lisbon	5		Analysis pending
Eureka	4		Analysis pending
Lemox (Triploid)	3		Analysis pending
Total Lemons	**30**		
Bears	5		Analysis pending
Total Limes	**5**		
Grand Total	**176**		

Table 6.9.7. Mother trees at the CFB indexed for CVd.

Cultivar	**Number of mother trees**	**Number negative**	**Number positive**
Nadorcott SL	3		Analysis pending
Empress	3		Analysis pending
Mandalate	3		Analysis pending
Nova mutant	3		Analysis pending
Tango	4		Analysis pending
Shani SL	3		Analysis pending
Sweet Spring	3		Analysis pending
Clara	3		Analysis pending
Gold Nugget	3		Analysis pending
Tacle	3		Analysis pending
Total Mandarins	**31**		
Clarke	3		Analysis pending
Lane Late California	6		Analysis pending
Fukumoto	6		Analysis pending
Glen Ora Late	3		Analysis pending
Lina	6		Analysis pending
Fischer	3		Analysis pending
Cara Cara	6		Analysis pending
Gerhard (Letaba Early)	4		Analysis pending
Witkrans 3	6		Analysis pending
Gloudie (Nuwe lande)	3		Analysis pending
Fukumoto 2	3		Analysis pending
Total Navels	**49**		
Delta	4		Analysis pending
Late	4		Analysis pending
McClean	4		Analysis pending
Midknight	4		Analysis pending
G5	4		Analysis pending
Bennie 2	6		Analysis pending
Alpha	5		Analysis pending

Midknight 1 Ruby Louisa Jassie Henrietta Premier	1 3 3 3 3 3		Analysis pending Analysis pending Analysis pending Analysis pending Analysis pending Analysis pending
Total Valencias	**47**		
Miho Wase Owari Bela	6 3 5		Analysis pending Analysis pending Analysis pending
Total Satsumas	**14**		
Basol Nules SRA 63	3 6 3		Analysis pending Analysis pending Analysis pending
Total Clementines	**12**		
Nartia	6		Analysis pending
Total Grapefruit	**6**		
2PH Eureka Genoa Eureka SL Limoneira Lisbon Eureka Lemox (Triploid)	3 3 6 6 5 4 3		Analysis pending Analysis pending Analysis pending Analysis pending Analysis pending Analysis pending Analysis pending
Total Lemons	**30**		
Bears	5		Analysis pending
Total Limes	**5**		
Grand Total	**194**		

3. ITSC-ARC and CRI collaborative work

Shoot tip grafting for the CIS is done at both the CRI and ARC-ITSC laboratories. To confirm the pathogen free status of new accessions prior to release to the CFB, a duplicate testing arrangement has been instituted between the two laboratories. Duplicate molecular testing done in this report period is summarized in Table 6.9.8.

Table 6.9.8. Numbers of new accessions subjected to duplicate testing for the various pathogens.

Pathogen	ARC-ITSC accessions	CRI accessions
CVds	24	15
CTV	28	5
CPsV	14	
Greening Disease	14	

4. Test optimization for direct testing of CFB multiplication blocks for viroids.

In order to use multiplication blocks for longer than 36 months, trees have to be tested to ensure viroid freedom. A sampling and testing protocol was required which is sensitive, but also enables pooling of samples from the various multiplication blocks to make the screening manageable. This optimization was done by the ARC-ITSC to determine the maximum number of samples that can be pooled and to determine which extraction protocols are best suited for a large screening.

5. *Ad*-hoc indexing

5.1. General indexing for growers

Citrus material submitted by growers or collected during visits, are indexed for specific diseases (Table 6.9.9). Once the results are available, they are communicated to the submitting parties.

Table 6.9.9. Indexing of material sent in by growers or collected during visits.

Disease	No of samples	Results
CTV	5	Communicate to clients
CVd	4	Communicate to clients
CPsV	9	Communicate to clients
ASGV	3	Communicate to clients

5.2. Nova ARC investigation:

The CRI virologists visited a Nova ARC planting in Marble Hall where severe gumming on Nova ARC trees was observed. Samples were analysed for the presence of viroids and *Citrus tristeza virus*. The samples tested negative for these pathogens. The absence of CTV was not unexpected as erratic transmission of the CTV pre-immunisation sources on soft-citrus was observed previously and is currently being investigated. There were thus no indications of viral pathogens and the symptoms seen were not typical of any citrus virus pathogen described to date.

A stress trial to evaluate symptom expression under specific stress conditions was initiated. The trial was completed this year and a final report was submitted to the relevant parties. The report is given below.

FINAL REPORT: Nova ARC stress trial

Introduction

'Nova ARC' mandarin was developed by irradiating 'Nova' mandarin with the aim to reduce the seediness of 'Nova' mandarin. A large number of buds of 'Nova ARC' were supplied by the Citrus Foundation Block (CFB) to nurseries and the first trees were planted in 2008. About three years later, die-back and excessive gum formation on the stems of the trees were reported. The reports came from different production areas *viz*. Citrusdal, Marble Hall and Ohrigstad. Tests for virus and viroid pathogens were negative.

During the annual evaluation of mother trees at the CFB, it was observed that the 'Nova ARC' mandarin mother trees also displayed severe gumming and dieback. Subsequent inspections of the multiplication block revealed that most of the trees were free of gumming or die-back, however, patches of trees showed gum formation. The water supply for irrigation of these trees was all from the same point of exit on the main irrigation line, indicating that either excessive water or a deficit of water was involved at a stage. An experiment was initiated to investigate which of the two extremes might have been involved.

Aim
To investigate if excessive or deficit water stress will induce leaf drop, gum formation and die-back.

Procedures
Buds of 'Nova ARC' mandarin from the virus-free nucleus source at CRI and from healthy looking 'Nova ARC' mandarin multiplication trees at the CFB were budded to healthy Carrizo citrange rootstocks in the glasshouse. The potting mixture for the rootstocks was a decomposed bark and top soil (3:1) mixture which drain well. Four pots with three plants each were budded for each bud wood source and the buds allowed to grow.

When the first growth cycle of the scions has hardened off, two pots of each bud wood source were transferred to rooms at 24-26°C and 30-32°C respectively. One pot at each temperature range of each budwood source was stressed by reducing watering to once a week and the other one got normal watering (± twice/week). Hereafter the plants were monitored regularly for abnormal symptoms.

Results
After six weeks the water stressed plants of both bud wood sources, at both temperature regimes, showed severe drought symptoms and the watering was changed to that of the control plants. Two weeks later the leaves of trees which received the reduced watering dropped. Gum started to ooze through the bark of trees from both bud wood sources but only at the high temperature (Fig. 6.9.1). Die-back also started near buds (usually beneath the bud) and at the shoot tips only at the high temperature (Fig. 6.9.2). Gumming and die-back did not occur on any plants at the low temperature or any of those that received normal watering. Following on the normal watering procedure, new shoots developed on all the stressed plants and the trees recovered.

Conclusion

The experiment showed that stress of water deficit and high temperature can cause gumming and die-back of 'Nova ARC' mandarin trees. High temperature with sufficient irrigation did not cause any die-back or gumming. After rectification of the drought stress the trees with gumming and die-back recovered. Stress factors or combinations thereof that may cause gumming and die-back of 'NovaARC' mandarin are drought, cold, hail, chemical applications and micro-element deficiencies/excesses.

Figure 6.9.1. Gum oozing through the bark of a 'Nova ARC' mandarin twig after water stress and at a high temperature.

Figure 6.9.2. Die-back starting below the buds of a 'Nova ARC' mandarin after water stress at a high temperature. Note the gumming at the die-back areas.

5.3. Glen Ora Late Investigation:

Claims have been made within the South African industry of improved fruit quality and yields with certain citrus varieties infected with viroids (pre-scheme material) and that the viroid-free material supplied by the Citrus Improvement Scheme (CIS) is not equivalent in these regards. Researchers at Citrus Research International have started to investigate these claims to see what data are available to substantiate this and have also initiated research trials to scientifically investigate this issue.

Glen Ora Late navel is a late bearing navel and yield concerns were expressed in certain commercial settings for trees made from CIS material. The initial evaluation of this cultivar was done on pre-scheme daughter trees established on Rough lemon in Burgersfort in the Limpopo province as a semi-commercial trial. The pre-scheme material contains CVd-III and HSV (a non-cachexia variant), in addition to a mixture of *Citrus tristeza virus* (CTV) strains. The material supplied from the CIS is viroid-free and is pre-immunized with a specific CTV source. The only other semi-commercial Glen Ora Late orchard established on Rough lemon in South Africa is found in the Sunday's River Valley in the Eastern Cape. As there is no single site available where both the viroid-free and viroid-infected material are planted on the same rootstock, a clear comparative analysis of the two sources could not be made due to the different locations. However, data is presented from these two sites to compare fruit bearing potential over time (Table 6.9.10). Data were supplied by the two producers at the different sites. Fruit size and internal fruit quality data obtained from the two sites was similar. Both producers employed fruit set manipulation treatments, but as these, as well as climate, soil and other factors could influence yield, we present the yield data relative to production in year 9 at the same site. The Sunday's River trees produced heavier yields, which can be attributed to many other factors. With regards the early-bearing potential, it was not evident from this comparison of Glen Ora Late on Rough lemon that the viroid-free trees came into bearing any later than the viroid-infected trees. Similarly, when the Glen Ora Late yields in Sunday's River Valley were compared with those of other viroid-free late navel cultivars on the same rootstock over a 9-year period, delayed production of the viroid-free Glen Ora Late was also not evident.

Table 6.9.10. Production relative to yield in year 9 for the two production sites of Glen Ora Late navel on Rough lemon rootstock. Plant material at the Burgersfort site was derived from the original source and is viroid infected (CDVd and HSV). The Sunday's River Valley plant material was supplied by the CIS and is viroid-free.

	Production relative to yield in year 9 (%)					
Tree age	**4**	**5**	**6**	**7**	**8**	**9**
Burgersfort	14%	46%	59%	81%	69%	91 kg/tree
Sunday's River Valley	16%	39%	51%	93%	112%	124.3 kg/tree

5.4. Viroid field trial

In the Citrus Improvement Scheme (CIS) all graft transmissible agents, including CVd, are removed from all cultivars by shoot tip grafting (STG) but then re-inoculated with a selected *Citrus tristeza virus* (CTV) source for cross protection since this virus is endemic due to the abundance of aphids. Recently claims were made by cultivar owners and agents that the removal of CVd from some cultivars by STG, changes the horticultural characteristics. These characteristics include productivity, fruit colour and size and it was requested that CVd infected material be maintained and supplied by the CIS. No empirical evidence support these claims and it is necessary to evaluate these cultivars with and without CVd to substantiate the approach of the CIS. Project 1074 was registered to compare STG and old clone material. Indexing to determine the CTV and CVd status of various sources of three cultivars to be used in the project was done. The procedures and results are given in the Annual Report of Project 1074.

5.5. Delta Valencia

Biological and molecular indexing was conducted on a field sample submitted from the Limpopo province displaying psorosis-like fruit symptoms. The sample indexed negative for CPsV with PCR, but oak-leaf symptoms developed on the biological indicator indicating a probable viral-like disease. Similar symptoms were reported on the same cultivar at a different location. No pathogen has been identified yet. A source obtained from the submitted sample is being maintained and further analysis will be done if the problem persists in the next season.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy bud wood to the industry and is the primary objective of this project. Additionally, diagnostic services were provided and analysis of industry problems and concerns relating to graft transmissible diseases were addressed.

Technology transfer

Cook, G., Joubert, J., van Vuuren, S.P. & Fourie, P.H. 2014.Citrus Viroids. Friend or Foe? *S.A. Fruit Journal*. 13(1). 80-84.

Further objectives and work plan

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2014 and Jan-Mar 2015

- Propagate virus-free indicator plants, for the different graft transmissible diseases.
- Bud any bud wood send in, to these indicator plants.
- Keep plants in the glasshouse according to the disease diagnostic requirements.
- Use ELISA and PCR for detection of the presence of a pathogen.
- Discussion of results to the party involved.
- Improvement and validation of diagnostic techniques and development of new techniques for indexing for the various graft transmissible diseases (on-going)
- Source virus-free seed of various herbaceous plants for a host range study. Determine optimum growing conditions and maintain seedlings, mechanically inoculate various "psorosis"-sources. Submit certain samples for electron microscopic examination.
- Perform double-stranded RNA isolations and poly-acrylamide gel electrophoresis (PAGE).

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6.10 Citrus Biosecurity activities

Feedback from the biosecurity activities that are funded / coordinated from CIS in support of DAFF are briefly discussed here.

African Citrus Greening surveys

Western Cape

- **Knysna magisterial district:** As a follow up to previous years, orchards on the farm Candlewood were surveyed and six samples were collected. At the farm Portland Manor, neighboring farm five samples were collected from a neglected orchard. The results were negative for both sites; however, the Candlewood grower was removing the old Clementine orchards.

- **Malmesbury magisterial district:** Five positive trees were found in a farm in the Malmesbury district; destruction orders were issued and trees removed. Delimiting surveys will be conduicted in 2014.
- **Piketberg magisterial district:** In the Piketberg area five farms and seven home gardens were surveyed. No psylla was found but six samples were taken from trees with discoloured leaves on farms; these tested negative.

Eastern Cape

- **Fort Beaufort magisterial district:** A survey was conducted; all samples collected tested negative.
- Except for two magisterial districts in Transkei, the Eastern Cape is still Greening-free. To ensure its freedom the regulated Control Measures (R.110) under the Agricultural Pest Act was amended to include buffer zones protecting the Eastern Cape from the Western Cape and KZN where African Greening does occur. These buffer zones include the greening-free Knysna and Uniondale districts, and the Alfred Nzo and Oliver Tambo districts south of the KZN border, where greening is under eradication. These buffer zones will be regarded as greening-free and more intensive surveys will be conducted to ensure its sustained pest freedom.
- An provincial working group will be established between DAFF, the Eastern Cape department of agriculture, CGA and CRI to drive the required awareness campaigns, delimiting surveys and eradication in Transkei.

Asiatic Citrus Greening (HLB) and Asian Citrus Psyllid (ACP) surveys

A national HLB/ACP action plan was developed by CRI and approved by DAFF. Important actions are contingency surveys and awareness programmes. These are coordinated between DAFF, CGA and CRI at bi-annual Greening Stakeholder meetings. Given the seriousness of this risk to the southern African citrus growers, it has to be acknowledged that not enough resources are being allocated to these actions.

KwaZulu-Natal

- **Durban:** The survey in KZN focussed on Asiatic Citrus Greening (HLB) and its vector, *Diaphorina citri*. In order to improve awareness and increase the scope and effectiveness of the survey, schools were targeted and learners requested to brings samples from home. 106 samples were collected; all tested negative for HLB, except 26 positive for African Greening. Yellow sticky traps were also placed in the different areas around Durban and in the botanical garden to determine the possible presence of *Diaphorina*; only the African triozid, *Tryoza erytreae* was caught on the traps.

Citrus biosecurity in Africa

Angola

In the recent past, CRI became aware that citrus nursery trees were being imported from Brazil. Forunately, these trees originated from excellent nurseries in Sao Paulo state, which has a mandatory citrus improvement scheme. This reduced the risk of inadvertent import of exotic diseases; the practice is nonetheless risky, especially considering *Citrus leprosis virus* that is vectored by the *Brevipalpus* mite. New evidence has emerged that Brazilian trees were imported into Angola from Minas Gerais province. As this Brazilian state does not have a formal citrus improvement scheme, these imports are extremely risky as many of the feared citrus diseases that occur in Brazil, and are exotic to Africa, could have been exported with these consignments.

CRI invited a high-level Angolan ministry of agriculture (MINADERP) delegation to SA-CIS and CRI facilities. The delegation consisted out of Dr Adelino Rodrigues (Director: Plants and Livestock), Julio José Nascimento (Head of Department: Plant Protection) and Sidonio Mateus (Head of Department: Agriculture). They visited CFB, a certified nursery, packhouse, CRI facilities in Nelspruit and orchards. A meeting was held with SA-DAFF [Alice Baxter, Mashudu Silimela, Salomina (Inspection Services), Kgabo Matlala (National contact point) and Mike Holtzhausen]. The outcomes are briefly summarized below:

- MINADERP acknowledged the risk of propagation material import and indicated that they will immediately cease to authorize imports from Brazil
- MINADERP was encouraged to rather buy trees from CIS certified nurseries in South Africa. Feedback from nurseries indicates trade in citrus trees with Angola and other African countries.
- MINADERP will liase with DAFF to get information to get their import conditions in line with those of RSA
- This matter will also be raised on a workshop specifically aimed at actualizing a RSA-Angola bilateral protocol that has been signed years ago.

    - From this protocol, funding should become available to support training, translation, research, surveys, and services.
- On invitation from MINADERP, Hennie le Roux attended an Agricultural Trade Fair and presented a talk on Citrus Biosecurity (end-November 2013). Hennie's excellent presentation at Trade show was also televised. A report sent to MINADERP proposing follow-up surveys to be conducted. No feedback was received from Angola.

The initiatives in Angola will be continued in 2014.

Mozambique

Evidence of citrus development in Mozambique with Brazilian links raised concern that they might use Brazilian-made trees. MC Pretorius and Hennie le Roux arranged a meeting with Mozambique officials to discuss the citrus biosecurity risks and to promote the use of CIS nurseries. The meeting was postponed and will be held in 2014.

Challenges: Resource, time and personnel constraints. Given the capacity constraints within DAFF, a lot of these biosecurity actions are handled by CRI personnel. More resources are urgently required for the HLB/ACP surveys and African Biosecurity actions.

7 INTERNATIONAL VISITS

7.1 S.D. MOORE

7.1.1 REPORT ON A VISIT TO CROATIA ON 16-20 JUNE 2014 – 14th BIENNIAL MEETING OF THE INTERNATIONAL ORGANISATION OF BIOLOGICAL CONTROL WORKING GROUP ON INSECT PATHOGENS AND INSECT PARASITIC NEMATODES

INTRODUCTION

This visit took place in June 2013. The 14th biennial meeting of the International Organisation of Biological Control Working Group on Insect Pathogens and Insect Parasitic Nematodes took place from 16-20 June in Zagreb.

ITINERARY

Date/s	Destination	Meeting	Activity
15 June	Zagreb	-	Fly from PE via Jnb & Istanbul
16-20 June	-	IOBC Meeting – Insect Pathogens & EPNs	-
21-24 June	Dubrovnik	-	Holiday
25 June	PE	-	Return flight

PURPOSE OF TRIP

1. Familiarise myself with and report on the latest applied biocontrol research findings from around the world.
2. Meet and network with applied biocontrol researchers e.g. researchers developing new viruses, EPFs or EPNs with potential against pests which occur in South Africa, particularly in citrus.
3. Present recent research conducted by CRI and solicit opinions on work presented.
4. Establish valuable contacts to follow up with on relevant current and future biocontrol research and practices.

Trip details

IOBC MEETING

Key points from key papers

The theme of the congress was “Biological control – its unique role in organic and integrated production”. Strictly speaking, this is a Western European working group meeting, however, there were participants from Eastern Europe, Asia, South America and Africa. It was a small meeting with approximately 120 delegates from 25 countries. Most delegates were scientists (including 20 students), but with some representation from commercial biocontrol companies. Several of Europe’s (and the world’s) leading experts on microbial control were present. I have attended several Society for Invertebrate Pathology meetings – the main annual forum for insect pathologists – but found this IOBC meeting more valuable, in the sense that it was more applied in nature. There were 49 oral presentations, 41 poster presentations, a round table discussion and two workshops. All presentations were in English and have been published as full papers in a comprehensive 360 page book of proceedings.

Plenary address: Authorisation of biological control agents – theory and practice – Ralf-Udo Ehlers (e-nema, Germany).
Ralf reported on the work conducted within the REBECA (Regulation of Biological Control Agents) programme, with the objective of driving change in EU (and member state) regulations. Currently, registration requirements are prohibitive. Ralf stated that all BCAs (including semiochemicals) should be categorised as low risk. However, there was not yet a clear definition of “low risk”. This should mean that data requirements would be lower, registration would be prioritised and fast-tracked and that lower fees would be payable. Current EU legislation leads to more illegal use of plant protection products and the registration of BCAs as biostimulants or fertilisers, which is easier. The REBECA proposals have been published in a book, which I consider very useful for guiding the improvement of South Africa’s registration guidelines for BCAs.

Plenary address: Dynamics of baculovirus as insect biocontrol agent – Just Vlak (Wageningen, Netherlands)
58 baculovirus (BV) genomes are currently deposited in Genbank, including about 130 sequenced genes. In total, approximately 600 BVs have been identified. Examples of some of the more interesting genes which have been characterised are threonine, tyrosine and serine (genes that remove phosphite enzymes from proteins), ptp (leads to increased larval movement), egt (kills faster and causes upward movement of larva), phr (responsible for photolyase that can repair UV damage e.g. in TnSNPV and ChchNPV – mainly responsible for day/night biorhythm of host). According to EFSA (www.efsa.europa.eu), BVs are regarded as safe at the family level.

Plenary address: Insect pathogenic fungi: what was obtained and where to go? (Jorgen Eilenberg, University of Copenhagen, Denmark)
Metarhizium translocates nitrogen and can therefore also be used as a biostimulant. A recent EU supported project INBIOSOIL (2012-2015) has as one aim to study effects on target and non-target of novel formulations of Hypocreales. Recently the knowledge on mass production of Hypocreales was compiled (Jaronski & Jackson, 2012). Their book chapter contains the full information package needed for small scale to medium scale laboratory production as well as significant considerations about real mass production. A suggestion was made of applying EPFs and EPNs together as a means of more effectively getting EPFs into the soil.

Exploring synergistic effects of semiochemicals, entomopathogenic fungi and nematodes against root-herbivores – Michael A. Brandl, Mario Schumann, Stefan Vidal (Georg-August-University, Germany)
Control of western corn rootworm and wireworm larvae in maize is being investigated by combining semiochemicals, known as components in “attract” or “confuse” strategies, with *Metarhizium anisopliae* and *Heterorhabditis bacteriophora* known as kill components. The concept uses biological control agents in co-formulated capsules for preservation. Furthermore, either attractant or repellent semiochemicals, with regard to the targeted strategy, are added. CO_2 was used as the attractive component in the “attract & kill” strategy and botanicals as the repellent component in the “confuse & kill” strategy. To date, three different strains of *M. anisopliae* have been tested in bioassays and in the greenhouse. All strains were not as efficient as a standard insecticide.

***Beauveria bassiana* strain ATCC 74040 interferes with oviposition behaviour of Mediterranean fruit fly – Luca Ruiu, Giovanni Falchi, Edith Ladurner (Dept Agronomy, Italy)**
A commercial formulation of *Beauveria bassiana* (Naturalis) and different fungal preparations (pure conidia, hyphae, culture supernatants) were applied to orange fruits offered to ovipositing medflies. A significantly lower number of fly visits and oviposition punctures were recorded on fruits treated with Naturalis and with pure conidia than on control fruits (17.4 vs 5 visits and 8.4 vs 1.2 punctures). The physical and/or biochemical properties of the conidia appear to interfere with the female’s oviposition behaviour. The conidia impede the fruit fly’s ability to locate the orange odours. The same effect is observed with SDS and formic acid (?).

Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane – María Fernández-Bravo, Inmaculada Garrido-Jurado, Monica Oreste, Enrique Quesada-Moraga (University of Cordoba, Spain)
Optimum temperature for germination and growth ranged from 23.9 to 30.4°C. No significant relationship was detected between optimum and maximum temperatures for growth and habitat (soil or phylloplane). Also, no significant relationship was detected between humidity requirements among isolates from soil and phylloplane, with maximum values of colony growth and germination rate between 0 and 5 bars. None of the isolates grew above 100 bars. Delaying of germination and colony growth was directly proportional to UV-B radiation dose.

On the role of baculovirus photolyases in DNA repair upon UV damage of occlusion bodies – Magdalena A. Biernat, Primitivo Caballero, Just M. Vlak, Monique M. van Oers (Wageningen, Netherlands)
Photolyases are known to repair UV damage. CPD-photolyase (*phr*) genes were reported in *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV). Their role was tested by infecting larvae with UV-irradiated viral occlusion bodies (OBs) that were subsequently treated with visible light or kept in the dark. The observed mortality was the same for both treatments, indicating that these photolyases are not active as DNA repair enzymes in OBs, but may play a role in other aspects of baculovirus pathogenesis. It appears that the phr gene (only in plusiine larvae) is responsible for the circadian clock.

Effect of top spray drying and freeze drying on the photostability and insecticidal activity of a *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV 003) formulation – Mauricio Cruz, Martha Liliana Chaparro, Laura Fernanda Villamizar, Martha Isabel Gomez (Centre of Biotechnology and Bioindustry, Colombia)
Spray drying was a more effective method for removing moisture content than freeze drying. No obvious differences in the insecticidal activities were observed for both drying methods although a higher photostability (88.54%) was observed in the formulation prepared with spray drying than freeze drying (77.77%) and unformulated virus (15.62%) after 6 hours of UV radiation exposure.

Elucidation of a novel mode of resistance of codling moth against *Cydia pomonella* granulovirus by homogenization experiments – Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle (Institute for Biological Control, Germany)
Since 2005, codling moth populations with a reduced susceptibility to CpGV products have been reported from about 40 European orchards. The resistance could be traced back to a single, dominant, sex-linked gene. Currently, resistance management strategies are based on the application of improved CpGV products containing resistance-overcoming CpGV isolates. Recently, two CM field populations with a reduced susceptibility to even these improved CpGV products were found. First single pair crossing experiments between individuals of these resistant field colonies and a susceptible laboratory CM strain (CpS) indicated that the inheritance of resistance of these populations did not follow the previously described pattern of Z-linked, dominant resistance.

Natural populations of *Spodoptera exigua* are infected by multiple viruses: implications for the production and use of virus insecticides – Cristina Virto, David Navarro, M. Mar Tellez, Salvador Herrero, Trevor Williams, Rosa Murillo, Primitivo Caballero (University of Navarra, Spain)
A significant percentage of *Spodoptera exigua* adults were found to be co-infected with nucleopolyhedrovirus (NPV) and iflavirus. This finding has important implications for in vivo production of NPV, as iflaviruses do not cause lethal infection, but result in lower larval weight gain. A positive finding was that the presence of NPV appeared to negatively affect the spreading (including trans-generational) of the iflavirus covert infection.

Estimating the importance of maternal and paternal contributions to the vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) – Cristina Virto, Carlos A. Zárate, Rosa Murillo, Primitivo Caballero, Trevor Williams (University of Navarra, Spain)
Both males and females were able to transmit the infection to the next generation, although females infected a higher percentage of the offspring and female-mediated transmission was more consistent than that of males. Venereal transmission appeared to be half as effective as maternallymediated transmission, and the main route of transmission is likely transovarial rather than transovum. The prevalence of the infection in the offspring did not vary according to gender, therefore both males and females can be infected by their parents in similar proportions. Incorporating verticallytransmitted genotypes in biological insecticides might have the potential for reducing pest densities and extending periods between virus applications.

Baculoviruses for the biological control of cutworms (*Agrotis* spp.) – Jörg T. Wennmann, Gianpiero Gueli Alletti, Johannes A. Jehle (Institute for Biological Control, Germany)
Four different baculoviruses (3 NPVs and 1 GV) were identified infecting two species of cutworm. Bioassays were conducted, infecting larvae with different combinations of virus. NPVs were generally more effective than the GV. A combination of GV and NPV was marginally the most effective. Using multiplex PCR, the exact mortality causative agent could be identified and quantified. This study was sponsored by Andermatt, obviously indicating commercial intentions with these viruses.

The project ATTRACT: Protection of crops from soil-borne insect pests with a novel attract and kill strategy – Marina Vemmer, Wilhelm Beitzen-Heineke, Hubertus Kleeberg, Edmund Hummel, Stefan Vidal, Anant Patel (University of Applied Sciences, Germany)
This project is still in a very early phase. It plans the development of an attract-and-kill strategy for protection of crops against soil-borne insect pests. Novel formulations (capsules, granules) based on CO2 emitting sources will be developed and tested under practical conditions in order to lure larvae away from plant roots. In these attract formulations, plant-based environmentally friendly insecticidal compounds, such as neem and quassin will be incorporated in multiphase or multilayer systems with additives.

Compatibility of five different entomopathogenic nematode (Nematoda: Rhabditida) species with registered insecticides and fungicides under laboratory conditions – Žiga Laznik, Stanislav Trdan (University of Ljubljana, Slovenia)
Compatibility of five species of EPNs was tested with with 6 chemical, one plant-based and one bio-insecticide, and 13 synthetic organic and two inorganic fungicides under laboratory conditions. The effect of

direct IJ exposure to insecticides for 6 and 24 hours was tested in Petri dishes at 15, 20 and 25 °C. *S. carpocapsae* and *S. kraussei* were sensitive to all tested insecticides, while *H. bacteriophora* was sensitive only to abamectin and lufenuron.

Attract and kill against western corn rootworm larvae with entomopathogenic nematodes – Mario Schumann, Felicitas Kaemena, Anant Patel, Stefan Vidal (Georg-August-University, Germany)
Biocontrol with EPNs is effective but expensive. Using an attract and kill strategy (with CO_2) may improve the efficacy of EPNs and reduce the cost. In trials, a combination of EPNs (*H. bacteriophora*) and CO_2 was more effective than EPNs alone.

Impact of *Entomophaga maimaiga* on gypsy moth populations in Bulgaria – Plamen Mirchev, Andreas Linde, Daniela Pilarska, Plamen Pilarski, Margarita Georgieva, Georgi Georgiev (Forest Research Institute, Bulgaria)
The Entomophtorales fungus was introduced into three populations of gypsy moth in Bulgaria in 1999. After the first big epizootics were observed in 2005, the species was introduced into another six outbreak populations of gypsy moth in different regions of the country (2008 to 2011). The pest is now totally suppressed in Bulgaria. The fungus has naturally spread throughout the country and is now present in nearly all regions of the country in which the pest occurs.

Development of new formulations for soil pest control – Miriam Hanitzsch, Michael Przyklenk, Bianca Pelzer, Anant Patel (University of Applied Sciences, Germany)
Capsule systems were prepared by ionic gelation, thermal gelation, complex coacervation and additional bead coating. In selected capsules, lignin was incorporated. Capsules based on single biopolymers were able to form stable spherical capsules, e.g. alginate, pectin derivates and gelatin. Capsules based on combinations of polymers also showed stable capsule formation, e.g. alginate/gelatin, alginate/lignin and SEC/PDADMAC. Additionally, lignin was used successfully as an additive in SEC hollow beads. First experiments indicated significant differences in biological degradability and thus persistence in soil with different capsule systems. These novel capsule systems with increased persistence are suitable for delivery of BCAs (specifically EPFs) into the soil.

Pathogenicity of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* on larvae and adults of the sisal weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) – V. T. Gkounti, D. Markoyiannaki and D. C. Kontodimas (Benaki Phytopathological Institute, Greece)
Could a delayed response with a lower dose actually be beneficial for dissemination?

Colorado potato beetle (*Leptinotarsa decemlineata* Say) – control strategies in organic farming using biological insecticides (azadirachtin, *Bacillus thuringiensis* var. *tenebrionis*, pyrethrum and spinosad) – Stefan Kühne, Uta Priegnitz, Benjamin Hummel and Frank Ellmer (Julius Kühn Institute, Germany)
It is important that in a spray programme, one does not begin with Bt, as Bt stops feeding. Therefore, begin programme with Neem.

Exploiting vine weevil behaviour to disseminate an entomopathogenic fungus – Tom Pope, Charlotte Arbona, Harriet Roberts, Jude Bennison, John Buxton, Gill Prince and Dave Chandler (Harper Adams University, UK)
Using Exosect powder (carnauba wax) as a carrier for fungal spores to weevils. I will contact Pope towards the end of the year to find out what the results were.

Field persistence of *Metarhizium* spp. strains applied as biocontrol agents against ticks (*Ixodes ricinus*) – Maria Mitteregger, Sarah Sonderegger, Hermann Strasser (University of Innsbruck, Austria)
In open fields, survival of the EPF dropped dramatically withink 5 days, but some persistence was measured up to 44 days. Adjuvants – Neo-wett and Schaumstop had no detrimental effect on the fungus. In bioassays, all ticks were killed within 10 days.

FORMAL CONTRIBUTIONS BY SEAN MOORE TO THE IOBC PROGRAMME:

Characterisation of novel CrleGV isolates for false codling moth control - lessons learnt from codling moth resistance to CpGV

John Opoku-Debrah[1], Sean Moore[1,2], Martin Hill[1], Caroline Knox[3]
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Abstract: Recently some codling moth, *Cydia pomonella*, populations in Europe developed resistance to CpGV. In order to prepare for the possibility of a similar occurence with the false codling moth, *Thaumatotibia leucotreta*, in South Africa, a search was conducted for novel CrleGV isolates. Through overcrowding, outbreaks of novel isolates were recorded from laboratory populations of five geographically distinct host populations. The genetic novelty of these and two commercially available isolates was confirmed through restriction enzyme analysis and sequence analysis of the *granulin* and *egt* genes. Phylogenetic analysis showed the existence of two CrleGV-SA genome types. Significant differences in virulence were also shown between certain isolates against certain host populations.

Comments on presentation:
Jurg Huber: Did you determine whether the virus you obtained from dead larvae in bioassays was indeed the same virus with which it was inoculated? If not, can you reliably conclude that your virulence comparisons can be linked to the inoculated virus?
Have you tried to induce resistance in FCM to CrleGV in the lab?
Johannes Jehle: From the DNA profiles, all isolates look the same. The only differences appear to be in the sub-molar bands i.e. because of nucleotide differences. Refer to Jehle et al 2005 for more information on this. (This comment was interesting, as Jehle was an examiner on Opoku-Debrah's thesis and he did not make this comment then).
Just Vlak: Why did you use egt as your reference gene, as it is highly conserved. In future, rather use non-functional genes, as they have far more variation between isolates.

Subterranean control of an arboreal pest: EPNs and EPFs for FCM

Sean Moore[1,2], Candice Coombes[2], Aruna Manrakhan[1], Wayne Kirkman[1], Martin Hill[2], Ralf-Udo Ehlers[3], John-Henry Daneel[1], Jeanne de Waal[4], Jo Dames[2] and Antoinette Malan[5]
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Abstract: Control measures against the false codling moth (FCM), *Thaumatotibia leucotreta*, have traditionally ignored the soil-borne pupal stage. Recent trials with entomopathogenic nematodes (EPNs) and fungi (EPFs) have targeted this life-stage. Application of *Heterorhabditis bacteriophora* to a citrus orchard floor, reduced *T. leucotreta* infestation of fruit by up to 81%. Conservation of *H. zealandica* through non-usage of a nematicide also resulted in dramatically lower fruit infestation. Dose-response and exposure time-response bioassays identified the three most promising fungal isolates against pupating *T. leucotreta*. Orchard trials showed persistence of these fungi in orchard soil for at least six months.

Comments on presentation:
All extremely positive.

Both of these presentations were published as full articles in the proceedings of the meeting, along with those of all other presentations. A copy of the proceedings can be obtained from me.

Short course on Virus taxonomy and classification – Johannes Jehle
Family name must be written in italics.
When referring to virus species, use italics; however, when referring to the virus itself, then don't use italics. Therefore, the norm would be to use italics for the species only once, when introducing it, and thereafter in Roman letters. A virus abbreviation (eg CrleGV) is always in Roman.
All newly described virus use a four letter abbreviation (from host genus and species) before the virus genus e.g. CrleGV. Only some old viruses are allowed to maintain the original two letter prefix e.g. CpGV.
No longer use the abbreviations S or M for single- and multiple-embedded NPVs.
Anybody can make a new taxonomic proposal to the Invertebrate Virus sub-committee chair (Elizabeth Herniou or Peter Krell) of ICTV e.g if a new virus is found. However, not that just because it is the first time a virus is found in a particular species, it may not necessarily be a new species of virus i.e. it may be very closely related to an existing virus species and would then simply be viewed as a new isolate, rather than a new species. The ICTV does not involve itself in the naming used for isolates or strains.

For more details get hold of the Virus Taxonomy Manual by Andrew King et al. This refers to to criteria for nomenclature e.g. ultrastructure, type of nucleic acid, host range and molecular phylogeny. See also www.ictvonline.org but not Genbank.

Panel discussion: An integrative view on soil pest control – Stefan Vidal, Ralf-Udo Ehlers, Tariq Butt and Rod Blackshaw

Butt – See Ansari and Butt (2013, Biocontrol 58: 257-267) for a cost-efficacy assessment on the use of *Metarhizium* for black vine weevil control in strawberries. Efficacy of *Metarhizium* can be enhanced with an "efficacy enhancing agent". This could be a low dose chemical, EPNs or botanicals. For example a sub-lethal dose of chlorpyrifos enhances the efficacy of the fungus and enables application of the fungus at a much lower rate. Found that melanic form of insects can be tolerant to *Beauveria bassiana* e.g Galleria – see Dubrovskiy et al 2013.

Blackshaw – Influence of global warming e.g. Agrotis segetum in Denmark usually has 1 generation per year. However, with global warming this has increased to 1.5 per year. Therefore 2nd generation does not reach overwintering stage and dies over winter, leading to much lower pest levels. However, if global warming continues, then this will turn into 2 generations per season, which will result in much greater problems than previously.

VALUABLE DISCUSSIONS HELD WITH IOBC DELEGATES:

Johannes Jehle

Indicated a willingness to sequence any new viruses for us.

Juerg Huber

Suggested we pursue a study of the potential for development of resistance by FCM to GV by attempting to induce resistance in lab trials.

He also suggested that we test the virulence of our entire range of tortricid viruses (all CrleGV isolates, CpGV, litchi moth virus) against all tortricid pests that are relevant to us i.e. FCM, codling moth, litchi moth and macadamia borer.

Primitivo Caballero

We discussed the possibility of a collaborative project, genetically characterising a range of bollworm virus (HearNPV) isolates and comparing their virulence against a range of geographically distinct host populations in Spain and South Africa. We are planning to pursue this collaboration through the appointment of a post-graduate student at Rhodes University.

Jorg Eilenberg

Jorg is recognised as one of the leading experts on Entomophthorales fungi. He invited us or a student to visit his lab for 2 weeks to learn a range of techniques for working with these fungi. This would include isolation, culturing and bioassaying. He also mentioned a possible opportunity for us to collaborate with someone in Brazil (Sao Paolo), as they apparently have funding for sending out post-grads. One could possibly conduct a study at Rhodes.

Massimo Toni

Managing Director of MT Bio-consulting, but essentially works as an international product scout and procurer for De Sangosse, a French biocontrol company. It might be worthwhile for RB to contact him re potential products for the southern African citrus industry.

Tom Pope

Is using entostat to help apply EPFs for vine weevil control at Harper Adams University, Cambridge. He will have some results towards the end of the year and I will contact him again to find out what they are.

Albert Ester

Works on slugs and snails. Gave me some ideas as to how to manage snails more effectively. Also suggested a botanical derivative as a possibility for snail control.

VALUE AND SUMMARY OF VISIT

This was the most interesting and valuable scientific meeting I have participated in for years. My measure of a good meeting is the number of good ideas and valuable contacts one departs with after the meeting. I returned home with numerous new ideas for research projects and possible products. I have also established some potentially valuable relationships which can lead to collaborations and research guidance in the near future. The meeting was very applied in nature, without any compromise to the value of the

science being presented. Several of the world's leading experts on various aspects of insect pathology and microbial control participated in the meeting. I am very keen to again participate in this biennial meeting in 2015 and strongly recommend that River Bioscience send one of their staff to this meeting. It is a meeting in which both scientists and commercial representatives will feel comfortable and will benefit from.

FOLLOW UP ACTIONS TO BE TAKEN

1. Initiate research project on host range of tortricid viruses.
2. Initiate collaborative research project with Primitivo Caballero (Spain) on genetic and biological characterisation of bollworm virus isolates against different host populations.
3. Initiate research project (with Copenhagen University) on Entomophthorales fungi.
4. Initiate research project on synergism between EPFs and EPNs.
5. Contact Tom Pope later in the year on entostat results.
6. Follow up with Albert Ester on how to improve snail control, including potential botanical products.

ACKNOWLEDGEMENTS

River Bioscience is thanked for funding and facilitating the trip. River Bioscience's Directors are thanked for authorising the trip. CRI is thanked for supporting the application.

7.2 A. MANRAKHAN

7.2.1 REPORT ON IAEA EXPERT MISSION, MAURITIUS - 10-14 June 2013

INTRODUCTION

I was tasked by the International Atomic Energy Agency (IAEA) to conduct an expert mission on an IAEA project "Preventing the Introduction of Exotic Fruit Fly Species and Implementing the Control of Existing Species with the Sterile Insect Technique and Other Suppression Methods" (RAF5062 9003). I participated in a second project coordination meeting to finalize common protocol and modalities for management of fruit flies including a common emergency action plan for exotic fruit flies. The meeting took place between 10 and 14 June 2013 in Mauritius (See Annex 1 for meeting agenda).

OBJECTIVES OF EXPERT MISSION

The objectives of my mission were to:

1. Develop a draft action plan for invasive species for the Indian Ocean Countries (draft should be distributed to the meeting participants 2 weeks before the meeting).
2. Lead the discussion on the detailed revision of the draft and produce an advanced document by the end of the meeting.
3. Support the project RAF5062 work plan review.

DEVELOPMENT OF ACTION PLAN FOR THE INDIAN OCEAN REGION

An action plan for invasive fruit fly species was developed for the six Indian Ocean countries participating in this project: Reunion (Republic of France), Republic of Madagascar, Republic of Mauritius, Republic of Mozambique, Republic of Seychelles and the United Republic of Tanzania (see Annex 2 for plan). The plan was adapted from the South African action plan for the control of *Bactrocera invadens* Drew Tsuruta and White. Some of the annexes to the action plan have not yet been finalised as they are awaiting information on detailed control methods from participating countries. The action plan developed was adopted by all participating countries at the end of the meeting.

In the Indian Ocean emergency action plan, the post eradication monitoring was changed to 8 weeks compared to 4 weeks in the South African plan following suggestions from participants, with lifting of quarantine occurring only after 16 weeks of no exotic fly find in an area.
In the action plan developed, the Steering Committee will ensure financial and resource management and oversee communication, co-ordination of actions and decision making in response to exotic fruit fly detection. Notifications to the international community will be done by the National Plant Protection Organisation (NPPO) in accordance with the requirements of the World Trade Organisation Sanitary Phytosanitary Standards (SPS) Agreement, the International Plant Protection Convention (IPPC) and relevant International Standards Phytosanitary Measures (ISPM), with which the national phytosanitary standard and operating procedures for pest reporting are aligned. The Steering Committee will be informed of the notifications.

NEW PEST REPORT

Bactrocera dorsalis/*Bactocera invadens* specimens were detected in three specific areas in Mauritius since 8 March 2013. Between 8 March 2013 and 31 May 2013, 24 *B. dorsalis/B. invadens* males and 4 *B. dorsalis/B. invadens* females were detected in 17 traps. Thirty four *B. dorsalis/ B. invadens* adults were reared from four fruit types: *Citrus bergamia* Risso & Poit (Bergamote), *Terminalia catappa* L. (Indian Almond), *Psidium guajava* L. (Guava), *Malphighia emaginata* DC (Acerola) collected in April in one of the detection sites. In all detection sites, eradication actions were initiated following the first fly find. Eradication actions are carried out using bait application technique (weekly sprays of NuLure and Dipterex) and male annihilation technique (deployment of plywood blocks impregnated with methyl eugenol and malathion every 100 m).

FIELD VISIT

Eradication sites

We visited one of the towns where *B. dorsalis*/ *B. invadens* was detected and where eradication actions are being carried out (Fig. 7.2.1 & 7.2.2). We also visited a citrus orchard which was placed under eradication and quarantine.

Figure 7.2.1. Application of a mixture of protein hydrolysate and Dipterex using knapsack sprayer in a residential area

Figure 7.2.2. Plywood block impregnated with a mixture of methyl eugenol and malathion, nailed onto a tree.

Mass rearing facilities

The Sterile Insect Technique was developed for two fruit fly pest species in Mauritius: *Bactrocera cucurbitae* (Melon fly) and *Bactrocera zonata* (Peach fruit fly). We visited the mass rearing facilities for these two pests. For both pest species, SIT will be integrated with other approaches such as orchard sanitation, bait application and male annihilation techniques. The latter two techniques would be applied first to reduce pest levels to below 1 fly per trap per day before sterile flies are released. For *B. cucurbitae*, only sterile males would be released and for *B. zonata*, both sexes will be released.

Production of local protein autolysate

We visited a facility at the Entomology division, Ministry of Agroindustry and Food security where local protein autolysate is being produced. Waste from Mauritius Breweries is collected and heated to boiling point (100ºC) (Fig. 7.2.3). Thereafter the product is allowed to cool down to 70ºC, when the enzyme papain is then added. In order to store the protein autolysate, potassium sorbate is added at the rate of 2g per L of protein autolysate.

Figure 7.2.3. Production of local protein autolysate.

Annex 1

International Atomic Energy Agency
Regional Africa Technical Cooperation Project
RAF5062
Preventing the Introduction of Exotic Fruit Fly Species and Implementing the Control of Existing Species with the Sterile Insect Technique and Other Suppression Methods

"Second Project Coordination Meeting to Finalize Common Protocol and Modalities on Management of Fruit Flies"

10-14 June 2013, The Flying Dodo Brewing Company, Bagatelle, Mauritius

DRAFT AGENDA

SESSION I: TECHNICAL SESSION

MONDAY, 10 JUNE 2013

SESSION I (a): Opening		
Time	**Agenda item**	**Facilitator/Speaker**
08:30 - 09:00	Arrival and registration of participants	Mr. Sookar
09:00 - 09:30	Opening and Welcome	Mr.Permalloo
09:30 – 09:45	Goals of the meeting	Mr. Kiza
09:45-10:00	Administrative issues	Mr. Sookar
10:00-10:30	**COFFEE BREAK**	
SESSION I (b): Countries' Progress Reports & Experiences on Management Fruit Flies		
10:30 – 10:50	France's (Reunion) Report	Mr. Quilici
10:50 – 11:10	Madagascar's Report	Ms. Raoelijaona

11:10 – 11:30	Mauritius' Experience	Mr. Permallo
11:30 – 11:50	Mozambique's Report	Mr. Cugala
11:50-13:00	**LUNCH**	
13:00 – 13:20	Seychelles' Report	Mr. Stravens
13:20 – 13:40	Tanzania's Report	Ms. Mramba
13:40 – 14:00	BREAK	
SESSION I (c): Common Emergency Action Plan for Exotic Fruit Flies		
14:00 – 15:30	Presentation of the draft Common Emergency Action Plan for Exotic Fruit Flies	Ms. Manrakhan
15:30 – 16:00	Discussion and improvement of the draft Common Emergency Action Plan for Exotic Fruit Flies	All

TUESDAY, 11 JUNE 2013

SESSION I (c) (cont.): Common Emergency Action Plan for Exotic Fruit Flies		
09:00-12:00	Discussion and improvement of the draft Common Emergency Action Plan for Exotic Fruit Flies	All
	LUNCH	
13:00-16:00	Discussion and improvement of the draft Common Emergency Action Plan for Exotic Fruit Flies	All

WEDNESDAY, 12 JUNE 2013

09:00 – 12:00	Finalization of the draft Common Emergency Action Plan for Exotic Fruit Flies	All
12:00-13:00	**LUNCH**	
SESSION I (d): Project Activities Review		
13:00 – 16:00	Review the project status, work plan and implementation arrangements	Mr. Kiza

SESSION II: MEMORANDUM OF UNDERSTANDING
THURSDAY, 13 JUNE 2013

SESSION II (a): Official Opening		
08:30 - 09:00	Arrival and Registration of new participants	Mr. Sookar
09:00 - 09:15	Official Opening Statement	PS, Ministry of Agro Industry & Food Security
09:15 - 09:30	Statement from the IAEA	M.Kiza
09:30-10:00	Summary of Status of Fruit fly problems and management in the region	Ms. Manrakhan
10:00 - 10:15	**COFFEE BREAK**	
SESSION II (b): Presentation of Recommendations from Technical Group		
10:15– 11:00	Presentation of the Final draft Common Emergency Action Plan for Exotic Fruit Flies	Ms. Manrakhan
11:00 - 12:00	Presentation of the final draft MOU	Ms. Murugaiyan
12:00 13:00	**LUNCH**	
	SESSION II (c): Field Tour	
13:00 – 16:00	Field Excursions to the insect rearing facility and trapping areas	
18:00-20:00	**OFFICIAL DINNER**	

FRIDAY, 14 JUNE 2013
SESSION II (d) Signing and Launching Ceremony

09:00-09:30	Adoption of Common Emergency Action Plan for Exotic Fruit Flies	
09:30-10:00	Signing of the MOU	
10:00-10:15	**COFFEE BREAK**	
10:15-10:30	Statements from France (Reunion)	
10:30-10:45	Statements from Madagascar	Mr. A. R. Z. Tsitohaina, Directeur de la Protection des Végétaux
10:45-11:00	Statements from Mozambique	Ms. A. Mondjana. Deputy VC Eduardo Mondlane University
11:00-11:15	Statements from Seychelles	Mr. M. Naiken, CEO Seychelles Agricultural Agency
11:15-11:30	Statements from Tanzania	Mr. Futakamba, Deputy PS, Ministry of Agriculture
11:30-11:45	Statement from the IAEA Director Africa Division, Technical Cooperation Department	Mr. Kiza (Reader)
11:45-12:00	Remarks from FAO.	A. Huynh, FAO Representative a.i.
12:00-12:15	Remarks from the UN Resident Coordinator	Mr. S. Springett - UNRC
12:15-13:00	Statement from Mauritius, Launching of the Action Plan & MOU and Official Closing of the Meeting	Minister of Agro Industry & Food Security
13:00 14:30	**LUNCH**	

Annex 2

Indian Ocean Region Emergency Action Plan for Exotic Fruit Flies

GENERAL INFORMATION

1.1. Action statement

The Indian Ocean Region emergency action plan for exotic fruit flies is a common recommended protocol for all Indian Ocean member states for survey, containment and eradication of exotic fruit flies following detection in an area within an existing surveillance network. The recommended protocol is adapted from the IAEA/FAO *Bactrocera zonata* action plan and the South African action plan for the control of *Bactrocera invadens* Drew Tsuruta and White

Under the memorandum of understanding to strengthen sub regional cooperation for preventing the invasion of exotic fruit fly species and improving the control of existing species between the Republic of France and Republic of Madagascar and Republic of Mauritius and Republic of Mozambique and Republic of Seychelles and the United Republic of Tanzania, the Parties acknowledge that among the identified Tephritid fruit flies, the species with the highest risk of becoming pervasive are *Bactrocera invadens* recently introduced and existing in some parts of Sub-Saharan Africa, particularly, in some of the Indian Ocean Islands, in addition to *Bactrocera cucurbitae, Bactrocera zonata* and some *Ceratitis* species which are already pervasive in the region.

Under the Indian Ocean Commission Regional Fruit Fly Programme (1997-2000), *Bactrocera dorsalis* was considered at high risk of introduction for all member states of the Indian Ocean.

In the core of this emergency action plan for exotic fruit flies, recommendations were provided for *B. invadens* and *B. dorsalis* which respond to the male attractant methyl eugenol. Trapping densities and eradication actions recommended for *B. invadens* and *B. dorsalis* will be similar to other methyl eugenol responding species such as *B. zonata*. For other exotic fruit flies which do not respond to methyl eugenol and are at high risk of introduction to specific member states, supplementary recommendations on trapping densities and eradication actions were provided for two groups of exotic fruit flies: cuelure responding flies and trimedlure/enriched ginger root oil responding flies.

A Steering Committee should be set up in each country for detection survey and management of actions relating to exotic fruit flies. The Steering Committee will ensure financial and resource management and oversee communication, co-ordination of actions and decision making in response to exotic fruit fly detection. Notifications to the international community will be done by the National Plant Protection Organisation (NPPO) in accordance with the requirements of the World Trade Organisation Sanitary Phytosanitary Standards (SPS) Agreement, the International Plant Protection Convention (IPPC) and relevant International Standards Phytosanitary Measures (ISPM), with which the national phytosanitary standard and operating procedures for pest reporting are aligned. The Steering Committee will be informed of the notifications.

The Steering Committee should consist of officials from the NPPO, representatives from major affected industries, representatives of related ministries and other major stakeholders. The Steering Committee should be chaired by the authority at the NPPO.

1.2. Pest profiles of Bactrocera invadens and Bactrocera dorsalis

1.2.1. *Bactrocera invadens*

1.2.1.1. Origin and distribution

Bactrocera invadens originates from Asia and has invaded various parts of Africa, including recently some southern African regions. The pest is also present in three Indian Ocean countries/islands: Comores, Mayotte and Madagascar. *B. invadens* is closely related to *B. dorsalis*.

1.2.1.2. Host Range

B. invadens is a polyphagous species and has to date been recorded from a large number of host species. The host list is compiled Annex 1a and is mainly derived from field studies conducted in different countries in Africa. It must be noted that information on the maturity stages of the hosts listed were not always provided. In addition for some commercial hosts listed for *B. invadens*, information on the cultivated variety was not always stated. Commercial fruit which are affected by *B. invadens* in Africa include fruit for export such mango and citrus.

The hosts listed should be inspected and regulated in the case of a *B. invadens* find. The host list presented below is not exhaustive and can still expand.

1.2.1.3. Demography

The mean generation time for *B. invadens* was found to be 30.7 days at 28 ± 1º C (Rwomushana *et al*., 2008). However, generation time is largely dependent on temperature. In order to determine phenological events in the field for monitoring and eradication purposes, it is important to determine the temperature-development rate of the pest. The developmental rates of *B. invadens* were determined at five constant temperatures of 15°C, 20°C, 25°C, 30°C and 35°C and a photoperiod of L12:D12. . The table below gives the mean total pre-imaginal developmental time (egg to pupa) (days) obtained at varying constant temperatures for *B. invadens*.

Table 2. Mean total developmental time for immature stages of *B. invadens* (Rwomushana *et al*., 2008)

Temperature ºC	Mean total pre-imaginal developmental time, days (egg to pupa)
15	75.74
20	31.45
25	21.19
30	17.76

To predict the developmental rate of individual life stages, a temperature summation model can be used. This approach is based on the assumption that above some lower threshold for development, temperature-developmental rate relationships are linear and, therefore, a constant number of heat units, expressed as day-degrees above this threshold are needed to complete the development. To calculate developmental times in fluctuating daily temperature regimes, the number of day-degrees per day can be determined by the formula $(T_{max} + T_{min})/2 - t$ with T_{max} being maximum temperature, T_{min} minimum temperature and t, the lower development threshold. The lower development threshold of *B. invadens* was found to be 8.8°C, 9.4°C and 8.7°C for the egg, larva and pupa.

1.2.1.4. Attractants

B. invadens responds to methyl eugenol which is a parapheromone and attracts only males. Some attraction of both sexes of the fly to protein hydrolysate (such as Torula yeast) and the 3-component BioLure have also been reported.

1.2.2. *Bactrocera dorsalis*

1.2.2.1. Origin and distribution

Bactrocera dorsalis is part of the *B. dorsalis* complex. The *B. dorsalis* complex contains 75 described species which are largely endemic to Asia. *B. dorsalis* is distributed in several countries in Asia and is also established in Hawaii and some Pacific Ocean islands.

1.2.2.2. Host Range

B. dorsalis has a wide host range and was recorded on 124 species in 42 plant families. The host list is compiled in Annex 1b and is derived from published literature with host records from China, Hawaii and Pacific Ocean islands.

The hosts listed should be inspected and regulated in the case of a *B. dorsalis* find.

1.2.2.3. Demography

The mean generation time for *B. dorsalis* was found to be 33.4 days at a constant temperature of 29 ± 1º C (Vargas *et al*. 1997) and 55.1 ± 2.3 days at an alternative temperature regime of 29:18 ± 1ºC (maximum:minium at cyles of 9-h periods and 3 h periods of increase or decrease) (Vargas *et al*. 2000). The developmental rates of *B. dorsalis* were determined at four alternative temperatures (maximum: minimum) of 24:13, 24:24, 29:18 and 35.24 ± 1ºC, at 60 ± 10% RH and a photoperiod of L12:D12. . Table 4 below gives the published mean total pre-imaginal developmental time (egg to pupa) (days) obtained at varying alternative temperatures for *B. dorsalis*.

Table 4: Mean total developmental time for immature stages of *B. dorsalis* (Vargas *et al*. 2000)

Temperature ºC (maximum : minimum)	Mean total pre-imaginal developmental time, days (egg to pupa)
24:13	39.2
24:24	22.1
29:18	21.5
35:24	20.3

1.2.2.4. Attractants

Similar to *B. invadens*, *B. dorsalis* males respond to methyl eugenol. Females and males of *B. dorsalis* respond to both Torula yeast and the 3-component BioLure, with the pest species having a stronger attraction response to Torula yeast compared to the 3-component BioLure.

2. SURVEY PROTOCOL

2.1. Early detection

A regular detection programme throughout the year should be in place to detect any incursion of *B. invadens*/ *B. dorsalis*. Detection traps should be placed at points of entry such as border posts, sea ports and international airports as well as in production areas of known hosts and cities/towns/villages close to the points of entry. Surveillance traps can also be placed in production areas and marginal areas in other parts of the country. Trapping with Methyl Eugenol and Biolure (3-component) should be carried out to determine pest absence or presence. Fruit fly trapping guidelines provided as Annex 1 to International Standards on Phytosanitary Measures No. 26, Establishment of pest free areas for fruit flies (Tephritidae) should be followed for relevant trapping densities used in different locations (points of entry, production and marginal areas) and trapping procedures.

2.2. Delimiting survey

When one *B. invadens/B. dorsalis* is collected in an area, a delimiting survey should be implemented immediately. The area immediately surrounding each fly find will be a core area of a 1 km x 1 km square grid. Methyl eugenol baited traps and traps baited with food-based attractants (Biolure-3-component or protein attractants) will each be placed at a density of 10 traps per km^2 within the core area (Figure 1 & Table 5). Moving outwards from the core area, there will be three surrounding zones of sizes 8, 16 and 24 km^2. In each of the surrounding zones, the trapping density will be 2 methyl eugenol baited traps per km^2. Additionally, radiating transects of up to 100 km will be put into place from the third surrounding zone and will follow main road networks. Methyl Eugenol baited traps will be placed every 2 km for the first 10 km, every 5 km thereafter for the next 40 km and every 10 km for the 50 remaining km (depending on countries). Moreover, within 50 km radius of the core area, methyl eugenol baited traps will be placed in farms with orchards or fields containing host material. The density of traps in the farms will be determined by farm size, crops and extent of plantings. All traps will be serviced weekly, with core traps serviced daily for the first week. Traps will be maintained through three to four *B. invadens/B. dorsalis* generations (approx. 16 weeks) after the last fruit fly find.

If a fruit fly is found in an additional trap, a 1 km x 1km core area will be established around the fly find and traps will be placed at the same rate as mentioned above.

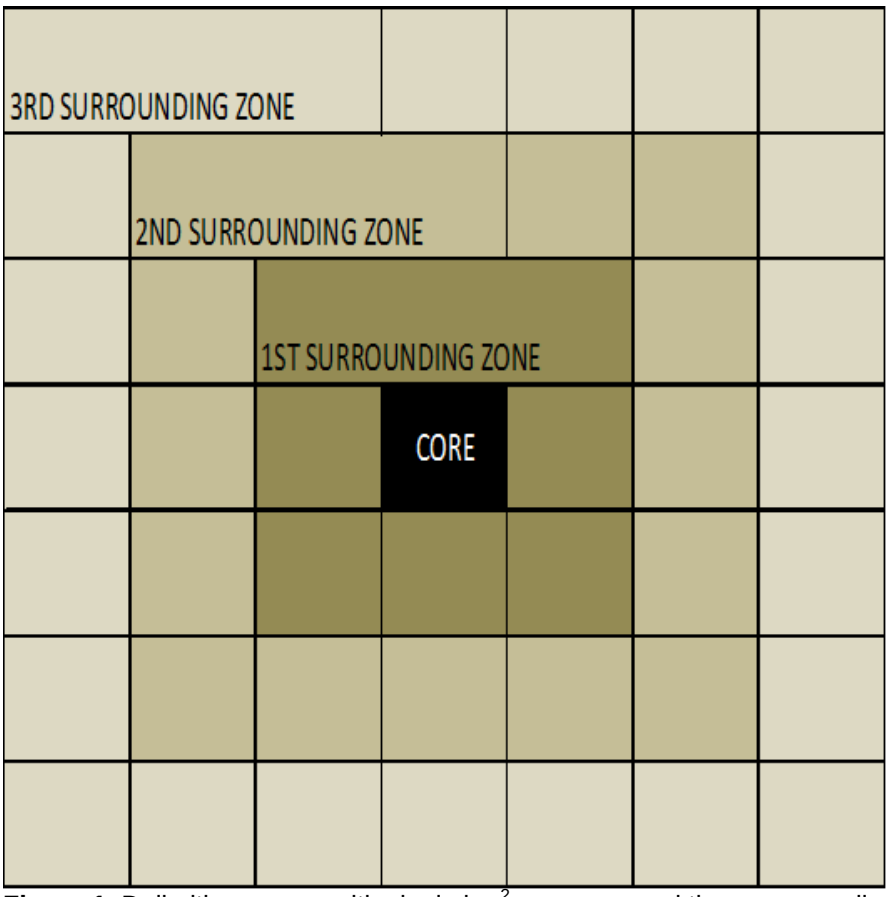


Figure 1. Delimiting survey with single km^2 core area and three surrounding zones

Table 5. Trap density in core and surrounding zones.

Zones	Area/km^2	Number of traps per km^2. Methyl Eugenol (ME) + food-based attractant (food-based attractant only in core area)
Core	1	10 food-based attractant +10 ME
1st	8	2 ME
2nd	16	2 ME
3rd	24	2 ME

Record keeping is essential in a delimiting survey. The geographical coordinates of all traps should be taken and incorporated in a geographical information system. The location of traps should be geo-referenced with the use of global positioning system (GPS) equipment. Records of all trap inspections should be kept by the NPPO and should include trap number, date of servicing, outcome of servicing (catch/no catch), status of trap and replacement of trap in cases where it is gone or damaged, replacement of lure (yes/no) (See Annex 2).

2.3. Fruit sampling

Host fruit from the delimited area will be surveyed, depending on host availability. Infested fruits will be collected and incubated for up to 6 weeks in pupating medium (sand or sawdust) in closed, aerated plastic containers in a facility within the area. Any adult should be killed following emergence and preserved in alcohol or mounted for identification.

3. QUARANTINE

Once a *B. invadens/B. dorsalis* sample is caught in a trap and the identification is done with reasonable confidence by a trained personnel, the area of the fruit fly detection is quarantined with immediate effect to restrict movement of host material, in particular fruits listed above as *B. invadens/B. dorsalis* hosts, cannery waste and soil, out of the area. The initial quarantine area will

extend to a circular area of 5 km radius from the trapping point. The delimiting survey will also be implemented immediately to determine the area of the infestation and therefore also any expansion of the initial quarantine area.
Movement of host material will be regulated in accordance with both relevant local legislation and international trade agreements.
Road blocks, where possible, could be implemented to regulate movement of fruits from the area. At any international point of entry or exit near a detection site, a random check of passenger baggage could be implemented.
All local growers in the area of the fruit fly detection, establishments within the area that handle fruits, cannery waste and soil, as well as the organs of state that would implement road blocks, should be notified of the threat posed by the fruit fly and actions (monitoring and control) that need to be taken.
An area may be removed from quarantine status by the NPPO after the pest has been declared eradicated or there has been no other B*. invadens/B. dorsalis* find for at least 4 generations (calculated from the local climate data, but generally around 16 weeks).

4. ERADICATION PROCEDURES

Eradication of *B. invadens/B. dorsalis* should be initiated following the second detection of *B. invadens/B. dorsalis* in the delimiting survey area. The total area of coverage will depend on the extent of spread. For each *B. invadens/B. dorsalis* detection, the area under eradication will be 25 km^2 surrounding the trap site. Duration of eradication measures should be planned for at least 2 generations of *B. invadens* (generation estimated based on local climatic conditions but generally should be estimated for about 8 weeks). Trapping to verify eradication should continue for at least two *B. invadens* generation (generally 8 weeks) after eradication measures have stopped (no more bait spraying and placement of fresh male annihilation blocks).
A combination of ground applied male annihilation treatments and air/ground applied protein bait treatments (air/ground application in orchards and ground application in residential areas) should be carried out. Fruit stripping should be considered as a contributory measure, where appropriate. Countries should ensure legal authorization of all products required for eradication.

Male annihilation Technique (MAT)
This will involve the distribution of square (5cm x 5 cm) 1.3 cm thick, wooden blocks (fibre-board/soft board/plywood) soaked in a mixture of methyl eugenol and insecticide at a ratio 3: 1 and placed at a density of 400-600 per km^2, either nailed to poles or hung from trees (10 000-15 000 blocks per 25 km^2 fly-detection unit). A single application of MAT blocks will cover a period of 8 weeks.

Protein baiting
Protein bait sprays should be carried out weekly (See Annex 3 for recommended products). For repeated applications, products/insecticides should be alternated to prevent development of resistance. Protein bait should preferably be applied on host trees. In production areas, aerial bait sprays can also be an option.
Optional eradication treatments
Fruit stripping. Fruit stripping is removal of all susceptible fruit on trees. If fruit stripping is undertaken in the core area, stripped fruits should be placed in plastic bags, fumigated if possible and removed for burial under at least 50 cm of fill. The burial site should be located within the quarantined area. Fruit should be left on a few selected trees as “trap” trees. This is a strategy to prevent dispersal of the pest outside of the quarantine area. Fruit lying on the ground under the “trap” trees should be removed, part incubated in the laboratory in the quarantine area and the rest disposed of properly. Additionally the soil under the “trap” trees should be treated with locally approved product (for e.g imidacloprid or entomopathogens such as *Metarhizium anisopliae*).
Treatment of hot-spots: In most eradication programmes there will be some areas where the pest species persists longer than in the main treated areas. This may be because conditions are particularly suitable for pest breeding, the treatments have not been uniformly applied or a host has not been detected and treated. These situations may sometimes be detected by persistent fly catches in the monitoring traps. Where a “hot spot” is suspected, the spray teams should apply additional treatments and the blocking teams check the distribution and placement of the MAT blocks. Where feasible, some sampling and rearing of hosts could also be conducted.

5. IDENTIFICATION & INFORMATION FLOW

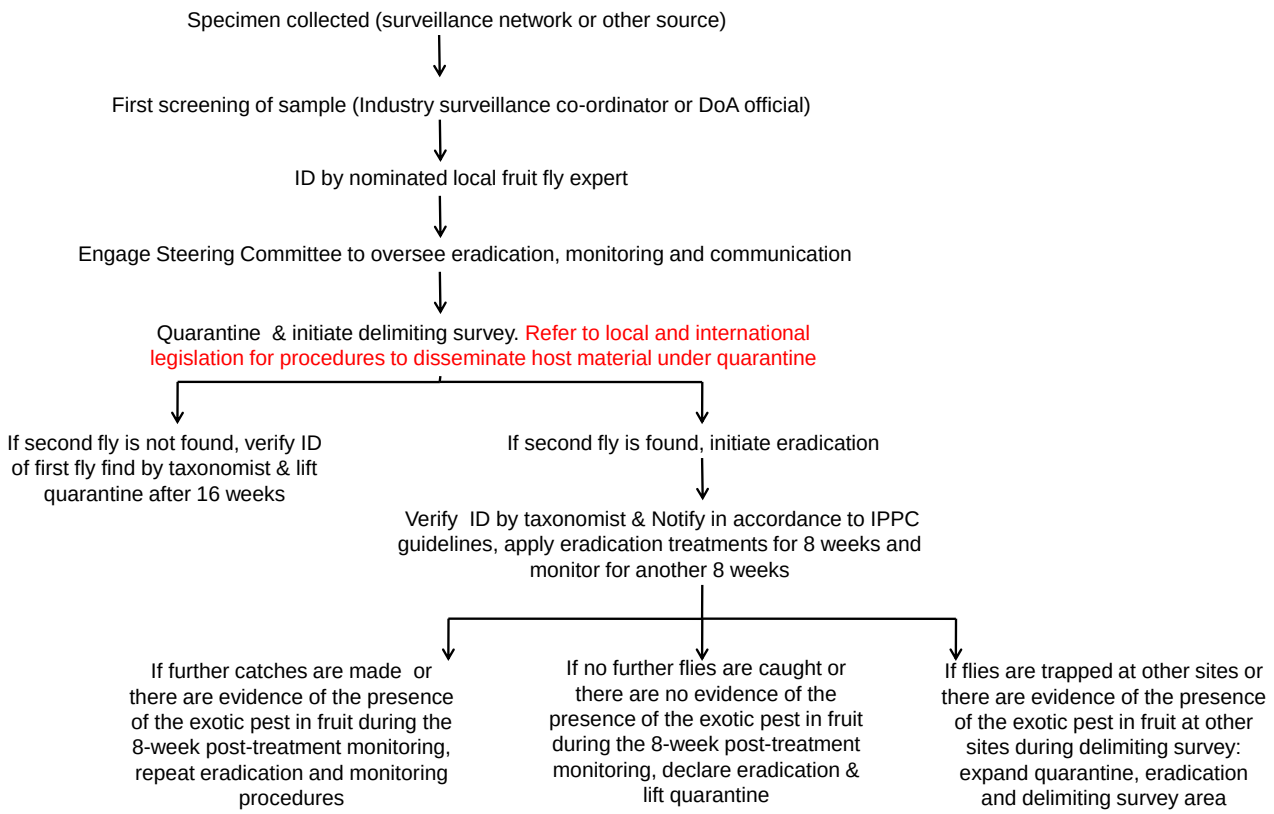
5.1. Identification

During detection trapping, specimens should be collected and first screened by a local designated identifier. Any suspect specimen should be forwarded immediately to the local fruit fly expert in vials of at least 70% alcohol for confirmation.

If a positive ID is obtained from the local fruit fly expert, a Steering Committee should oversee the implementation of the quarantine, delimiting survey and eradication measures as described above. The effectiveness of the programme should be monitored periodically by the NPPO through review of documentation and procedures.

For final confirmation of the fruit fly ID, the specimen should be sent to a fruit fly taxonomist. Care should be taken to ensure that reference samples are preserved in accordance with acceptable scientific procedures.

6. SEQUENCE OF EVENTS



7. TRAPPING DENSITIES AND ERADICATION ACTIONS FOR NON METHYL EUGENOL RESPONDING SPECIES

For exotic fruit flies of high risk to the Indian Ocean Region and which do not respond to methyl eugenol, recommendations on trapping densities and eradication actions are provided for two fruit fly groups: cuelure responding flies and trimedlure or enriched ginger root oil (EGO) responding flies.

7.1 Trapping densities during delimiting surveys for cuelure and trimedlure or enriched ginger root oil responding flies

Given that cuelure and trimedlure are less powerful attractants compared to methyl eugenol, a higher trapping density is required when using these two attractants during a delimiting survey EGO could be an alternative to trimedlure as it is attractive to the same Ceratitis species but it also attracts some other Ceratitis species that do not respond to trimedlure (e.g *Ceratitis cosyra*). The densities of cuelure and trimedlure/EGO traps required in the core and surrounding zones are provided in Table 6.

Table 6. Trap density in core and surrounding zones for delimiting surveys on cuelure and trimedlure/EGO responding exotic fruit flies

Zones	Area/km^2	Cuelure responding species	Trimedlure/EGO responding species
		Number of traps per km^2. CueLure + food-based attractants (food-based attractants sonly in core area)	Number of traps per km^2. Trimedlure (TML)/EGO + Biolure 3C (Biolure 3 C only in core area)
Core	1	20 Cuelure + 10 food-based attractant	40 TML/EGO + 10 Biolure 3C
1st	8	10 Cuelure	20 TML/EGO
2nd	16	6 Cuelure	10 TML/EGO
3rd	24	4 Cuelure	8 TML/EGO

7.2 Eradication actions for cuelure and trimedlure or enriched ginger root oil responding flies

The trigger, treatment area and duration of treatments for cuelure and trimedlure/EGO responding flies should be similar to those recommended for *B. invadens*/*B. dorsalis* (see section 4). Similar protein bait treatments should be implemented for cuelure or trimedlure/EGO responding flies. For cuelure responding flies, male annihilation treatments using cuelure should be combined with protein bait treatments. For trimedlure/EGO responding flies, protein bait treatments should be implemented. Moreover, male annihilation treatments with EGO (such as Last FF) can be used if available.

For cuelure responding flies, the male annihilation treatment will involve the distribution of square (5cm x 5 cm) 1.3 cm thick wooden (fibre-board/soft board blocks/plywood) soaked in a mixture of cuelure and insecticide at a ratio 3: 1 and placed at a density of 800 per km^2, either nailed to poles or hung from trees (20 000 blocks per 25 km^2 fly-detection unit). A single application of MAT blocks will cover a period of 8 weeks.

For both cuelure and trimedlure/EGO responding flies, weekly orchard/vegetable plantation and back-garden sanitation should be implemented in eradication area. Fruit lying on the ground or remaining on trees after harvest should be removed and buried in a designated site within the quarantine area. Optional eradication treatments as described in Section 4 could also be implemented.

8. STOCK OF MATERIALS REQUIRED IN PREPAREDNESS OF ERADICATION AND MONITORING

Materials should be kept in a designated facility in preparedness for a potential exotic fruit fly outbreak. The stock is essential to be able to initiate a delimiting survey and eradication procedures without delay. In the event of an incursion and eradication actions being initiated, replacement of such stock must commence immediately. In the absence of an outbreak, stock of attractants and insecticides should be replaced every 2 years.

For eradication, the quantity of materials to be stockpiled in preparation will be based on units of one fly detection site and 2 months of eradication (See Annex 4 for amount of materials to be stockpiled and conditions for storage of materials). The area of coverage around each fly detection site will be 25 km^2 as mentioned previously. The extent of stock piling (in multiples of single detection site units) is to be determined by the Steering Committee.

For monitoring, the amount of materials required would be based on one fly detection and 4 months of trapping. Four radiating transects will be calculated from the zone surrounding the core area.

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7.3 J.G. VAN ZYL

7.3.1 REPORT ON THE XII WORKSHOP ON SPRAY APPLICATION TECHNIQUES IN FRUIT GROWING (SUPROFRUIT 2013) IN VALENCIA, SPAIN 26-28 JUNE 2013

This past June I was given the opportunity to attend and present an oral presentation at the 12th Suprofruit congress in Valencia, Spain. The workshop was attended by delegates from all over the world, mostly agricultural engineers, plant pathologists and entomologists, focussing on agro-chemical application studies in a wide variety of orchard crops. Seven oral sessions, one poster session and a one day technical tour comprised the workshop. Presentations and posters focused on methods to assess and measure spray deposition and more importantly, especially in European countries, spray drift. This was followed by sessions focussing on dose adjustment and reduction of spray risks through the optimisation of application methodology and better implementation of legislation and EU regulations. New technologies in spray application was investigated and discussed to achieve improvement of application. Lastly a session on mutual worldwide problems of operator training, best practices and sustainability was presented for the improvement of these issues. The contacts made and the knowledge gained will be of great benefit for current and future research. With European legislation on spray application becoming stricter, the need for optimisation of spray application, especially spray volume, keeping phytosanitary restrictions in mind, is much needed for the South African citrus industry.

ORAL PRESENTATIONS

OPENING

Cross, J. et al. Orchard spray application in Europe – State of the art and research challenges.
An overview of spray application in Europe was given, focussing on current machinery used, air adjustment systems, atomisation/nozzles used, real-time canopy geometry and density sensing, drift mitigation, and dose expression and adjustment issues.

Machinery used: There is a gradual change in European countries in terms of machinery to more efficient, “fit for the job” machines e.g. high profile sprayers for larger canopies, multi-row sprayers for increased work rate etc. Still the change is in vain because of growers rarely making adequate adjustments to optimise sprayer performance and reducing run-off and drift. Regular maintenance of machinery is also a big issue. This resulted in implementation of legally required, mandatory machine inspections and grower training and certification across Europe.

Spray drift/atomisation: The biggest issue in Europe, especially countries with a large area of open water (e.g. the Netherlands). The success of drift mitigation through the use of low-drift air induction nozzles, implementation of “no-spray” zones and mandatory buffer zones where certain pesticides are not allowed to be sprayed, as well as the legal enforcement of these factors, were discussed.

Canopy geometry/density sensing for efficient calibration/dose rate systems, improvement of expression of dose rates on pesticide labels: An overview of current and new sprayer technology was given focussing on sensor systems to determine dose rate and sprayer calibration in real-time instead of pre-spraying manual adjustments. Real-time calibration management has the potential to realise chemical savings, reduce drift and exclude grower calibration mistakes but is very expensive to implement in relation to pre-calibration management which is simple to implement, low cost but most of the time neglected by growers, leading to spray misapplication etc. Agro-chemical companies are now being advised to register products in the way it has to be applied, taking in account factors that will influence the application such as type of crop, canopy geometry/density, type of nozzle/sprayer used etc.

Emphasis was given to the fact that application is far from “optimal”, even with state of the art European application tools and methods. Key challenges are:

1. Improving machine design and crop adaptation to improve deposition/ reduce losses including real time calibration
2. Understanding spray deposit/quality/cover and their effects on biological efficacy
3. Dose adjustment

Session 1: Field measurement of drift

Cotteux, E. et al. Comparison of vertical and horizontal collecting methods for spray deposits in crop canopy and airborne spray drift assessment.

Emphasis is put on plant protection products lost (over 50%) in high canopy crops (citrus/banana orchards) due to drift. In this study, a complementary method, consisting of fluorescent tracer and PVC string collectors, to the European standard method (ISO 22866) is described for measuring drift at different horizontal and vertical planes and distances. The method is evaluated and efficiency of measurement determined through a mass balance study.

Gregorio, E. et al. Spray drift measurement using a UV lidar system.

The relationship between spray drift measurements with an elastic-backscatter lidar system and passive collectors are studied. The lidar system proved to be a good system for fast, accurate drift measurement instead of tedious passive collectors such as water sensitive paper. Longer distance measurements (Further than 250m) can be a problem if the lidar is not range resolved. A number of experiments is also needed for quantification of tracer/pesticide with lidar backscattered signal.

Chueca, P. et al. Methodolgy for a fast, in field estimation of efficiency of anti-drift measures.

Different drift mitigation systems were compared to conventional spray methods in different orchard crops. Deposition quantity was measured over distance on the ground and in the air (above canopy). A drift reduction factor was calculated from data (anti-drift and conventional data) as a ratio. Distance of deposition away from the target (symmetry) was also included as a parameter. The symmetry coefficient of the two systems can be used effectively to determine buffer zones and save application methods near open water sources.

Van de Zande, J.C et al. Spray drift reduction of the KWH three-row orchard sprayer. Effect of variable levels of air assistance and nozzle type.

The Water Pollution Act (LOTV) in the Netherlands requires growers to reduce pesticide emission to surface water by 90%. This is very hard to achieve even with drift reduction protocol. Therefore additional regulations has been implemented to reduce drift such as no planting buffer zones that can vary from 3 to 9 m, depending on what drift reduction techniques are implemented. The KWH three row orchard sprayed has the potential to reduce drift and therefore minimise the buffer zone. Al combinations of drift reduction techniques reduced drift significantly in relation to control sprays over distances of up to 7 m at different growth stages. The denser the canopy, the higher the reduction of drift. Deposition in the canopy as well as biological efficacy still needs to be evaluated with this sprayer and drift reducing combinations.

Stallinga, H et al. Spray drift of an experimental mast sprayer spraying high nursery trees.

A Tower sprayer was designed and evaluated for spray drift in comparison to conventional axial fan sprayers used in high (6m) tree crops. Drift was significantly reduced (at 7m deposition measurement with 72%) by focussing spray directly into canopy foliage with the tower spraying in addition to flat fan nozzles and drift reducing flat fan nozzles in comparison to conventional hollow cone nozzles on the axial fan sprayer with spray directed upwards into the canopy. Incorporating a buffer zone 5m reduced drift with an additional 94%.

Future research will focus on incorporating canopy detection systems to switch nozzles on/off when not in direct alignment with the canopy.

Session 2: Evaluation of deposition

Codis, S. et al. EvaSprayViti: a new tool for sprayer's agro-environmental performance assessment.

A framework was designed to simulate vine structure and density at different growth stages with leaves being simulated by water sensitive paper. Although it is a highly efficient system to simulate and accurately replicate spray deposition for the development of leaf wall index calibration systems and sprayer tests, it is still an artificial simulator. The debate on accuracy of deposition on artificial targets vs. real targets (e.g. leaves/fruit) was raised and discussed.

Davy. A, et al. Multiplex: An innovative tool for real-time quantitative evaluation of spray deposit.

A portable sensor was developed to quantify spray deposits in field on artificial and real targets by measuring intensity of fluorescent pigment sprayed. The measurements are still qualitative. Quantification is done by comparing measurements to fluorescent washings since the sensor does not take leaf size or area spray deposited vs. not deposited into account.

Dekeyser, D. et al. Effect of spray application technique on spray deposition and coverage in artificial pear trees.

Different spray techniques were evaluated in terms of deposition quantity on artificial pear trees in an indoor test. Indoor test on artificial trees allows for manipulation of a controlled environment. Again the debate on tests on artificial targets was raised. Sprays were also done statically, which is not comparable to moving sprays in field. However it was still interesting to see the different swath patterns different sprayers realise vertically at different setups.

Llop, J. et al. Spray distribution produced by a hand-held trolley boom sprayer in greenhouses.
Greenhouse pesticide applications will always have a risk of contamination to the operator. Accurate deposition is also required especially under strict MRL legislation with fresh market vegetables. A manual/automatic trolley was developed to optimise greenhouse application and reduce operator exposure. Different nozzles were evaluated with the system. No statistical difference could be observed in terms of deposition between different nozzle systems tested, however, in uniformity, differences were observed, especially between flat fan and hollow cone systems.

Session 3: Dose adjustment

Solanelles, F. et al. Reducing pesticide residues in fruit crops by means of improving spray application techniques.
The program Fruit.Net was launched in and around Catalonia (Spain) to focus on reducing/optimising the use of PPPs. This is done by organising workshops focussing on grower training on how to effectively calibrate sprayers. Apart from compulsory sprayer checks, growers are advised to do this regularly themselves. The program DosaFruit was developed and distributed to help growers determine PPP dosage with concentrate sprays, taking into account orchard characteristics. The system was evaluated over 1 season in pear orchards. Overall, the program realised a reduction of 17% of PPP use throughout the district as well savings of up to 40% per farm. Residue call-backs were also reduced to zero during the season.

Walklate, P.J. and Cross, J.V. Dose adjustment of different types of orchard spraying product.
This talk presented the development of a regulated dose adjustment model for more sustainable and effective use of PPPs. The operational limits/constraints of spray GA (maximum ground area) calibration/dose rate expression was discussed as well as LWA (Leaf Wall Area) dose rate systems for more efficacious calibration and PPP use. With the use of developed equations to determine safe dose rates from GA labels, the incorporation of LWA dose rate calibrations and target density calculations, a 59% reduction in pesticide was realised and the risk of under-dosing reduced by calibrating wrongfully whilst achieving effective biological efficacy.

Garcera, C. et al. Relationship of actual copper deposition and control of the peacock spot disease in olive trees.
Amount of copper deposited after sprays is evaluated and related to biological efficacy. Copper residues were taken from the spray tank as well as from leaves at different canopy positions. Higher copper concentrations were found on lower canopy positions/leaves (run-off and targeted low profile application) than in other canopy positions. Copper residues in the tank and on leaves correlated well (R^2 = 0.90). However, a negative relationship was observed between bio-efficacy on leaves and the amount of copper deposited on leaves, indicating other influencing factors may be affecting efficacy.

Contador, F. et al. Product deposition and distribution on citrus from application with different equipment and the effect of air and water volumes.
Emphasis was put on the citrus tree canopy structure/density/composition, classing it as one of the most difficult targets to effectively apply PPPs to. It was also stated that modern technology and methodology exist to tackle this problem; however, growers are still using traditional methods (new sprayers, air volume and volume change) to solve persistent problems. These parameters were investigated and quantified in terms of tracer deposition. Different combinations of air and water volume and sprayers were evaluated in trial sites worldwide. Significant differences were observed in deposition. Lower deposition quantities were mostly observed in problematic areas such as the centre and top of the canopy, depending on sprayer type (low or high profile). Increases in water (carrier) volume did not improve deposition in these problematic areas. Interestingly, the highest deposition quantity used was 3000 l ha^{-1}, with the volume determined by canopy volume. Results discussed also showed better air movement and canopy penetration in well aerated, pruned canopies, with air volume similar to canopy volume, improving carrier movement into the canopy. However, it was also stated that too high air volume negatively affected deposition and product impingement on outer canopy leaves. Thus, a 2/3 ratio of air volume to canopy volume seem too effective for adequate deposition. Application quality is highly dependent on operator knowledge and professionalism.

Garcera, C. et al. Rationalized pesticide applications against *Aonidiella aurantii* Maskell in citrus.
European directive 2009/128/EC enforces calibration to be done by taking target pest, characteristics of PPP and vegetation surface/volume to be treated. Amount of active deposited, method of deposition, effect on

pest population were evaluated to model and validate above mentioned relationships for the control of California red scale. This was done to provide data for rational dosage of organophosphate+mineral oil and a method for effective assessment of theoretical biological efficacy. Efficacy vs. deposition data showed that lower deposition quantities (µl cm^2) are needed at younger stages of CRS. With adult stages, higher deposition quantities are needed. Data showed that the model can improve calibration and dosage determination and realise pesticide savings of up to 40%.
Fourie, P.H. et al. Optimisation of spray application in South African citrus orchards: challenges and progress.
I presented a talk on application in South Africa, giving a general overview of current application methods, the use of deposition benchmarks for biological efficacy, optimal use of spray adjuvants and applicators, and the challenges producers are facing with PPP application.

Session 4: Reduction of risks
Nilsson, E. How equipment and spray technologies are regarded in National action plans on sustainable use of pesticides.
European directive on sustainable use of pesticides lays down minimum requirements to be implemented (action plans) by European member states. The talk presented these requirements and enforcement of it by local authority. This included application of PPP and application technology (equipment) used; inspection of equipment; aerial application, open water protection; operator safety; handling of PPP, storage and proper disposal. This talk was very interesting in terms of stringent implementation of equipment and calibration checks by local officials. Fines and strikes are given to producers which do not comply. Random spot checks are done year-round. If this type of system was to be implemented in South Africa, the implications would be disastrous for producers.

Wenneker, M., de Bruine, A., Kruijne, R., van de Zande, J.C. How pesticides get into surface water: a case study of emission pathways in fruit growing in the Netherlands.
Reduction of pesticide exposure to open water sources in the Netherlands was a regularly discussed topic at the symposium. Due to the vast amount of open water in the country, and the close proximity of it to orchards, elevated drift reduction to be an enforced law. Drift through regular PPP application has to be reduced by 90%. This is difficult to achieve, and high concentrations of pesticide actives is still found in ground water. Methods were discussed to reduce drift and also why some of the implementation of drift reduction methods is not effective enough such as operator neglect and insufficient enforcement.

Black, C., Shaw, A., Borges, Ofelio, B., Holheisel, G. Assessing interest in new woven pesticide applicator protective garments with repellent finish in Washington State.
Pesticide labels were evaluated (USA) to establish what type of garment/clothing/chemical protectant clothing is needed when applying what pesticide and with what type of machine (tractor – with or without canopy). Also, surveys were made in Washington State to evaluate what is being used by applicators. It was found that few pesticide registrations require adequate protective clothing. Also, most producers do enforce applicators to wear applicable clothing (long shirt/pant/respirator) but not chemical protective clothing (due to apparent heat stress observed due to non-permeability of chemical resistant clothing. Research is being conducted on new more applicator friendly materials/textiles to reduce operator exposure.

Session 5: Drift Assessment
Salcedo, R., Granell, R., Garcera, C., Palau, G., Molto, E., Chueca, P. Validation of a CFD model of the effect of an orange tree canopy on the air flow produced by an air-blast sprayer.
Computational Fluid Dynamics (CFD) is an effective numerical system to map/study the airflow to/through canopies. Citrus canopies are usually large/dense which creates high resistance to air penetration when sprayed with air blast/shear systems. This in turn complicates trajectories of spray droplets and therefore impaction on target and off target drift. In this study, Airflow was measured actively with anemometers throughout the canopy on the inside and outside as well as in orchard rows adjacent. The data was subjected to CFD and airflow modelled with different constants. It was found that most low profile airblast sprayers caused vortex on top and behind canopies, negatively affecting deposition and penetration. This model and CFD method used was very effective and it would be interesting to apply such technology in South Africa to evaluate canopy and spray machine air flow.

Salyani, M., Larbi, P.A. Model to predict spray deposition and losses in citrus applications.
An empirical model was designed to predict deposition and losses when applied with certain spray applicators. The model takes sprayer design, operating variables, tree geometry/density and climatic conditions into account. It was very interesting to observe that the study found that target canopy distance is the most important factor leading to variation in deposition, with canopy density, and volume following. Sprayer speed and air speed was less/ to not significant in very dense canopy situations. The model has the potential to help plan accurate spray parameters to optimise deposition.

Duga, A.T., Defraeye, T., Hendrickx, T., Dekeyser, D., Nuyttens, D., Nicolai, B., Verboven, P. Sprayer-canopy characterization using field experiments and CFD modeling.
Four different types of canopies were sprayed with three different spray machines and deposition evaluated and compared on leaves, stems and ground. This was done at the same tractor speed and spray volume (interestingly 6.2 km h^{-1}, 500 l ha^{-1}; "high speed, low volume). The CFD model used takes into account air speed, airflow, air jet velocity, droplet movement and nozzle characteristics. Deposition was measured with metal tracers. Good correlation was made between deposition in different canopies with different types of machines, if the model was adequately corrected with parameter changes. The CFD model approach was again proven to be a useful tool in spray machine development and canopy/ or trellising systems.

Garcera, C., Molto, E., Salcedo, R., Chueca, P. Influence of wind in citrus drift measurements using ISO 22866.
ISO 22866 – Legislation that depicts the standards for measuring drift generated by "equipment for the application of plant protection products". All newly designed machinery must adhere to this legislation to be built, distributed and used. Wind is one of the major causes of drift (whilst spraying) and is one of the factors that is often neglected or not focussed on enough when implementing said ISO law. Citrus trees are also bigger and denser than other crops which in turn influences drift in other ways. This ISO law cannot always be accurately applied on machines designed to spray citrus. The talk focused on amendment of the ISO 22866 to be used more effectively for measuring drift in citrus. The study showed that the legislation do not take wind speed and direction properly into account and gives solutions to how it can be effectively measured to reduce drift.

Balsari, P., Marucco, P., Tamagnone, M. Proposal of a methodology for assessing spray drift from air-assisted sprayers to enable their classification according to drift risk.
The implementation of ISO 22866 to test applicators was labelled as redundant and expensive since it generalised over different types of application on different crops. Potential drift generated by spray machines was evaluated by measuring spray deposits at different height and length intervals when used with drift mitigation strategies e.g. anti-drift nozzles, buffer zones etc. The test benches/collectors used was more efficient in collecting deposits than that proposed by ISO 22866. Testing potential drift with the proposed method has the potential to classify machines on drift potential quickly and easily.

Planas, S., Solanelles, F., Torrent, X., Camp, F., Gregorio, E., Rosell, J.R. Comparing standardized methods of potential drift assessment.
Different drift assessment protocols used in Europe have been evaluated. Some of these legislations enforced by local authorities differ from country to country in Europe. In Spain, these legislations have been introduced. The presentation was more of a discussion between countries on the harmonisation of assessment protocols throughout Europe to ease parallel trade and mutual recognition. Simple drift reducing nozzles was used to explain how the different regulatory systems differ from each other, showing the differences in deposition when measuring drift reducing potential with the same nozzle with different regulation methodologies. It would be a catastrophe if such a regulation system should be implemented in South Africa, as well as the enforcement of it.

Session 6: New technologies on spray application
Nieuwenhuizen, A.T., Van de Zande, J.C., Wenneke, M. Autonomous precision spraying in fruit orchards.
Orchard spraying can be very arduous, often being the reason for treatment failures since operators lose focus after a while. It can also be costly in terms of labour for the time spent spraying. An autonomous spraying system was developed to replace the human applicator. A tractor was adapted with a teach and playback system, with the applicator or farmer only spraying an orchard once, the system learning the route, and then for the next spray doing it automatically. If the system encounters a human shape or deviate from the drive plan it will automatically stop. The sprayer also is automotive, spraying only when the canopy detects foliage/fruit. It also calibrates spray volume and chemical concentration to canopy specifics through real time imaging and LIDAR readings.

Zhu, H. et al. Development of variable-rate precision spraying systems for tree crop production.
The presenter stated that, referring to the presentation given on the first day by Jerry Cross, spray application in Europe is still very inefficient. The presentation focussed on two newly developed sprayers: a variable rate hydraulic boom sprayer and a variable rate air-assisted sprayer. Both machines used ultrasonic sensing together with the variable rate nozzles – It detects the canopy size and volume as well as the tractor speed, and calculates the flow rate of the spray mixture accordingly. The air-assisted sprayer had laser scanning sensors which helped for more accurate density calculations for determining air speed/flow. With this type of variable rate system, spray volumes were reduced by 86% for the hydraulic system and 70% for

the air-assisted sprayer, in relation to no variable rate systems. Uniformity of deposition was also greatly improved with both systems.

Llorens, J., Landers, A. Precision fruit spraying: digital canopy measurement for air and liquid control.
The presentation focussed on research in precision spraying; real time measurement of the canopy and subsequent real-time adjustment of liquid and air flow on the sprayer. A manual louvre has been designed to adjust the air leaving the sprayer. The problem is it has to be manually adjusted by the operator – increasing operator exposure. Landers stated that manipulating volume by adjusting forward speed is not recommended since penetration and coverage is affected. A system was developed and installed consisting out of ultrasonic-sensors, each connected to a group of four different flat fan nozzles (13 groups-installed in tower form) (interesting that they use flat fan nozzles) that is controlled by pneumatic manifolds – separately or individually. Depending on the canopy, nozzles are switched on and off (a specific nozzle or group of the four nozzles) to manipulate flow rate. Studies are underway to connect the system to the louvre to manipulate air flow automatically.

Holowinicki, R., Doruchowski, A., Godyn, A., Swiechowski, W. Variable air-flow discharge system for orchard sprayers.
The presentation was about the development of an energy efficient variable air-flow discharge system for continuous independent (left and right hand of the tower) airflow adjustment on the sprayer. Preliminary results have shown that the design of the turret enables to manipulate airflow/wind speed to the canopy. The system is also implementable on “smart sprayer” systems.

Triloff, P., Knoll, M., Lind, K., Herbst, E., Kleisinger, S. Low loss spray application: a concept for more efficient and safer crop protection in tree fruit.
Low volume, low drift spray application has been the norm for spraying in Europe since the 1990s. The concept of low loss crop protection consist out of low loss spray application (Optimisation of air and spray liquid distribution; individual sprayer testing and adjustment with air and spray liquid test standards; canopy adapted, low loss/low volume application) + canopy adapted dosing and application + training. All these factors were evaluated to and formulated into a reaction plan to improve drift mitigation. All sprayers to be sold in regions where this plan is implemented needs to be tested and “certified” before sale. For example, machinery with poor air distribution (photo below) that increases spray drift will not be certified for sale.

Fig. 7.3.1. Axial fan sprayer with poor air distribution.

Tamagone, M., Balsari, P., Marucco, P. Tank agitation device performance: overview of present situation in some new sprayers.
Certification of new sprayers also requires tests to evaluate tank agitation to ensure that the pesticide sprayed, stays in solution uniformly throughout the spray tank. It was found that most agitation systems performed poorly (keeping copper oxychloride in suspension), especially at higher capacity tanks (3000 l tanks). Mechanical stirrers were found to be inadequate (67% mean deviation from reference concentration), whilst horizontal injectors and pump back flow was found to be the best (20% mean deviation from reverence concentration), but still not good enough. An investigation into South African sprayers into what type of agitator is used will be interesting.

Session 7: Train operators to promote best practices and sustainability
All five presentations were in relation to TOPPS-prowadis (Train Operators to Promote best Practices and Sustainability) (www.topps-life.org). This included how the program was started, how study material was

decided on, how to implement the training programs locally etc. Training includes guides to water protection, point sources management, diffuse sources management, the use of sprayers in optimised manner and remnant management. The implementation of TOPPS during extension is a way to refresh growers on the influences of spray application and will be discussed at the pest and disease management workshops.

Technical day tour

On the technical day tour we visited IVIA (Instituto Valenciano de Investigaciones Agrarias) outside of Valencia to attend a sprayer demo attended by some local and international sprayer manufacturers. These machines were all low profile axial fan sprayers, not suitable for the South African market. However, the designs of the machines were interesting, especially the machines where air speed could be adjusted in real time. Very low volumes (in South African context) were applied (2000 to 4000 l ha^{-1}).

Fig. 7.3.2 and 3. Hardi (left) and FEDE (right) axial fan spray machines demo.

Fig. 7.3.4 and 5. Variable air speed fans of FEDE (left) and Martinez-lozano (right) machines that is adjusted in real-time to adjust spray-plume to canopy density/volume.

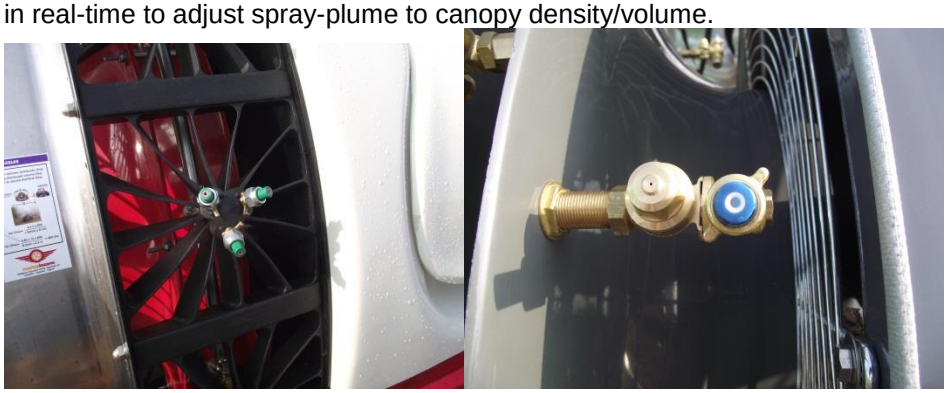


Fig. 7.3.6 and 7. Low drift nozzle configurations (ATR/TVI/AVI-ceramic) is a common sight on all sprayers.

After the demo, we departed to Vicente Gandia for a wine tasting and lunch after which we went to the FEDE plant to go and look at some more sprayers. The most interesting machine was the low profile axial-fan sprayer “Frontier” with an ultrasonic sensor for canopy detection and real-time dose adjustment (Fig. 7.3.8).

Fig. 7.3.8 and 9. Ultra-sonic sensor (left) and real-time control unit (right) of the Frontier sprayer.

Conclusion

This was by far the most informative and involved workshop/conference that I have ever attended. The amount of knowledge gained and contacts made at the conference is a great input to the spray application research program. The biggest topics that got my attention were: the amount of attention researchers, chemical companies etc. put into operator training and safe and concise application; the amount of attention given to drift and pollution mitigation; optimal spray application; spray application technology and; the enforcement of legislation by authorities in terms of spray application. It is of great concern to me that if European spray legislation is to be implemented (by Global Gap etc.) that South Africa will not be able to adapt fast enough which in turn will affect market access dramatically.

Listed recommendations

- Continuing the study of spray application for the improvement of safe and efficient application in South Africa.
- Promoting good practice and understanding of spray application through regular grower/industry meetings/engagements/extension.
- Getting the basics right: Equipment, calibration, operators and application are not up to the standard that it has to be in South Africa – This step will improve disease/pest control dramatically and prime industry for change to more optimal application protocols in the coming years.
- Systematic focus change to above mentioned "optimised application study/methods/equipment" e.g.
    - Reduced volume application calibration study and implementation
    - The use/study of niche equipment/technology
    - Anti-drift technology
    - Operator safety, exposure and pollution management
    - Optimising integration of spray application into IPM

All PowerPoint presentations and the book of abstracts are available in PDF format. For any information contact me (gideonvzyl@sun.ac.za).

7.4 P.H. FOURIE

7.4.1 ATTENDANCE OF THE INTERNATIONAL SOCIETY FOR CITRUS NURSERYMEN (ISCN) CONGRESS IN MAZATLÁN, MEXICO, 14-19 FEBRUARY 2014

Since 1981, the ISCN has been a forum of international citrus nurserymen where technical and scientific matter pertaining to citrus tree production is discussed. South Africa has been involved in the ISCN since inception, most notably through the leadership of citrus nurserymen, including Patrick Niven, who was a founding member, past-president and patron of the society.

ISCN2014 was held in Mazatlán, Mexico, with pre- and post-congress tours in Florida and California, respectively. Due to other commitments, I could not attend these tours, but realised that this was indeed the most valuable part of this congress; especially for nurseries. Although long, the tours visited various nurseries where operational matters were demonstrated and discussed.

The congress involved to days of lectures, a workshop on nursery practices and a field trip. The lectures were mostly practical and are listed below. Unfortunately, quite a number of the lectures were in Spanish with simultaneous translation. Highlights from these presentations are briefly noted. Many cultivars were introduced, but these are not discussed in this report.

Selected highlights from congress programme

- *Overview of the citrus nurseries in Mexico* - Dr. Javier Trujillo, General Directorate of SENASICA

Mexico has 850 thousand hectares citrus, of which 61% is planted to oranges, 19% to lemon/limes and 20% to soft citrus. Initially they did not have an improvement scheme, and the industry was established with cultivars introduced from USA (California, Texas, Florida) and Spain. Some diseases were inadvertently imported and only since 2001 did Mexico get a certification scheme. Presently, their scheme is compulsory and serves 52 nurseries producing 10 million trees per year. They have one germ plasm bank, 5 foundation blocks, 20 bud producers, 13 seed producers. Of the 52 nurseries, 44 are producing trees in insect protected structures. Diseases in Mexico include psorosis, cachexia, exocortis, tristeza (CTV), leprosis and recently also huanglongbing (HLB; Asiatic citrus greening). Production loss from HLB is estimated to be 14% within 1 year and 35% in 5 years. Their control strategy for HLB involves a clean stock programme, regional psyllid control and eradication of infected trees.

- **Keynote**. *Overview of new varieties and the accompanying commercialization models of different entities around the world* – Etienne Rabe

Dr Rabe presented an overview of commercialisation models and the various aspects involved private cultivar management. He also gave some overview of some new varieties from Biogold and indicated certain gaps where new varieties are needed; these included early navel, productive late navel, seedless Valencia, early and mid-season mandarin cultivars.

- **Keynote.** *The future of the global citrus industry: new scions, rootstocks and approaches*. Dr. Fred Gmitter

Dr Gmitter from University of Florida gave an excellent talk about new cultivars and the challenges of cultivar breeding. His talk focussed on latest trends in breeding, as well as his projected needs for new cultivars. A major challenge for breeders is the lack of fruit quality markers that can be used to screen breeding lines prior to cropping; naturally such markers will fast-track selection. New cultivar needs were summarised as sweet oranges for fresh and orange juice use, seedless easy-peeling mandarins, 'healthy' grapefruit with lower furanocoumarin content, new citrus types, stress and disease resistant rootstocks. On the latter need, and in the USA-Florida context where their citrus industry is being devastated by HLB, he noted that it is "disease resistance or extinction". He gave an overview of the origin of citrus, and also some new cultivars emanating from their breeding programme.

- *Chilean Citrus planting trends, challenges and nursery business* - Montserrat Pérez

Chile produces 21600 ha citrus of lemon (7500 ha), oranges (7500 ha) and mandarins (600 ha). Recent growth in mandarin plantings (W.Murcott and Tango) was described as massive. Given their remote location, Chile is relatively free from citrus diseases and pests. Chile's certification scheme was established in 1990 and was a voluntary scheme. The industry abandoned the scheme in 2005 and since has started to cut budwood from orchards. This is a matter of concern, and the industry is criticised for being in a false sense of security. Import of new cultivars into Chile is described as very difficult with strict bureaucratic inspectors. Imported cultivar introduction is not via shoot-tip-grafting and occurs via government or private quarantine facilities, with government facilities taking 3 years before cultivars are released.

- *Advantages and challenges of centralized propagation material supply experience from the Southern African Citrus Improvement Scheme* - Dr. Paul Fourie

I presented an oral presentation giving an overview of the SA CIS, highlighting the advantages and challenges of centralised budwood supply. The abstract of my presentation is shown below:

The objective of the southern African Citrus Improvement Scheme (SACIS) is to increase the profitability of the southern African Citrus Industry by ensuring that growers are supplied with nursery trees of the highest possible quality made from the best genetic citrus material and being free from any harmful pathogens. The SACIS is presently a voluntary private scheme operated by Citrus Research International (Pty) Ltd (http://www.citrusres.com/cis/about) for the southern African Citrus industry, the largest exporter of shipped fresh citrus fruit. Although voluntary, the SACIS has >95% participation amongst the 22 certified nurseries and growers. The SACIS is operated from the Citrus Foundation Block (CFB) biosecure multiplication source and is the primary supply of rootstock seed and citrus budwood in South Africa. Centralised budwood supply has many obvious advantages, most importantly the assurance of biosecurity and traceability, and economy of scale. Budwood supply from certifiable trees in nurseries is authorised as secondary supply source, but only in cases when the CFB is unable to timeously supply demand. Over the past few years, seasonal budwood supply in the SACIS ranged between 2.5 and 3.2 million buds per season. Over the years, CFB's ability to supply demand has declined from 100% to 62%; as a result, a larger proportion of budwood was authorized for cutting in certified nurseries. It is clear from these figures that the dynamics of budwood supply have changed significantly (2003-2013). This change is largely

ascribed to cultivar popularity, the increase in number of cultivars supplied, as well as the proportion of privately managed cultivars. The latter ranged from 19.1% to 47.5% of the 67 to 192 cultivars supplied in the 2003-2013 period. Budwood supply dynamics are clearly changing and are indicative of the rapid changes in demand for cultivars dependent on the success or failures of cultivars in overseas fresh fruit markets. The challenges and future strategies to improve the CFB's ability to timeously supply demand will be discussed.

- *Management of a certified organic citrus nursery in Peru* - Klaus Bederski

Klaus owns and manages Topara nursery, a certified organic nursery in Peru. They have no Phytophthora or nematodes, no HLB, canker, blight, leprosis or CVC, but they do have endemic CTV. The nursery is an open nursery, and only imported material is kept in closed quarantine structures. The EU-organic certified Topara soil mix consists of sand and a compost consisting of cow manure and chicken feathers. They are also investigating a new plant medium grown from *Azolla fuliculoides*, which shows slow water release properties and takes up 200% of its weight in water.

- *Thermotherapy for Huanglongbing-free (HLB or Greening) sweet orange propagation*. Jose Lima

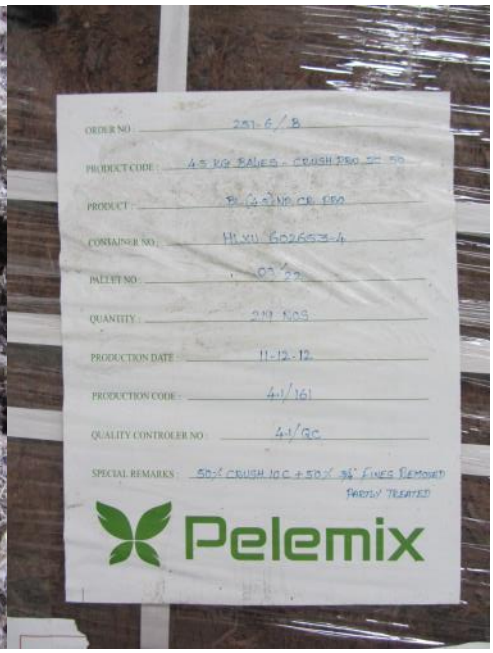
Jose Lima presented a very interesting talk on steam treatment of budwood for HLB protection. Higher temperatures reduced bud take, but take was >90% at 51°C for 10 min or 3 x 2 hours at 48°C. Both regimes appeared to eliminate HLB from the budwood based on PCR assays.

- *Genetic diversity of tristeza virus from cross-protected and unprotected citrus challenges after 20 years of natural challenge in Peru* – Klaus Bederski

Peru started their cross-protection programme after CTV caused the total death of trees on sour orange rootstock from 1950 to 1965. Surviving trees that were CTV tolerant were used as interim parents. CTV cross-protection was studied with help from Chet Roistacher and CTV strains from California. Various CTV strains and rootstocks are being evaluated and cross-protection and tolerant rootstocks have indeed saved their industry.

- **Keynote.** *Technology and citrus nurseries.* Roger Smith

Roger Smith from Tree Source Nursery in California presented an overview of technologies that can reduce the cost, time and labour needed in citrus tree production. His advice was for nursery operators to be open-minded, test and evaluate the technology in small- to large-scale trials, and to calculate the return on investment (RoI) before implementation. He believes the RoI should be 2-3 years. Many menial and labour intensive nursery tasks can be mechanised with tools such as seed extractors and planters, pot fillers, track or trolley tree moving systems, steam sterilisers of pots. Technology can also improve the precision management of tree production: environments systems measuring temperature, RH, CO2 and light (trees in California must be grown in insect-proof structures where measurement of environment in important), as well as inventory management by means of barcoded systems and stock management software. Container technology improved from bags / sleaves to plastic re-usable containers with open slots in sides and bottom to promote drainage and air-pruning of roots. Tree Source Nursery has been working with Proptek in the development of a citrus pot that fits into a raised 8-pot tray. The trays slide into a racking system in their nursery, thereby raising the post to waist level. The criteria for a suitable substrate or plant medium includes total porosity (50-70%), air-fill porosity (20-30%), bulk density (0.5-0.8 g/cm^3), fertility, water holding capacity (40-60%), pH (5.5-6.5), low dust fraction, EC level, pH and carbon level. Most nurseries in the USA have changed over to using coco-peat or coir as substrate. Technologies linked to this new substrate include coir-mixers, pot-fillers and conveyor belts (see photos below).



- *Intensive production of citrus plants in containers Agrinet* - Horacio de la Concha

The HDPP tree pots by Agrinet used less medium per pot, and realised faster tree development. However, pots have to be retrieved from growers and cleaned before re-use. See pictures of Agrinet pots below.

- *Container root systems and options for improvement.* John Cooley

John Cooley from Proptek presented a talk on the Proptek pots, which they developed with Tree Source Nursery. Their pots are ribbed and have slide and bottom slots to reduce root circling and promote air pruning. Other products include air-pots for larger trees and 'Ellie' pots for seedlings. See photos

below. 

- *Defining the right coir with suitable irrigation and fertigation strategy. Roy Peleg*

Roy Peleg, a technical consultant to Pelemix who is one of the largest coir suppliers to citrus nurseries, presented a talk on coir as citrus nursery tree substrate. Roy confirmed that an ideal substrate needs 30% air/water ratio and a high cation exchange capacity (CEC). Coir is made from coconut fibres and the major suppliers are in India and Sri Lanka. In his opinion, other suppliers' coir is ground too fine. Coir contains micro-capillary fibres and therewith a high degree of porosity and water holding capacity. It is a stable medium as fibres consist of 85% lignin and hemi-cellulose. Other characteristics are weight/volume of 75 kg/m^3, balanced pH of 5.8-6.2, CEC of 140-200 meq/100 gr, high in potassium and low in nitrogen, uniform, good rewetting ability and importantly also free from pests, diseases and nematodes.

The coir “Citrus mix” (middle photos) contains ¾-inch coir (top photos) mixed with crush (bottom photos) with no dust fraction (photos from Roy Peleg). Irrigation in coir is dependent on the physical properties of the coir mix; the finer the mix, the slower the vertical movement of water and drainage but with better horizontal movement, and vice versa. Other factors, such as dripper spacing and container size should also be considered. Based on trials in citrus nurseries, they saved 60-100 days in tree production cycle when using the “Citrus mix” coir.

- *Good quality and cost-effective alternative substrates for citrus container nursery stock growth.* Deng Ziniu

China produces 75 million citrus trees per year, of which 70% is open field and 30% in containers. For the latter, 320,000 m^3 is needed annually. Medium cost makes up 30% of tree cost, compared to a quoted 2% in USA. Hence, finding an alternative cost-effective and sustainable substrate is a priority. Of various substrates investigated, bio-charcoal from citrus canes showed most promise.

New cultivars

- *Effect Investigation of Huanglongbing Prevention and Control by Planting Virus-free Citrus Seedlings in Guangxi*. Deng Chongling.
- *Two new IVIA triploid hybrids of mandarin*; Alborea and Albir - Pablo Aleza.
- *Changye Cheng – a new bud mutant super orange* – Wu Houjiu.

Other presentations

- *Development and cycle duration of citrus seedlings cultivated using different substrates, water management and irrigation system under protected cultivation, in state of São Paulo, Brazil.* Fabian Goncalves.
- *Evaluation of mobile bar system for citrus nursery automated irrigation*. Dr. Sergio A. de Carvalho.
- *Daily citrus fruit growth rate in Baja California Sur* - Dr. José Ramírez.

GENERAL RECOMMENDATIONS

At this congress, the Citrus Foundation Block joined the ISCN. The ISCN is a very dedicated and applied forum specific to the needs of citrus nurserymen. The openness with which information was shared is commendable. It is highly recommended that South African nurseries join the ISCN and participate in future congresses. The next ISCN congress will be held in Australia in 2017. Participation of this congress is also

recommended. South Africa is poorly represented in ISCN; to my knowledge only one SA nursery is a member.
In terms of global citrus biosecurity, citrus nurseries and improvement / certification schemes are at the forefront of preventing disease incursions and spread. Moreover, once disaster has struck (for example, HLB in Brazil and Florida), or is imminent (for example, the HLB in California), nurseries are vitally important in managing the threat. The ISCN is therefore in my opinion a vitally important forum of which global citrus biosecurity should be a core objective.

7.5 J.J. BESTER & P.J.R. CRONJÉ

7.5.1 REPORT ON A TECHNICAL VISIT TO CALIFORNIA 2-17 MARCH 2014

The purpose of this technical visit to California was to investigate the latest pre- and postharvest technology in orchards and packhouses, manipulation and pruning techniques on navels, late mandarins and clementines, traceability from orchard to shelf, as well as alternative extension models and the latest trends in research. The aim of this document is to report back on the highlights experienced during this trip from a technical point of view. See itinerary attached.

It was mind boggling to experience the large scale on which crops are produced in California, with the citrus industry valued at close to $2.1 billion. Over the last years the main citrus production regions moved from the southern areas around Riverside to the central regions of California, where grapes, stone and pome fruit are also produced. This move was primarily driven by the ever increasing pressure of urbanisation in areas close to Los Angeles.

Fig. 7.5.1. Nules Clementines (5 km long rows) at Paramount Citrus

The soils in the Central Valley have a pH of 7.5 – 8.0 and are deep homogenous alluvial soil types with good drainage, which explains why *Phytophthora* root rot is not a big concern. In addition, the absence of fruit fly results in very low levels of pre- and postharvest decay. Low humidity results in very low *Penicillium* inoculum in the orchards, therefor orchard sanitation is non-existent.

California is experiencing a major drought at the moment. Though the main production region is a semi-desert with scrub like vegetation, the area is very much dependent on high rainfall in the far northern parts and high snowfall on the Sierra Nevada Mountains during winter months. In addition to the constant supply of water via the snow cap, it is believed that the snow and the resulting cold winds from these mountains is the main reason why citrus fruit can be hung late in the harvesting season, while maintaining internal quality. Severe losses were suffered during the current season due to a freeze in December, resulting in a 30-40% loss in volume, affecting supply to such an extent that the producers

of navels and mandarins cannot fulfil the current domestic market demand. As a result of that, the price for mandarins is almost unreasonably high at the moment, compensating to some extent for the loss in production. Installation of expensive gas driven fans at $30 000 per 4-5 ha is the only viable option in addition to increasing the humidity prior to the expected cold temperature by means of irrigation.

The main focus in the San Joaquin (Central Valley) of citrus production is on soft citrus, especially mandarins, followed on a much smaller scale by navels, Valencias and lemons, with only the odd grapefruit plantings in certain areas. The production of soft citrus is consolidated to a large extent and the three largest producers, i.e. Paramount Citrus, Sun Pacific and Fowler Packing, account for 80 percent of soft citrus production in California. The total plantings of Paramount Citrus alone, which is 20 000 ha, is equivalent to a third of the total South African citrus production and consist of 3000 ha Navels, 1000 ha Valencias, 3000 ha Nules Clementine, 3000 ha
W. Murcott (Nadorcott), 3000 ha Tango, 3000 ha grapefruit (Rio Red, mainly in Texas), 1500 ha lemons and 300 ha Minneolas. To extend the soft citrus season, experiments where W. Murcott and Tango are left on the trees well into flower and fruit set, to determine if such a practise could further stretch the harvest window, are underway. This whole enterprise belongs to one single grower in a fully integrated company from sourcing of new cultivars, propagation in its nursery, production, packing and marketing and under its own brand.

The California Citrus Mutual's 2014 Citrus Showcase
This mini one-day symposium was well attended by the major role players in the citrus industry. It consists of various workshops, running in parallel, and advertising booths similar to the CRI Citrus Research Symposium, but on a much smaller scale. The focus of the day was Huanglongbing (HLB), as this is being seen as California's major threat at this point in time.

During the inaugural breakfast session, "Water with Breakfast", Felicia Marcus (Chair of the State Water Resources Control Board) gave a key note address. Similar issues and challenges to our industry were briefly discussed. ***Availability of water, contamination and salinity should be carefully managed and researched for sustainability of our industry. More attention should be given to recycling of water.***

Huanglongbing (HLB) and Asian Citrus Psyllid (ACP)

The following workshops were attended at the CCM 2014 Citrus Showcase:

- Fighting Back Against ACP: Presenters – Nick Hill (Chair of the Citrus Pest and Disease Prevention Program), Dr Mary Palm (USDA) and Mike Sparks (President of Florida Citrus Mutual).
- Is There Hope for Resistance in Citrus to the ACP and HLB: Presenters – Fred Gmitter (University of Florida), Ed Stover (USDA-ARS) and moderator Mary Lou Polek (CRB).
- Progress on Pre-Symptomatic Detection of HLB: Presenters – Carolyn Slupsky (University of California, Davis), Alexander Aksenov (University of California, Davis), Wenbo Ma (University of California, Riverside) and moderator Georgios Vidalakis (University of California, Riverside).

In Florida $70 mil has already been spent fighting HLB. The disease moved 30 miles up north within two years, and during the last 14 years Florida suffered a 40% reduction in production and 8000 job losses. Citrus Health Management Systems (CHMS), consisting of the University of Florida, state agents, USDA-APHIS and growers, were established to coordinate control measures. At least eight sprays per year are applied, to control the vector, at a cost of up to $2000 per acre per year.

Although HLB was detected only in March 2012 in the Hacienda Height area of Los Angeles County in the south of California, the ACP was detected further to the north as well. An HLB Multiagency Coordination Group (MAC), including federal, state and industry representatives, was established for high level of coordination in combatting HLB. Since backyard trees planted in urban areas are a high risk of inoculum, programmes were put in place to educate the public on the symptoms and control of the disease through training of rare fruit growers, master gardeners (mostly retired teachers), landscapers and retail nurseries. They make use of various communication channels like magazines, newsletters, bookmarks, radio & television, presentations at seminars and garden shows, and various websites, even You Tube videos. The federal government made $25 mil per annum available for five years to fight the disease, a total of $125 mil. It became compulsory for citrus nurseries to put up greenhouses to ensure HLB-free trees are planted in the orchards.

Because the ACP was found in residential areas, repeated chemical treatments were not sustainable. Therefore a biological control program was developed to control the psyllid using the parasitic wasp *Tamarixia radiata*. Biocontrol rearing facilities are currently being built on a large scale to rear the wasp. The focus of plant breeding is on host resistance to *Candidatus Liberibacter asiaticus* (CLas), both for scions and rootstocks. Screening of scions showed certain varieties to be less affected than others. Highly affected areas in China were visited to look for natural selection for resistance against HLB, especially where only one unaffected or escape tree was found amongst heavily affected trees, to be used for identifying resistance genes. Seventeen rootstocks were identified in the trials that are less affected and three large rootstock trials will be planted out soon to screen for tolerance against HLB. Although most of these rootstocks are new, it was mentioned in a personal conversation that X639 is one of those rootstocks showing good tolerance. Thermotherapy on trees, at 40˚C for 48 hours, seems to eliminate CLas, but the problem is that the roots remain a reservoir for the bacterium.

Further research focus on the development of early detection tests for CLas. Since there is a lag phase between infection and symptom expression, the ACP can spread HLB long before visual symptoms are apparent. One method is to detect metabolite changes before leaves start to test positive with PCR. Metabolites are formed in reaction to CLas infection and clusters of these metabolites are closer in infected than in clean material. A profile for any type of citrus can be generated in metabolic test kits. A second method is detection of plant diseases by smell or volatiles. A device, called the VOC Sniffer, was developed by UC Davis to detect not only HLB in individual trees, but also CTV, and can even distinguish between the two. The third method is detection of HLB using secreted proteins of CLas. Branch imprinted assays can allow growers to detect HLB in the orchard prior to symptom development.

It is very clear from lessons learned in Florida that HLB, specifically CLas, is a devastating disease that must be taken up very seriously. The growers, retail nurseries and public, especially the Indian population in KZN who travel to and from the Indian subcontinent, should be trained on the symptoms and effects of HLB, as well as the potential dangers of bringing in plant material. Under no circumstances can we afford to underestimate the potential threat of introduction of HLB into SA and the subsequent disastrous consequences. Importation of citrus trees from Brazil into Angola should therefore be stopped with immediate effect! It became evident that phosphate plays a role in the expression of the symptoms. Foliar applications of phosphate don't cure the plant, they only mask the symptoms for some time. The effect of phosphate foliar applications on African greening should be investigated.

Cryopreservation is a method whereby single buds from plant material can be frozen and stored for long periods of time. This method is currently developed by Dr Gayle Volk of the USDA Plant Germplasm Preservation Research Unit in Fort Collins in collaboration with Dr Georgios Vidalakis. This method should be refined to make a back-up of all our varieties, which could enable us to make a back-up of our whole gene bank in a single freezing unit.

Pre-Harvest Practices and Technology

Many orchards were visited mostly at Paramount Citrus and Fowler Packing, as well as a few at Mulholland Citrus. By far the most were mandarins, planted since 2000 and therefore still fairly young. In general all the orchards are very well managed. Almost all the trees are planted on ridges, which is not really necessary on those deep alluvial soils. The orchard floors are kept clean of any weeds or grass to assist in managing freeze damage. The small trees are hardened off before the winter by reducing water and nitrogen. Large air fans, one per four hectares, are installed to circulate air during cold spells, preventing the formation of inversion layers and subsequent cold damage during winter. Trees are irrigated well before expected cold fronts to increase the humidity in the orchards.

Irrigation in all orchards is scheduled with different measuring instruments. The deep soils are well drained and over-irrigation and *Phytophthora* root rot problems are therefore almost insignificant, as is *Phytophthora* brown rot. Fertigation is the general way of applying fertilisers to the soil. Foliar application on the mandarins is an important way of manipulating the crop and up to eight foliar applications per season is not unusual. The nitrogen application on late mandarins seems to be higher (at app 2.6%+) than in South Africa, something to take note of. Nitrogen application amounts to approximately 180 kg/ha for Nadorcott and Tango, and 130 kg/ha for Clementine.

Nadorcott (W. Murcott) and Tango are the most planted soft citrus varieties for the last couple of years, certainly for the enterprises visited. General tree spacing is in the range of 2.5 x 5.0 metres. The only difference between the two, except for seed content, is the fact that Tango is ten to fourteen days

earlier than Nadorcott. The size is somewhat smaller than the most desired marketing counts and can only be manipulated by means of fertilisation, irrigation and pruning, since the use of Corasil and Maxim is not allowed for use on citrus in the USA. Due to very strict regulation of agrochemicals in the USA, none of the auxins currently available to the SA citrus producers will be available for use in California for the foreseeable future. Production of Tango on Sour Orange rootstock is more consistent than that on Carrizo in the same orchard and Sour Orange seems to be a good option as rootstock with good internal quality. Although Tango trees on C35 are smaller than on Carrizo, production on C35 is higher. C35 seems to be a very promising rootstock in combination with Nadorcott and Tango, to control vigour and improve production. External fruit colour of trees on Australian trifoliate is very good, but fruit size is too small. Most mandarin plantings, however, are on Carrizo.

The Nadorcott trees are netted for five to six weeks during the blossom period to prevent pollination by bees. The cost of the nets is $5000 per hectare, but it lasts for five to six years. The cost of labour for pulling it over and removing it is $2000 per hectare. One tractor with its team of 4 people can net six hectare per day.

Another very promising variety in California is the Daisy SL, which was displayed at the Citrus Showcase. It must be kept in mind, however, that *Alternaria* is not a problem in California. The colour and internal quality is excellent. The Gold Nugget also has a very good taste and flavour, but the rind is somewhat rough. A variegated lemon with a pinkish flesh was also at display, but the marketability of this cultivar is doubtful.

Fig. 7.5.2. Tango trees with excellent crop.

Fig. 7.5.3. Herbicide applicator.

Pruning is a very important practice to manipulate the crop. The ideal is to set a year on year crop on soft citrus of 60 – 65 tons per hectare of the desired fruit size in order to optimise returns. Citrus is seen as income sensitive, and not cost sensitive, meaning that sensible spending on production will ensure better returns. Pruning starts off directly after harvest, followed by midsummer regrowth control (thinning and topping of vigorous shoots), to create more bearing points. Crop load is still the most effective way of controlling vegetative growth. These varieties are terminal bearers and fruit are normally set on wood older than six months. These mandarins are pruned at least three times per season to control tree size and manipulate crop load and fruit size. Drastic intervention is needed on very large trees and what is called “table top cut” and “stag horning” is under investigation in some of the older orchards to maintain tree size and productivity of the orchards. Nules Clementines are performing well under those climatic conditions, mostly in combination with Carrizo. Clementines are pruned more heavily than in SA and that is something that should be addressed by CRI Extension.

Orchard sanitation is not an important practice in California as in SA, since there is no FCM or fruit fly and the inoculum of *Penicillium* is very low. Lots of fallen or stripped fruit were lying in some of the mandarin orchards, but no green- or blue mould was observed whatsoever. A few fruit were seen with mould in one navel orchard, but it was white mould and didn’t seem to have sporulated. The humidity is very low and the fruit can hang much longer on the trees without getting soft or puffy. Before netting the Nadorcott trees, the area between the rows is cleaned with an implement called a “sweeper” to remove twigs and leaves and prevent damage to the nets. The “sweeper” could be a very useful implement to remove leaves, twigs and fruit from underneath the trees into the rows to get mulched or removed in order to control CBS.

Fig. 7.5.4. Sweeper removing twigs and leaves. **Fig. 7.5.5**. Forklift used in orchards.

No picking platforms or any mechanical harvest techniques were used for harvesting the fruit on any of the farms visited. It is the personal view of Etienne Rabe that he will wait for the deciduous industry to sort out a mechanical harvester before they will consider it. The fruit is harvested from the ground or with the use of picking ladders where necessary. Tree height is maintained at 2.5 - 3.0 m for optimal management and ease of picking. Cost of labour is very high and farmers are either making use of brokers, or paying minimum wages plus incentive, per picking team. To minimise costs, systems were developed whereby picking bins are placed within very small walking distances from the pickers. When filled, a modified forklift with bigger wheels will collect the bins and put it together next to a road for loading it onto trucks, to be transported to the packhouse.

Pre-harvest technology to be developed and tested in South Africa includes:

- The sweeper for removing CBS infested leaves under the trees.
- The modified forklift to speed up and simplify harvesting.
- The stem applicator, which is a boom mounted onto a tractor with an electronic eye or toggle switch that gets activated by the stem for delivering a specific dosage of chemicals onto the stem. Bayer has the patent for the latter in Australia, but this should be fairly easy to develop locally.
- The modification of herbicide application fixtures to the tractors, half round metal cover over the nozzles, enable the herbicides to be applied under the foliage without damaging leaves.
- The electrostatic spray machines are rated very highly in California and these should be further evaluated locally.

One of the most prominent nurseries in the Central Valley, Mulholland Citrus, is trying out a new potting mix which looks very promising. This potting mix consists of 25% fumigated sandy soil, 25% decomposed saw dust and 50% peat. The seven litre bags used in this nursery are open at the bottom and stand on bare soil. Apparently *Phytophthora* root rot is not an issue in the Central Valley.

Postharvest Practices and Technology

From very poor packhouses, with up to 100% resistance against Imazalil and TBZ, to probably the most advanced packhouses in California, at Fowler Packing and Paramount Citrus, were visited. Since there is nothing relevant to report on poor packhouse practices, only the practices at the best packhouses will be discussed.

The low humidity and subsequent low levels of inoculum of *Penicillium* and *Phytophthora* makes control of decay somewhat easier than what we are used to. *Rhizopus* was the biggest problem on soft citrus during the current season. Fruit are picked and delivered to the packhouse, normally within 12 hours. The most favourable treatments and handling practices at the packhouses are, with a few exceptions, fairly similar to CRI-recommendations. Fruit is entering the packhouse through a dry dump with chlorine (ORP of 850mV at pH 6.5 – 7.0), running over brushes while washed with alkaline soap (pH 8.8 - 9.0), rinsed under sprayers with clean water and then dried with sponge rollers and fans before it gets waxed (storage wax with 3% solids plus imazalil) and dried in the drying tunnel. Chlorine at low concentration, or paracetic acid, can be used in the dump tank for treating organic fruit.

Fig. 7.5.6. Fans drying fruit before waxing.

Fig. 7.5.7. Fixed sliding curtains on trucks.

When limes are getting waxed before storage, 250 ppm Gibb is added to the storage wax to keep the fruit green for longer periods. Limes can then be stored for three weeks before they start to colour up. Yellow lemons can be stored for up to 12 weeks when 75 ppm Gibb is added to the storing wax. Gibb at 75 or 250 ppm is also applied on well coloured oranges early in the season to allow longer storage. After storage, fruit will be put through the packline again and then be treated with the appropriate fungicide, depending on market requirements, in the flooder. The flooder is seen as an improvement on the fungicide bath as application of the fungicide can be better managed and allows for much more flexibility compared to a fungicide bath.

Pre-sorting into different categories by size, colour, blemish (visible light technology) and internal sugar levels (NIR), followed by re-binning, are a common practise in California. Advantages of pre-sorting where fruit is separated by colour before degreening, allow for different exposure periods as well as an immediate culling of fruit not viable for export. This practise could lead to saving on wax and fungicides and release the pressure on the rest of the packing facility.

Guazatine is not allowed on citrus in the USA. It was mentioned at one of the packhouses that brown rot can be controlled postharvest with potassium phosphate at 4000 ppm. Sodium bicarbonate is widely used in packhouses in California to control blue- and green mould. After treatment with sodium bicarbonate, the fruit is rinsed with clean water to prevent any rind burn. Since application of chemicals can be better managed with the flooder system, it might be worthwhile to do further research in SA on the use of sodium bicarbonate as a GRAS chemical for the control of moulds.

Although inoculum levels of fungi are fairly low, packhouse sanitation is done on a daily basis with either a QAC, chlorine, alcohol or a chlorine degreaser. Waste water is running into a reservoir where it is cleaned up by the county officials for recycling. Heating up of the waste water from the fungicide bath or flooder will kill all blue- and green mould (10 min at 60°C). Packhouses are using the JBT bin scrubber with chlorine at ORP of 850mV to clean and sanitise the bins.

Fig. 7.5.8. Bulk bin for industrial fruit.

Fig. 7.5.9. Automatic bagging carousel.

The new packhouses are well automated. For example, the Paramount Citrus packhouse for lemons, oranges and Minneolas packs 60 000 cartons (40 lbs/18 kg) and 110 000 bags (automated bagging carrousel) in three shifts per day, with only 500 people. The mandarin packhouse packs 70 000 cartons (3500 bins) and up to one million bags in two shifts, of 10 hours each, per day, with 644 people. These packhouses have an operating efficiency of more than 90%, but continuous improvement projects are undertaken to further reduce the reliance on humans by optimising the automation processes.

Solar panels on the roofs supply more than 50% of the energy needed to run the packhouses, something that is lacking in our industry. Another way of saving energy is by using a flooder instead of a fungicide bath, as well as using air-knives or fans, installed above sponge rollers, to dry the fruit before waxing.

Technology that could add value to our industry is as follows:

- The automated bin scrubber from JBT which effectively cleans the bins with the use of multiple directional brush heads and disinfectant.
- Installation of solar panels at all packhouses to save energy.
- Fans or air knives to dry fruit instead of the first drying tunnel in the packline.
- A forklift with a device that can pick up a bin of fruit and dump it into any other container or bulk-transport vehicle.
- The tri-wall bulk bins used in California are designed in such a way that the cores in the corners are not required to stabilize and support the bins, which can make it cheaper than the bulk bins in our industry.
- Harvest Mark has developed a software program whereby a unique code per pallet allows individual growers to trace their fruit, with a cell phone, per scan point through the logistical chain, from the packhouse right to the country of destination.
- NIR technology is used in new packhouses to differentiate between fruit based on Brix° levels.
- Equipment to measure the density of the fruit is also available and can be used for sorting and culling fruit that is dried due to frost damage, sunburn, etc., or fruit with low juice content. Fruit density is a function of fruit volume and weight.

NIR technology is, however, a constant process of calibrating the software with samples from the orchards that will be packed, as large variation between areas, cultivars and picking dates will negatively influence the accuracy of NIR measurements. Optical sorting for decay is not adequately sorted out. Sorting of fruit in UV light for decay are used for all cultivars, except Nadorcott and Tango, as decay in these cultivars do not show the same sort of fluorescence, which is probably to do with an aspect around rind biochemical composition. The use of NIR to sort fruit according to sugar content should be implemented, as supermarkets in the US are already pushing for guaranteed higher Brix° levels after being made accustomed to minimum Brix° guarantees.

Extension

The University of California plays a significant role in extension in the Californian citrus industry through the division of Agriculture and Natural Recourses. Researchers and extension specialists at UC Riverside, and UC Davis in particular, are spending quite a lot of time to train other role players, like UCCE farm advisors, county ag commissioners, state and local inspectors, pest control advisers, citrus growers, nurserymen, packers, master gardeners and rare fruit growers.

Pest control advisors play almost a similar role to private consultants in SA. It is compulsory for them, however, to attend a certain number of training courses each year in order to keep their status. They have to make the recommendations for fertilisers and pest and disease control products, or have to sign off any recommendations made by somebody else. Farmers are not allowed to use any fertilisers or chemical products unless signed off by a pest control adviser.

Websites play an integral role and are powerful tools in extension, but the cost of setting up and maintaining a website is expensive, since a couple of fulltime employees are needed to keep all the information updated. Other extension channels include online courses, seminars, workshops, field days, grower magazines, newsletters, the UC Citrus Production Manual, demonstration projects and social media, depending on the target audience. Growers can also get warnings, weather data, important dates and other critical information via their cell phones.

Research

Researchers funded by the citrus industry via the Citrus Research Board (CRB) have to do an oral presentation on their research proposals twice a year at an open meeting to the research committee. The committee will then normally call a meeting a week later to decide on which proposals to fund, or continue funding thereafter, and researchers are informed accordingly.

It is quite evident that very few young researchers are coming through the UC system in all disciplines. However, the large pool of postgraduates from China, India, Mexico, etc., is used to identify promising researchers such as Dr Georgios Vidalakis and Dr Wenbo Ma. It was evident that research and training of plant physiology/horticulture is not a current focus in California, with only Drs Carol Lovatt and Mary Lu Arpaia left in the UC system and both will retire in the next few years. Bigger enterprises like Sunkist, Paramount and Fowler are employing or training their own technical people. The funding and support from the federal government on a major threat like HLB is astounding and stands testament to the effective lobbying done for the citrus industry in Washington. The evident disaster due to the severity of this disorder, as well as the various errors made in Florida, could also have resulted in improved focus by the California industry.

During discussions with the various researchers and technical personnel the following topics for potential future research were identified:

- There are a few options that should be tested in the orchards to manipulate the vegetative and reproductive balance in order to decrease the impact of alternative bearing, as well as improve colour and fruit size. Prof Lovatt is of the opinion that the use of ABS (Protone from Valent Bioscience) could be developed into a valuable management tool. She also said producers should be aware of build-up of uniconazole (Sunny) in the soil with possible negative side effects.
- Sodium bicarbonate should be evaluated to use as a GRAS chemical in the flooder for control of *Penicillium.*
- Cryopreservation is a technique that can be useful to safeguard the South African citrus gene pool by storing a back-up of all varieties.
- Has the role of phosphate as foliar application on the symptom expression of African greening been investigated?
- Quanti-gene is a new technique that can have up to thirty six beads for detecting viruses in plant material.
- The VOC sniffer can most probably be modified to detect African greening and CTV in orchards.
- Techniques for early detection of HLB should be followed up on.

Conclusion

The value of a very successful and insightful technical visit like this lies in the experience gained first hand and new ideas that can be tested and implemented over time in our industry to add value to citrus growers and packers and the industry as a whole. Highlights of the trip include improved and more precise manipulation of the late mandarins, new ideas to be tested by horticultural and pathological research, new equipment that can be either developed locally or imported for use in our industry, and to have experienced the awareness, co-operation and serious approach towards devastating threats to a valued citrus industry.

After visiting a large first world country like California, we realised that we have every reason in the world to feel proud of the high level and quality of citrus research and technology transfer that is being done in the South African citrus industry. This is mirrored in the successes achieved by a couple of South Africans in the Californian citrus industry. Etienne Rabe is not only employed in a senior position in the biggest single citrus farming enterprise (Paramount Citrus) in California, but is also chair of the Citrus Research Board. Henk Du Plessis is production manager of Fowler Packing and Pierre van Rensburg is production manager of the northern part of Paramount Citrus.

Itinerary

Date	Time	Visit / Event	Contact
Tuesday 4 Mar 14	8:00	Riverside UC	Prof. Carol Lovatt Dept of Botany, Univ of California, Riverside
	11:30	JBT/Riverside	Charlene Jewell Postharvest Technical Manager,JBT FoodTech Riverside, CA
	14:00	Corona College Heights Packhouse	John Demshki Charlene Jewell & Tom Gillum
Wednesday 5 Mar 14	8:30	Riverside	Dr Tracy Khan Curator, UC Riverside Citrus Variety Collection UC Riverside Campus
	10:30	Riverside UC	Dr Georgios Vidalakis Dept of Plant Path & Microbiology Univ. California, Riverside
Thursday 6 Mar 14	8:00	2014 Citrus Showcase Visalia (Mini Symposium) www.cacitrusmutual.com/	Prof Etienne Rabe Citrus Research Board & Paramount Citrus
	17:00	Citrus Research Board	Ken Keck (CEO)
Friday 7 Mar 14	3:00	Harvest Watch Traceability Systems, Harvest Mark	Tony Luna & Juan Garibay Redwood City, CA
Monday 10 Mar 14	10:00	Parlier UC Davis, Lindcove	Dr Beth Crafton-Cardwell & Dr Mary Lu Arpaia Univ Calf. Davies, Parlier Calf
	14:00	Mulholland Citrus	Tom Mulholland
Tuesday 11 Mar 14	8-9:00	Compact technology (Visalia) Vision sort (Visalia) – Fancher Creek Packhouse	Charlene Jewell, Don Lawrence & Don Armson Darryl Parker & Jeff Pilegard
	14:00	Paramount Citrus	Pierre Van Rensburg
Wednesday 12 Mar 14	8:00	Fowler Packing (orchard systems, harvesting and mechanisation)	Henk du Plessis
Thursday 13 Mar 14	10:00	Paramount orchards & packhouse	Prof Etienne Rabe Paramount citrus Bakersfield, California Freddie Hernandez & Tarcisio Ruiz
Friday 14 Mar 14	10:00	Paramount orchards & packhouses	

8 EXTENSION 2013-14

By Hennie le Roux, Hannes Bester, Keith Lesar, M.C. Pretorius, Dawid Groenewald, Andrew Mbedzi and Melton Mulaudzi (CRI)

Die 2013 Seisoen

Die finale volumes sitrus wat die afgelope seisoen verpak is, was 17.9 mil kartonne pomelo's, 8.4 mil sagtesitrus, 10.6 mil suurlemoene, 25.4 mil nawels en 51.0 mil Valencias, om ŉ rekordtotaal van 113.3 mil kartonne te gee. Met die uitsondering van pomelos kan dit as die beste sitrusuitvoerseisoen ooit beskou word.

Die seisoen is deurgaans deur die EU se onwetenskaplike standpunt teenoor sitrusswartvlek oorheers, maar gelukkig het die vrese dat uitvoere na die EU vroeg gestop sou word, nie gerealiseer nie. Uitvoere na die EU is uiteindelik gestop vir 2013 na 36 onderskeppings. Dit het egter eers in werking getree nadat die plulseisoen verby was.

FCM was die grootste rede vir afkeurings deur PPECB. Suurvrot het sporadies voorgekom, maar andersins was die bederfsituasie goed onder beheer. Die interne gehalte was besonder goed. Die voorkoms van bederf was effens hoër as die vorige seisoen, maar aangesien die mark sterk was, is minder eise deurgegee. Skildefekte was volgens die ETP soortgelyk aan verlede seisoen. Vruggrootte was, veral op Valencias, oor die algemeen klein, maar het goed verkoop danksy die sterk mark.

Die 2014 Seisoen

Die skatting vir die verskillende sitrus tipes vir 2014 is 15.8m kartonne pomelo's, 9.2m sagtesitrus, 12.1m suurlemoene, 25.8m nawels en 47.0m Valencias, om ŉ totaal van 110.0m kartonne te gee.
In die meeste noordelike areas was dit droog tydens blom waarna die algemene reënval bo gemiddeld was, met uitsonderlike hoë neerslae teen die einde Maart. Heelwat bewolkte dae het voorgekom gedurende die tydperk Januarie to Maart. ŉ Bruinvrot-waarskuwing is per Snykant uitgestuur. Hael en windskade is ook vanuit verskeie gebiede gerapporteer. Alhoewel hierdie klimaatsuiterstes skade aangerig het, is produsente oor die algemeen tevrede met die oes, met die uitsondering van sekere Delta Valencia boorde wat agv die droeë klimaat tydens blom min tot geen oes op het nie.

Tydens die Xsit roadshow is die konsep van vrylating van steriele valskodlingmotmannetjies aan produsente verduidelik met die doel om Xsit se dienste in die noordelike produksie areas uit te brei. Die boodskap was baie duidelik dat FCM as fitosanitêre plaag ŉ groot uitdaging vir ons bedryf kan inhou in die toekoms.

ŉ Besoek aan Swaziland deur Chris Kellerman en Hennie le Roux het weereens bevestig dat Swaziland se sitrusareas aan die afneem is agv die kompetisie met suikerriet en piesangs en die swak vooruitsigte in die mark vir pomelos. Indien daar nie ŉ heelwat soeter pomelo ontwikkel word nie, sal hierdie tendens voortduur. Pomelos is, van al die sitrustipes, die tipe wat die meeste onder druk is.

CRI-PTF (CRI Na-oes Tegniese Forum)

Geakkrediteerde kartonvervaardigers en die toets van hulle kartonne: Hierdie proses is volgens skedule gedoen. Deel van die proses was ook om volledige inligting te verstrek oor die papier wat gebruik word. Sappi se SANAS geakkrediteerde laboratorium het al die toetse op SAPPI se kostes gedoen wat baie deur die bedryf waardeer word.
Dit het vroeg in die jaar aan die lig gekom dat twee kartonvervaardigers, wat nie geakkrediteer is nie, begin het om kartonne aan produsente te verskaf wat vervaardig is met papier wat nie aan die CRI-PTF se spesifikasies vir papier voldoen het nie. ŉ Kort berig daaroor is in die 31 Mei uitgawe van die CGA se CEO nuusbrief geplaas. In die berig is daar ŉ beroep op produsente gedoen om kartonne slegs by geakkrediteerde verskaffers te koop.

Beskikbaarheid van plaaslik vervaardigde papier was kommerwekkend gedurende die afgelope jaar. Dit het veral oor "Virgin Liners" (VL) gegaan. Wat plaaslik vervaardigde VL betref is die bedryf 100% van Sappi afhanklik. Baie hoë vlak samesprekings met Sappi het op ŉ gereëlde basis plaasgevind en tydens gesprekke in Mei het Sappi se topbestuur weer opnuut die versekering gegee dat hulle nie sal ophou om VL vir die Sitrusbedryf te vervaardig nie. Daar is begin met navorsings- en ontwikkelingswerk op kartonne, wat met ander tipes/meer koste-effektiewe papier vervaardig kan word, sonder om die stapelsterkte van die reeks uitvoerkartonne nadelig te beïnvloed. Tesame hiermee het ondersoeke voortgegaan om van meer ingevoerde papier gebruik te maak. Die wisselkoers het dit egter moeilik gemaak. In Mei is samesprekings

gevoer met twee senior beamptes van Billerudkorsnäs, Swede. Hulle “Semi-chemical fluting” is van hoogstaande gehalte. Proewe om met hulle Billerud Fluting te loop, is beplan.
As deel van voortdurende werk op meer koste-effektiewe kartonne, is ŉ proef met A15C kartonne met korter flappe by Schoeman Boerdery gedoen. Dit was ongelukkig nie suksesvol nie. Openinge tussen die flappe word te groot en dit kan beserings aan vrugte veroorsaak en die vou en lym van die kartonne word bemoeilik. Dit mag probleme gee met flappe wat oopgaan.

Op 24 Mei is al die koelkamers in Durban besoek. Die doel van die besoeke was om eerstehandse inligting te kry oor die toestand van kartonne en palette. CRI-PTF was aangenaam verras oor die algemene toestand van kartonne en palette. Die kartonne en die palette het baie goed vertoon. Die enkele palette wat nie goed gelyk het nie, was veroorsaak deur rowwe hantering. Die paletvervaardigers verdien spesiale vermelding. Die verbetering in die kwaliteit van die palette vanaf 2012 is dramaties.

Sekere konsultante in die Wes Kaap is betrokke by ondersoeke/projekte in die vrugtebedryf. Ongelukkig bestaan die neiging dat sagtevrugte, druiwe en steenvrugte met sitrus vergelyk word. ŉ Projek is in die Wes Kaap geloods om meer vrugte in “Hi-cube containers” te laai. Dit kan met sagtevrugte, druiwe en steenvrugte gedoen word, maar nie met sitrus nie, aangesien die maksimum vraggewig van 25,9 ton reeds in die oorgrote meerderheid van die gevalle met sitrus oorskry word.
Hoë verhogings in die prys van papier: Suid-Afrikaanse papiervervaardigers het aangekondig dat die prys van hulle produkte verhoog gaan word. Die verhogings was effektief vanaf 1 Oktober 2013. Die verhoging vir Sappi se Virgin Linerboard (VLB) is 18% en Fluting (F) 14%, Mpact Paper is 15% vir Wit Linerboard (hulle vervaardig glad nie bruin VLB nie) en 13% vir F. Gebaseer op Sappi se prysverhogings is die A15C karton se prys (slegs die papier komponent) met R1,10 per karton verhoog.
Lomold Plastics het groot vordering met plastiese palette gemaak en die eerste gietstuk is nou op bestelling. Hulle beplan om redelike grootskaalse proewe met die 2013/2014 druiweseisoen te doen. Ongelukkig is die plastiese palette op hierdie stadium aansienlik duurder as hout palette en daarom sal plastiese palette oorsee verkoop moet word om dit enigsins kompeterend met hout te maak.

Anti Split Plates. (ASP) word op die punte van hout pale en balke gebruik om te voorkom dat die hout kraak. Twee baie kleinskaalse proewe is by Schoeman Boerdery en Bosveld Sitrus op palette gedoen, waar die ASP aan die onderkant van die palette op die vier hoeke ingekap is. Dit maak die palette stewiger en sal tot ŉ mate ook die deurbuiging van palette in rakke verminder. Die grootte wat gebruik is was 100X70mm. Die lysprys vir hierdie grootte is R1,43 stuk, dus ŉ addisionele koste van R5.72 per palet vir die materiaal. Arbeidskoste om die ASP op die palette aan te bring moet nog bygesit word. Sitruspalette wat volgens spesifikasie vervaardig word, veroorsaak egter nie probleme nie, en daarom sal dit moeilik wees om die addisionele koste te regverdig.
Daar is ook weer gekyk na die moontlike gebruik van “Bliss Style 400x300mm Telescopic Carton”, met sykante en bodem in enkelwand bord met twee dubbelwand kopkante. Ondersoek is gedoen maar ongelukkig oorheers die nadele die voordele. Dit is dus nie ŉ opsie as ŉ meer koste-effektiewe karton nie.

Sappi het gedurende die jaar amptelik aangekondig dat hulle met die produksie van Sappi se Stackraft High Performance Liners staak. Kartonvervaardigers is nou verplig om ŉ swaarder VLB te gebruik. Proewe is gedoen en die resultate toon dat die swaarder VLB dieselfde stapelsterkte gee as die ligter Stackraft. Ongelukkig gaan die swaarder VLB die kartonne ietwat duurder maak, maar die kartonvervaardigers wat Stackraft gebruik het, het met Sappi onderhandel om hulle tegemoet te kom met prys.

Sappi is nie meer met Tree Wrap (TW) betrokke nie. River Bioscience was op ŉ stadium betrokke by die verspreiding van TW. Daar word verneem dat daar nie meer enige voorraad van TW by Sappi beskikbaar is nie. Intussen het ŉ maatskappy met die naam van Rotopak begin om TW te vervaardig en hulle maak gebruik van Obaro om die TW te bemark. Die Sappi TW is verkoop teen ŉ prys van R0,17 stuk en na verneem word is die huidige prys nou R0,42. In samewerking met Houers word die moontlikheid ondersoek om die papier in rolvorm aan die produsente beskikbaar te stel. Houers gaan “reel ends” gebruik, dit in ŉ wydte van 210mm sny, en dan sny die boer dit net in lengtes van 380mm. Hierdie opsie sal op een of ander wyse aan die boere bekend gemaak moet word. Dit sal aansienlike besparings meebring.

DAFF het ŉ skrywe uitgestuur dat slegs A15C Supervent en Oop Vertoon-kartonne vanaf 2014 na EU Lande uitgevoer mag word. Dit het ongelukkigheid veroorsaak onder produsente wat T64 en “Bulk Bins” (BB) gebruik. Volledige proewe is gedurende die 2012 seisoen met T64 Supervent kartonne gedoen en die resultate het baie duidelik getoon dat daar geen verskille in die tempo van verkoeling tussen die standaard en die eksperimentele T64 Supervent kartonne is nie. Die standaard T64 karton is gevolglik ook op die lys van goedgekeurde kartonne vir EU lande geplaas. Alle opsies om kostes te verlaag word deurgaans ondersoek. As deel hiervan word die moontlikheid van die uitvoer van groter volumes vrugte in Bulk Bins

ondersoek. Die koste van die pakmateriaal, 70 A15C kartonne teenoor BB, 2-hoog gestapel, lyk soos volg: Kartonne: R878,22. BB: R501,07. Hierdie kostes sluit slegs pakmateriaal in.
Papier vir toedraaipapier (wrappers) word nie meer plaaslik vervaardig nie. In samewerking met die Sappi Technology Centre se personeel is begin met ŉ ondersoek om te kyk of ŉ geskikte papier nie by Sappi se Enstra Meule vervaardig kan word nie. Die plaaslike toedraaipapier was voorheen deur Nampak Tissue vervaardig. CRI-PTF is tans besig met ŉ ondersoek om te bepaal hoe ingevoerde produkte met die vorige plaaslike produk vergelyk.

Gedurende Oktober is vergaderings met APL Cartons, Corruseal, New Era, Sunnypacks, Houers, Mpact en Nampak gehou. Karton-spesifikasies vir 2014 is onveranderd gehou vir "Virgin Linerboard" en "Semi-chemical Fluting".

Na die skerp prysverhogings in papier is die bedryf nou onder meer druk. Gedurende die 2013 seisoen is verskeie proewe gedoen waar die middelste "liners" op die A15C binnestukke met ander tipes papier vervang is. Daar is onder andere gekyk na Bayplex, Superflute, Nampak se fluting asook Mpact se H-pact en Nampak se High Performance liners. Laasgenoemde twee liners is "waste based" produkte. Die resultate was baie wisselvallig en daar is eenparig besluit dat hierdie navorsingswerk in 2014 voortgesit moet word en dat die spesifikasies op hierdie stadium geensins kan verander nie.
Die 2014 Pakmateriaal Spesifikasies en Palettiserings Protokol dokument is opgestel en gedurende die eerste week in Maart aan al die produsente, pakhuise en ander belanghebbendes uitgestuur.

Na verskeie samesprekings met senior personeel het Sappi besluit dat hulle weer sal voortgaan met die toetse op kartonne, op Sappi se koste. Dit het die hele akkreditasie proses gered en groot dank en waardering is teenoor Sappi uitgespreek. Vir volledige inligting sien CRI Snykant Nr 173 wat op 20 Februarie 2014 uitgestuur is. '

Sappi het begin met die vervaardiging van ŉ nuwe riffelmedium en die eerste eksperimentele A15C en oop vertoon-kartonne met die nuwe produk is op 10 en 11 Maart 2014 by Houers vervaardig. Die eerste laboratorium resultate is belowend en proewe sal so spoedig moontlik gepak en uitgevoer word. Op hierdie stadium word beplan om Suurlemoene in die A15C kartonne en die eerste Sagtesitrus in die oop kartonne te pak. Die huidige "fluting" se basiese massa is 175g/m² en dié van die nuwe eksperimetele produk 165g/m². Dit hou ŉ besparing vir die produsente in, maar op hierdie stadium is daar nie sekerheid hoeveel dit gaan wees nie.

Die Verpakkingswerkgroep se pakmateriaal spesifikasies vereis dat slegs "Virgin Linerboard" en "Semi-chemical" fluting gebruik mag word. Nampak se Rosslyn meule vervaardig "fluting". Dit is nie ŉ "Semi-chemical fluting" nie, maar hulle beweer dat dit geklassifiseer kan word as ŉ "high performance fluting". Nampak het verlede jaar op kleinskaal kartonne met hierdie produk verskaf. Dit is nie vir die verpakkingswerkgroep aanvaarbaar nie en dit is ook so aan hulle oorgedra. Na verskeie gesprekke met Nampak se senior bestuur is daar in Februarie 2014 ooreengekom dat gekontroleerde proewe in samewerking met die CRI-PTF gedoen sal word.
DAFF het gedurende Januarie 2014 ŉ skrywe uitgestuur waarin die gebruik van enige plakker op uitvoerkartonne verbied word (Superimposing). Daar is begrip vir die verbod op "PUC, PHC and Orchard" plakkers, maar daar is geen rede waarom "Category 2 & Variety" plakkers nie gebruik mag word nie. Op versoek van Steve Turner is daar volledige inligting, ter motivering waarom produsente wel toegelaat moet word om bogenoemde plakkers te gebruik, aan hom asook Anton Kruger van FPEF deurgestuur. Volgens die nuutste skrywe van DAFF mag "Category/Class" nou gebruik word, maar Variëreit plakkers is nog steeds verbode. Die versoek dat Variëtiet plakkers wel toegelaat moet word, is weer na Anton Kruger gestuur en hy sal dit nou met DAFF opneem. As hierdie plakkers nie gebruik mag word nie, gaan dit geweldige hoë finansiële komplikasies vir die produsente inhou.

Sekere uitvoeragente het weer gevra om betrokke te raak by die sogenaamde "misleading logos/slogans" wat op kartonne gedruk word. Hulle wil sekere inligting op hulle kartonne, asook op toedraaipapier, druk en wil nou by DAFF hoor wat toelaatbaar is en wat gesien word as "misleading'. Teen die einde van 2013 is DAFF versoek om ŉ lys te gee van wat as "misleading" gesien word, maar tot dusver was daar geen reaksie nie. Hierdie tipe van optredes veroorsaak baie ongelukkigheid onder produsente en uitvoer-organisasies.

Gedurende 2013 het Prima Box in Somerset Wes die CRI-PTF genader vir akkreditasie. A.g.v. sekere aspekte en o.a ook die gebruik van die verkeerde papier kon hulle nie daarin slaag om akkreditasie te bekom nie. Intussen het Corruseal ŉ belang in Prima Box gekoop en sedert Februarie is Corruseal nou betrokke by pogings om Prima Box geakkrediteer te kry.

CRI Na-oes Voorligting

Die 2013 seisoen is afgesluit met ŉ suksesvolle rondte van besoeke/konsultasies aan 70 pakhuise wat omtrent 60% van die totale uitvoere verteenwoordig. Pakhuise is op ŉ een-tot-een basis besoek en die houding en terugvoering is weereens baie positief, met goeie interaksie en samewerking met die pakhuise. Pakhuisbestuur is meer tegemoetkomend en gewillig om hulle idees en vertroulike informasie ivm. terugvoering oor bederf, residu-resultate ens. te bespreek, en die pakhuise is bereidwillig om die nodige aanbevole veranderinge aan te bring. Dit was baie positief om te sien dat waar daar aanbevelings tydens die 2012 pakhuis besoeke aanbeveel is, die veranderinge aangebring is, met ŉ positiewe uitwerking op die pakhuis stelsels.

Die 2013 seisoen het in die Noorde goed begin, veral wat betref skilgehalte, in vergelyking met die 2010-11 seisoene. Dit het met die satsumas, oor die algemeen, goed gegaan, behalwe vir ŉ bietjie brand hier en daar. Bederf was meer van ŉ probleem, in sekere van die Noordelike streke, veral op die vroeë kultivars (satsumas en clementines) en op die nawels, weereens as gevolg van swaar reënval voor die plukseisoen, en wisselvallige reënval tydens pluk. Haelskade in sekere streke het ook hoë bederf op die vroeë nawels veroorsaak, en baie bederf is in die uitskot vrugte waargeneem. Die meerderheid bederf was as gevolg van die wondpatogene, groen en blouskimmel en suurvrot. Die latentepatogene het ook voorgekom, veral op die suurlemoene. Diplodia stingelent verrotting was hoofsaaklik die probleem.

Die aantal groen/blouskimmel vrugte na ontgroening, wat nog steeds in die vrugwas-stelsels gedompel word en nie gesorteer word nie is steeds ŉ groot probleem in baie van die pakhuise wat ontgroening in die Noorde doen. Kraakskil op nawels, was weereens ŉ problem. Twee pakhuise het onderskeidelik 2 en 12% uitskot van hulle vroeë nawel-oes agv kraakskilvrugte gehad.
Swak paletisering is steeds ŉ probleem in heelwat pakhuise. Sekere palette staan regop en lyk asof dit reg gedoen is, terwyl ander 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, en ander hoekstukke eindig by die onderste karton en ander raak aan die begin van die voetstuk van die palet. Te veel palette met gebuigde hoekstukke is ook waargeneem.

Dit is bevredigend om waar te neem hoeveel van die pakhuise nou poog om hulle pakhuis se kritiese-beheerstelsels reg te bestuur, veral die sanitasie komponent wat noemenswaardig verbeter het.Dit gaan hand in hand met die bestuur van pakhuisbehandelings en die toediening van die regte konsentrasies en residuladings. Die gevaar van bestandheid teen die klein hoeveelheid swamdoders, wat die afgelope paar seisoene beklemtoon is, het beslis ŉ verskil in bestuurspraktyke veroorsaak.

Daar is egter steeds pakhuispraktyke waar toestande gunstig is vir die ontwikkeling van hoë bederf. Die 2013 seisoen oor die algemeen nie ŉ “bederf probleem jaar” nie, maar daar was sporadiese gevalle van ŉ toename in bederf.
Die gebruik van ongeregistreerde middels in die sitruspakhuise is steeds kommerwekkend. Dit sluit hoofsaaklik “saniteermiddels” en benatters in, wat deur die chemiese verskaffers versprei word, sonder om eers vir CRI te raadpleeg of die middels goedgekeur word, of nie. Hierdie is aan die einde van die 2012 seisoen genoem en dit bly nog steeds ŉ bekommernis.

Skilprobleme was wel ŉ probleem op sekere vroeë sagte sitrus kultivars en ook op van die middel- en laat-seisoen Valencia kultivars. Sekere boord en na-oespraktyke, soos die vroeër pluk van vrugte met sensitiewe skille vir vroeër toegang tot die markte, wat te lank in ontroening gestaan het, het bygedrae tot swakker skille en ŉ korter raklewe. Die skildefekte wat meestal waargeneem is, is “pitting” en koueskade.

Die 2014 sitrus seisoen het in die Noordelike produksie gebiede teen ŉ stadige pas afgeskop in vergelyking met 2013, as gevolg van die hoë reënval. Die eerste Satsuma besending hierdie seisoen was vanaf die Burgersfort gebied en het goed verloop. Die interne gehalte was aanvaarbaar, maar daar is ligte fitotoksisiteit (brand) op ŉ klein persentasie vrugte na ontgroening waargeneem. ŉ Residu oorskryding (>10mg/kg) van pyrimethanil op Satsumas in ŉ opgegradeerde voorontgroening storting (“drench”) is ook gerapporteer. Daar word vermoed dat dit ŉ probleem met die meng van die mengsel was. Die eerste suurlemoene vanaf Hoedspruit, en die eerste Star Ruby pomelos en suurlemoene vanaf Tshipise, se algemene gehalte is goed, maar opvolg reën het verder pluk vertraag.

Die eerste Satsumas vanaf Citrusdal, en die eerste Satsuma besending vanaf Clanwilliam se algemene gehalte was “goed”. Die omgewingstoestande het weer ŉ rol gespeel. Die dag temperature het van 36°C tot > 40°C gestyg, en die nag temperature het slegs tot 23-25°C gedaal. Dit het veroorsaak dat die produsent opgehou pluk het en dat die vrugte nie op die bome behoorlik opgekleur het nie.

Die eerste latente patogeen infeksies (Antraknose, Alternaria kernvrot en Diplodia stingelent verrotting) het in die Swellendam gebied voorgekom na ontgroening. Dit is ook ŉ gebied waar swaar reënval en oorstromings voorgekom het. ICA het die bedryf in kennis gestel van die algehele tekort aan tiabendasool (TBZ) vir ten minste die eerste helfte van die seisoen. Die fabriek in China, waar die TBZ vandaan kom, het nog nie begin vervaardig nie. Die eerste besending TBZ word eers die einde Mei, of begin Junie verwag. ŉ Verdere bekommernis is gerugte dat sekere pakhuise die guazatine konsentrasie in die "drench" gaan verminder agv. die "moontlike" risiko vir brand, dit na al die reënval i, en die hoë risiko van suurvrot en groen/blouskimmel infeksies.

Die finale verslag na die afhandeling van die vier na-oes tegnologie oordragings projekte, die "Compendium of Postharvest Citrus Diseases", die "Citrus Packhouse Checklist for Auditing", die "CRI Produksie Riglyne Vol IV en die "SOP's for Citrus Packhouses" is vir die PHIP program ingedien.

CRI Werkswinkels

Die CRI Produksie Werkswinkels is gedurende Mei en Junie in vier van die vyf groot streke aangebied. KZN se werkswinkel is ter elfder ure gekanselleer agv ŉ gebrek aan belangstelling. Die onderwerpe wat gedek is, het gefokus op bemestig en besproeiing, boom- en vrugmanipulasie en kulltivars. Die bywoning was bo verwagting goed en die getalle is reeds ongeveer 60-70% van dié van die CRI Na-oes Werkswinkels. Die uitgestrektheid van Mpumalanga noodsaak dat twee werkswinkels in die toekoms daar aangebied sal word. Op versoek van die Groblersdal area en die Winterveld is ŉ verkorte weergawe van die afgelope CRI Produksiewerkswinkels gedurende Augustus daar aangebied
Die vyf CRI Plaag en Siektebestuur Werkswinkels is gedurende September afgehandel. Daar is drie in plaas van twee werkswinkels vir Limpopo en Mpumalanga aangebied, maar agv die ondoeltreffende bywoning van KZN in die verlede is hulle versoek om by die ander werkswinkels in te skakel. Die fokus was veral sterk op die fitosanitêre probleme, asook eksotiese siektes, lenteplaagkompleks en bespuitingstegnologie.

Die datums waarop die CRI Na-oes Werkswinkels gedurende 2014 aangebied is, was 28-29 Januarie vir Limpopo in Polokwane, 30-31 Januarie vir Mpumalanga by Loskopdam, 11-12 Februarie vir KZN & Swaziland in Durban, 13-14 Februarie vir die Oos-Kaap in Port Elizabeth en 18-19 Februarie vir die Wes-Kaap in Stellenbosch. Citrosol/Wenkem was weer die hoofborg vir hierdie werkswinkels gewees. Die bywoningsgetalle het ongekende groei getoon teenoor verlede jaar, met bykans 900 persone wat dit in totaal bygewoon het. Dit is sowat 50% meer persone as wat die vorige jaar se werkswinkels bygewoon het. Dit bring mee dat daar in verskeie areas na groter lokale gesoek sal moet word vir volgende jaar se werkswinkels.

Transformasie

Die Limpopo Departement van Landbou kom nie hulle deel van die samewerkingsooreenkoms met die CGA na nie. CRI, deur Andrew Mbedzi, breek homself om die beste moontlike hulp aan die LDA en produsente te verleen. Die Departement se personeel beskik nie oor die nodige hulpmiddele om hulle taak effektief te verrig nie. Interne veranderinge binne die LDA maak dit ook moeilik om ŉ langtermyn verhouding met die Departement aan te knoop. Elke nuwe Hoof van die Departement (HOD) het sy eie idees. Voordat dit egter geïmplementeer word, word hy/sy bevorder of verplaas en kom al die idees tot niksNa vele frustrasies is die samewerkingsooreenkoms tussen die CGA en die Oos Kaap onderteken. Hopelik sal dit lei tot nouer samewerking tussen Melton Malaudzi en die Oos Kaap se Departement van Landbou se voorligters

ŉ Inligtingsdag is op Addo vir opkomende produsente van die Oos-Kaap aangebied. Klem is hoofsaaklik op vrugmanipulasie en kultivars gelê. Die Koöperasie het as hoofborg opgetree en elkeen wat bygewoon het, het ŉ snoeiskêr ontvang. Dit het weer duidelik uitgekom dat hierdie produsente swaar leun op die ondersteuning van CRI en CGA, en CRI se voorligter betrokke by transformasie het groot lof en waardering ontvang.

19th Conference of the International Organisation of Citrus Virologists (IOCV)

South Africa was proud to host the 19th Conference of the International Organisation of Citrus Virologists (IOCV) from 28 July to 2 August 2013 at Skukuza. This memorable event was the first time that southern Africa had hosted this meeting in 41 years since the meeting in Swaziland in 1972 and was sponsored by the Citrus Growers' Association of Southern Africa and organised by a committee from the Agricultural Research Council, Citrus Research International, the University of Pretoria and Going Africa Conferencing. The conference was a platform for discussions, sharing of ideas and forging collaborations between researchers in a truly African setting. It was attended by 80 delegates from 13 countries representing North and South

America, Europe, China, Africa, Australia and New Zealand. The scientific programme covered the latest research on citrus graft transmissible diseases and included a mid-week field tour to visit orchards where our international guests could see African Citrus Greening and how the industry control the disease where it is endemic. The conference was opened with a plenary talk by Professor Vaughan Hattingh, CEO of Citrus Research International, on the health status of the southern African citrus industry and the role that research has played in the current status. A number of representatives from international improvement programmes attended which provided added benefit to our industry by allowing the sharing of approaches on a number of matters relevant to our industry's scheme. Local industry representatives and the Registrar of Plant Improvement, Directorate Plant Production attended making it an industry applicable event. Talks were presented by some of the most prominent scientists in this field such as Professor Bill Dawson (University of Florida, USA) and Professor Duran Vila (IVIA, Spain).

A hallmark of the IOCV is the passion that is carried by members who continue to add value to citrus research many years after retirement. Special mention is made of Professor Bové of INRA France who attended. He is the world authority on Citrus Greening/ Huanglongbing and added great value to the mid-week tour. However, the person that South African citrus is indebted to for being integral in the establishment of the Citrus Improvement Scheme and for many years of research in graft transmissible diseases, is Dr Fanie van Vuuren, who was recognised at this conference for his longstanding service to citrus virus research by the IOCV.

A whirlwind post conference tour of citrus in South Africa followed the meeting. From Skukuza the tour went by coach to Burgersfort to visit the farm Naranja. This farm is situated in an area where most of the citrus was destroyed by African Greening in the 1970s. Since the development of systemic insecticides, the use of disease-free trees certified by the SA Citrus Improvement Programme and the eradication of infected trees in the orchards, this district is today again back in citrus production. After visiting the Nadorcott orchards to discuss the open hydroponic systems, rootstocks, cultivars, planting densities and the control of citrus greening, the packhouse was also visited. The group then travelled to Port Elizabeth and then to Uitenhage to visit the CRI foundation block where the budwood multiplication for the citrus industry is done. Dr Paul Fourie and his team received the group at the foundation block and presented an overview of the SA Citrus Improvement Scheme and its' activities. Paul's wife, Sonja, prepared a traditional South African braai and it was a very festive visit after which the tour continued to the Sundays River Valley and made a stop at Apapansie Nursery. The tour continued to explore the Citrusdal area, the largest citrus growing area in the Western Cape. The first stop at Citrusdal was XSIT, a facility where a granulo viruses is produced for the control of false codling moth. From there the group was escorted to ALG Farms where citrus orchards and a packhouse were visited before having lunch on the farm. Organisers of the conference wish to thank those that hosted the post conference tour including Andrew Cooper of Indigo Citrus, Paul and Sonja Fourie and team at the Citrus Foundation Block, Nelis Meiring of Apapansie Nursery, Gerhard and Lizette van der Merwe of ALG Farms and Sampie and Marieta Groenewald of XSIT. Ethne Cameron and team of Going African Conferencing made the event the best IOCV ever and an almost impossible standard to equal.

Biosekuriteit en Marktoegang

Angola: Die grootste risiko wat die suider Afrikaanse sitrusbedryf tans bedreig tov eksotiese sitrussiektes is die inbring van sitrusplantmateriaal vanaf Brazilië, veral vanaf gebiede soos Minas Gerais waar geen plantverbeteringskema bestaan nie. Die afgelope jaar het dit gebeur dat bome van hierdie gebied in Angola ingekom het. On sweet dus nie of Asiatiese vergroening (Huanglongbing), Citrus Verigated Chlorosis, Leprose virus, Sudden Death of enige ander bedreiging moontlik reeds hier in Afrika gearriveer het nie.

CRI het 'n suksesvolle besoek aan Angola gebring in 'n verdere poging om die verdure inbring van eksotiese sitrussiektes vanaf Brasilië na suider Afrika stop te sit. Die volgende punte is van belang:

- Hierdie tipe besoeke aan Angola is feitlik onmoontlik sonder die hulp van die Angolan Alliance (Japie Krynauw, Nellus de Waal en Rui Lopez.). Hulle is Portuguees magtig en ken die regte persone in die regte kringe binne die Angolese regering.
- Dr Adelino Rodriques is ons sleutelpersoon in Luanda sonder wie niks sal gebeur nie. Hy is die Nasionale Direkteur van Landbou vir die Direktoraat Landbou, Diereproduksie en Bosbou.
- Die Angolese regering kry 98% van sy inkomste van olie. Die landbou is vir die Angolese regering van geen belang nie. As dit moet, sien hulle kans om al hulle voedsel in te voer.
- Dit is onwaarskynlik dat die Angolese Departement van Landbou in die nabye toekoms enigsens daarin sal slaag om 'n Plantkwarentynstelsel in plek te kry. Hulle wil egter volgende jaar 'n opvolgafvaardiging na Suid Afrika stuur om hierdie saak te ondersoek en het Angola Alliance aangestel om vir hulle die nodige strukture saam te stel en aanbevelings te maak.

- Daar was ŉ aankondiging in die Angolese medium dat daar ŉ sitrusprojek in die Cuanza Norte provinsie van stapel gestuur gaan word vir prosesering. Dr Adelino-hulle dra geen kennis rakende die betrokke persone nie maar probeer uitvind en sal ons op hoogte hou. Dit is belangrik om hiervan kennis te neem en hierdie groep te ontmoet omdat hulle waarskynlik van plan is om hulle bome van die buiteland (Brasilië ?) te kry.
- Daar is ŉ sitruskwekery sowat 10 km uit Luanda, Afroplant, wat sitrusboompies verkoop wat afkomstig is van Brazilië of wat van Braziliaanse plantmateriaal afkomstig is. (Eienaar: Clemes Alves Fernandes. E-pos: clemes@afroplant.com.br MSN: clemes.clemes@hotmail.com Skype: clemes.clemes Brazil: +55 19 9674 2222 www.afroplant.com.br). Hierdie kan die sleutel wees tot die invoer van plantmateriaal uit Brazilië.
- Daar is ŉ suksesvolle vergadering gehou met die Angolese Ministerie van Landbou om die gevaar van sitrusplantmateriaal uit Brasilië aan hulle te verduidelik. Behalwe vir dr Adelino was die volgende persone teenwoordig wat ŉ mate van Engels kon bemeester: Toscana Sebastiao Sengo (Chefe de Secretaria da Direccao Nacional da Agricultura, Pecuaria e Florestas - MINAGRI), Alfredo Amindo F. da Cunha, (Chefe de Seccao de Agricultura), Silvio Virgilio ((Head of the Department of Livestock).
  Na die gesprek met die Ministerie van Landbou is daar besluit om die sitruslesing by die landbouskou van derde spreker op te gradeer tot openingspreker. Die landbouskou, Agroangola 2013, 1 Salao International de Agricultura, Pecuaria Alimentacao e Florestas de Angola, was die land se eerste landbouskou sedert die revolusie.

Toekomstige optredes:

- Dit is uiters belangrik dat daar ŉ sitruskwekery, wat deel van die SACIS is, in Angola gevestig word, sodat bekostigbare bome geproduseer kan word. Die vervoerkostes om bome van Suid-Afrika na Angola te stuur, is te hoog.
- Dr Adelino sal oortuig moet word dat ŉ Suid-Afrikaanse afvaardiging Angola ssm moet besoek om Afroplant kwekery en ander plekke waar Brasiliaanse bome uitgeplant is (bv buite Sumbe), te monster vir eksotiese siektes uit Brasilië. Die protokol is reeds opgestel en ook beskikbaar in Portugees. Vir so ŉ besoek is minstens een patoloog van CRI en Japie Krynauw (of ander lid van Angola Alliance) nodig sowel as een of twee van die Angolese Ministerie van Landbou. Ons kan die CRI personeel en ontledings se koste dra en hulle die res. Die Angola- Alliance is die regte mense om die Angolese van hierdie besoek te oortuig. Hulle moet ook Afroplant besoek en probeer opvolg waar die res van die bome vanaf Minas Gerais uitgeplant is. Tydens hierdie besoek moet boorde wat buite Luanda, naby Huambo en naby Humpata, geplant is met bome vanaf Sao Paulo ook besoek en geïnspekteer word. Hierdie besoek sal ongeveer een week duur.

- Die tweede besoek wat belangrik is, is een waar daar ŉ kits opname gemaak moet word van wat die huidige situasie is tov peste, siektes en variëteite in bestaande boorde of agterplaasbome in Angola. Hierdie opname moet vergelyk word met die Mosambiekse opname sowat vyf jaar gelede. Om hierdie opname te doen sal daar ŉ plantpatoloog, ŉ entomoloog en ŉ kultivarspesialis ingesluit moet word in die span. Hierdie ondersoek sal met CRI voertuie onderneem word en sal vanaf die suide tot in die Uige provinsie gaan. Volgens Alfedo da Cunha is daar veral in die Uige provinsie nog boorde uit die koloniale dae wat uitstekend vaar en hulle sal graag van hierdie materiaal ook deur GPE wil neem en beskikbaar kry vir toekomstige aanplantings, omdat hulle weet hierdie lemoene aard goed in Angola. Hierdie opname sal die ruggraat vorm van enige "Pest Risk assessment" wat vorentoe deur die Angolese regering tov sitrus gedoen kan word en daarom moet die Angolese regering vir al die kostes verantwoordelik gehou word, behalwe die CRI personeel se tyd.

As CRI nie die inbring van sitrus plantmateriaal vanaf Brasilië na Angola aktief teëstaan nie sal dit nie gebeur nie.

<u>Huanglongbing (Asiatiese Vergroening)</u>: Tydens ŉ onlangse besoek aan California deur Hannes Bester en Paul Cronje, is ŉ gesamentlike mini-simposium van die Citrus Research Board en Citrus Mutual in Visalia bygewoon. Die aanbiedinge en werkswinkels is absoluut deur Huanglongbing (HLB) oorheers. Die omvang van die geweldige skade en verliese wat reeds deur Florida gelei is, is ŉ groot bron van kommer in California. Die Federal Government het $125 mil oor die volgende vyf jaar beskikbaar gestel om hierdie siekte in die VSA te bestry, wat wys hoe ernstig hulle daaroor voel. As gekyk word na die erns waarmee HLB deur al die betrokke rolspelers (soos produsente, USDA, CRB, Citrus Mutual, universiteite, verskeie regerings-instansies en privaat instansies) benader word, is dit kommerwekkend om te dink aan die impak wat HLB op die Suid-Afrikaanse sitrusbedryf sal hê. Dit wil egter voorkom of DAFF nie die gevaar wat HLB vir die suider Afrikaanse sitrusindustrie inhou, besef nie.

Suid-Afrika kan onder geen omstandigdhede bekostig dat HLB die land binnekom nie. **Die realiteit is dat indien ons biosekuriteit nie dramaties opgeskerp gaan word nie, HLB ons baie vinniger gaan tref as wat ons dink.** Net so maklik as wat HLB vanuit Suid-Amerika na die suide van California oorgekom het via immigrante, net so maklik kan HLB Suid-Afrika binnekom vanaf Angola via bome wat vanaf Brazilia gekoop word, asook via plantmateriaal wat vanaf China en India deur die Durban-lughawe ingebring kan word, soos ons reeds vroeër gewaarsku is. Sou dit wel gebeur, mag dit beteken dat die Suid-Afrikaanse sitrusbedryf sy deure binne ŉ paar jaar gaan sluit, aangesien ons nie naastenby oor die regerings-ondersteuning, kundigheid en kapasieteit beskik om dit te beveg nie. Daarom sal biosekuriteit opgeskerp **moet** word om alles moontlik te doen dat HLB nie die land binnekom nie.

Swartvlekvry areas: Volgens internasionale protokolle moet areas wat vry van ŉ sekere organisms veklaar is, op ŉ roetine basis geïnspekteer word vir die betrokke organisme. Om te verseker dat sekere distrikte in die Noord-Kaap en Vrystaat steeds as swartvlekvry geklassifiseer kan word, moes hierdie distrikte sowel as aangrensende distrikte vir die voorkoms van swartvlek gemonitor word. Die ondersoel is onder leiding van DAFF gedoen en beide Elma Carstens en Hennie le Roux was betrokke namens CRI en Liezl van Rooyen en Mamarutsi namens DAFF. Die dorpe wat gemonitor is het ingesluit: Phillipollis, Fauresmith, Luckhoff, Rust, Van der Kloof, Koffiefontein, Jakobsdal, Kimberley, Boshoff, Warrenton, Christiana, Jan Kempdorp, Hartswater, Taung, Gasnspan, Delportshoop en Barkley Wes. Dooie blare is versamel wat deur DAFF in Stellenbosch getoets sal word vir *Phylosicta citricarpa.*

8.1 TRANSFORMATION: EXTENSION COORDINATOR'S REPORT

Introduction

Training is the best investment. It is a vital component of ensuring the longevity of the citrus industry and the agricultural sector as a whole. Training facilitates knowledge transfers. It is important to share knowledge among ourselves. Spreading knowledge around is a way of diversifying the investment in the citrus industry.

The training of the government extension officers and the citrus growers about the South African Citrus Industry is a way of investing for the industry. The training workshops are held annually and topics to be discussed as well as the district where the training is going to take place are chosen by the officers and the growers themselves.

Training Workshops

1. Pest and Disease Management Training Workshop

The Limpopo Department of Agriculture extension officers could not attend the CRI Citrus Pest and Disease Management workshop that was held on 5 and 6 September 2013 at the Eiland Resort because of their new financial procurement policy challenges. A two day training workshop was therefore held from 10-11 October 2013. The workshop venue was the Department of Agriculture Boardroom at Makwarela. The focus of the workshop was the Citrus Pests and Diseases Management, Research and Extension needs of farmers. This training workshop was facilitated by Andrew Mbedzi. Dr. Hennie le Roux and Dr. Tim Grout presented the citrus diseases and citrus pest management respectively.

2. Citrus Safety Training Workshop

On 28-30 October 2013 a Citrus Safety Training Workshop was held at the Tzaneen Country Lodge for the 30 Limpopo Department of Agriculture (LDA) extension officers. The first two days focussed on theoretical work and the last day focussed on the visits to the Du Roi Nursery and Mahela Packhouse. The focus of the theoretical work was on citrus plant safety, the citrus value chain, worker training and development, citrus postharvest diseases and the packhouse process flow. The training workshop was facilitated by Carol Harington from Cabeton consultants and Candice Burgin from the Citrus Academy. Dr. Hennie le Roux presented citrus plant safety and Arno Erusmus on the packhouse process flow.

3. Farm Profiling Training Workshop

The Citrus Growers Association (CGA) and the Department of Agriculture Rural Development and Land Administration (DARDLA) held a MoU implementation meeting on 2 October 2013 at the Lowveld College of Agriculture-Marapyane Campus to discuss the establishment of citrus at Marapyane and the profiling of Mpumalanga citrus growers. The CGA was tasked to come up with an assessment template to ensure that the assessment is uniform and gives a detailed analysis of the respective farms/projects.

The training of the Mpumalanga DARDLA extension officers took place on 5 November 2013 at their boardroom in Jones Street, Nelspruit. Twelve (12) extension officers who were nominated to coordinate citrus in Mpumalanga attended this training session. The focus of the training was on the Overview of the Citrus Industry and the Profiling of Citrus Farms.

CRI Citrus Extension Regional Workshops

The Citrus Research International started with the hosting of the three citrus extension regional workshops in 2013. The three workshops are the packhouse, production and pest and disease management.

1. CRI Citrus Packhouse Workshop

The CRI Citrus Packhouse Workshops were held on 28 and 29 January 2014 at the Ranch Hotel and 30 and 31 January 2014 at the Loskop Dam in Limpopo and Mpumalanga respectively. Ten small citrus farmers in Limpopo attended the packhouse workshop.

2. CRI Citrus Production Workshop

The CRI Citrus Production Workshops were held on 28 - 29 June 2013 at Eiland and on 30 - 31 June 2013 at the CRI Boardroom in Limpopo and Mpumalanga respectively. Ten developing citrus farmers and 13 government extension officers attend the CRI Citrus Production workshop that was held at the Eiland Spa in Limpopo. Three (3) Citrus Academy students attended the one that was held at the CRI Boardroom in Nelspruit, Mpumalanga.

Four (4) EC DRDAR extension officers, Sixteen (16) citrus growers and eleven (11) beneficiaries attended the Citrus production Workshop at Sundays River Valley. The Perishable Product Exchange Control Board (PPECB) paid registration for sixteen small farmers. The Total number of none commercial farmers and Extension officers were thirty-one (31).

The Perishable Products Exchange Control Board (PPECB) requested CRI to hold a Production workshop on 20- 21 August 2013 for the (sixty) 60 Winterveldt citrus growers. Twenty six farmers from Winterveldt United Farmers Association (WUFA) attended the workshop. The workshop was funded by PPECB and two (2) beneficiaries from Brits who were funded by the CRI also attended the workshop.

3. CRI Citrus Pest and Disease Management Workshop

The CRI Citrus Pest and Disease Management Workshops were attended by emerging citrus growers from Moletele in Hoedspruit and Letsitele. One person representing Champagne and five students from Lowveld College of Agriculture (Nelspruit campus) attended the workshop that was held at the CRI Boardroom in Nelspruit.

Three (3) EC DRDAR extension officers from Cacadu District attended the CRI Citrus Pest and Disease Management workshop, twenty (20) citrus growers and eleven (11) beneficiaries attended the workshop at Sundays River Valley. PPECB paid the registration fees for thirty-two (32) farmers. The total number of none commercial farmers and Extension officers together with the Citrus Academy students were forty-five (45).

Agricultural Leadership Development Programme (ALDP)

The Agricultural Leadership Development Programme came into fruition after organised agriculture in South Africa identified the need for such a programme. This programme aims to provide and develop management skills as well as analytical and creative decision-making competencies for managers and individuals who function at a strategic management level. The programme focuses on strategic thinking and leadership within the changing and competitive agricultural and business environment. Delegates will acquire the competencies needed to deal with contemporary South African challenges.

The following farmers and officer were nominated by the Citrus Academy to participate in this programme representing the Citrus Industry:

Province	Name of Delegate	Company/Farm	Contact Details	
			Cell phone:	E-mail Address:
Limpopo	Jonas Mogale	Mariveni Farm	0718995217	Jonas@marivenifarm.co.za
Limpopo	Andrew Mbedzi	CRI-Nelspruit	0836936917	am@cri.co.za
EC	Luyanda Kutta	Nkomoshe Farm	0812711151	llkutta@telkomsa.net
EC	Manene Titimani	Cragheid Farm		nthanda.agribusiness@gmail.com
EC	Siyabulela Katyana	Battlesdan Farm	0734420446	siyarhee@gmail.com
EC	Headman Manyonta	Oakdene Farm	0732519233	hmanyonta@fortcox.ac.za
KZN	Simon Xulu	Umhlabawethu Farm	0833464167	zsxulu@csnet.co.za

The Citrus Field and Growers Days

1. The Citrus Field Day

This year (2013) the Citrus Field day was hosted by Vhembe district and was held on 15 August at the Botanical Garden in Thohoyandou. The theme of this year's event was *"Commercialization of Small/Emerging/Developing Citrus Growers"*. Ninety five (95) people attended which represented 2.5 million cartons. The following topics were presented:

- The Citrus Industry by Israel Nemaorani (A farmer and CGA Board member)
- The Importance of Accreditation Schemes by Andrew Mbedzi (CRI)
- Farm Business plans by Mmbodi (LDA)
- Impact of Bactrocera invadens in Province by Khorommbi (LDA)
- Granorpassi Fruit Processing Factory by Belemu N.D. (Granorpassi Rep.)
- Citrus Growers Development Chamber (CGDC) by Mankhili N.T. (A farmer and Deputy Chair: CGDC)

2. The Growers' Day

The growers' day was organized by Citrus Research International (CRI), supported by Citrus Growers Association of Southern Africa (CGA) and the Eastern Cape Department of Rural Development and Agriarian Reform (DRDAR). A total of 58 delegates attended the growers' day and the attendance was low because some of farmers were attending presentation on recapitalisation funding in East London on the same day. The presentations at the growers' day were as follows;

- Pruning and Manipulation of flowers and fruit size by Hannes Bester
- Crop insurance by Bennie Conradene
- Information Packs on energy efficiency by Rene De Kock from Eskom
- CGA Cultivars Development by Jonathan Robert
- Humandorp Coop: Andriaan Mooleman

The Citrus Study Groups

The conducting of the citrus study group sessions went well during the 2013/2014 season in Limpopo, Eastern Cape and KwaZulu-Natal provinces. The following table indicates the dates, venues, number of attendances, presenters and the topics that were presented in the different study group sessions.

Date	Study Group	Venue	No of People	Presenters	Topics
02/04/2013	Waterberg Citrus Study Group	Suggest Farm, Lephalale	10	Andrew	• Rootstoks and Cultivars
				Andrew	• Leaf and Soil Sampling
				Andrew	• FF, FCM and BI
12/04/2013	Vhembe Citrus Study Group	Danstaat Farm, Weipe, Musina	70	LDA	• Leaf and Soil Sampling
				Andrew	• FF, FCM and BI
				Andrew	• Orchard Management
				Rabelani	• Fruit Quality
17/06/2013	Mopani Citrus Study Group	Mariveni Farm, Letsitele	13	Samson	• Outline of Chamber Structure
				Andrew	• Overview of Citrus Industry
				Andrew	• Postharvest diseases
18/06/2013	Vhembe	Mutale	47	P.H. Dube	• Importance of Fertilization

	Citrus Study Group			LDA	• Postharvest disease
				Mafa	• Importance of Irrigation
04/07/2013	Waterberg Citrus Study Group	Morajomo, Radium, Bela Bela	22	Andrew	• Timing of Fertilizer Application
				Andrew	• Pruning of citrus trees
				Andrew	• Organic Fertilizers
				Andrew	• Cultivars and Rootstocks
19/09/2013	Waterberg Citrus Study Group	Sunningdale Mokgopong	26	Andrew	• Situational Analysis
				Andrew	• Citrus Spraying Programme
				Andrew	• Spring Pest Complex
19/09/2013	SRV Citrus Study Group	Willow Tree, SRV	12	Combrink	• Spring Pest Complex
				Melton	• Feedback on Recap Funding
20/09/2013	Patensie Study Group	Tobacco Office Patensie	5	Melton	• Spring Pest Complex
				Melton	• Feedback on Recap Funding
16/10/2013	KZN Citrus Study Group	Thulwane Farm, Nkwaleni	16	Melton	• Spring Pest Complex
				N. Siphiwe	• Bactrocera invadens (BI)
				Mzo	• Feedback on Chamber meeting
18/11/2013	Sundays River Valley Study Group	Lutando Farm, SRV	9	Melton	• CBS Interceptions
				Melton	• Health and Safety
				Ivy Mzamo	• Feedback on Chamber meeting
20/11/2013	Vhembe Citrus Study Group	Botanical Garden, T/Ndou	63	Protek	• Handling of Chemicals
				Andrew	• Citrus pests and diseases
				DAFF	• Responsible use of Chemicals
26/11/2013	Patensie Study Group	Boplaas Patensie	5	Melton	• CBS Interceptions
				K. Katoo	• Feedback on Chamber meeting
05/12/2013	KAT River, Study Group	Cape College KAT River	12	Melton	• CBS Interceptions
				Nohamba	• Feedback on Chamber meeting
10/12/2013	Waterberg Citrus Study Group	Gillimburg, Mokopane, Mohalakwena	15	Andrew	• Pruning of citrus trees
				Manager	• Fruit Enlargement
				Andrew	• Application Foliar Sprays
				Andrew	• Cultivation Practices
27/01/2014	Sundays River Valley Study Group	Luthando Farm, SRV	8	Melton	• 2014/15 Activity Plan
				Melton	• FF, FCM and BI
				Melton	• Leaf and Soil Sampling
28/01/2014	KAT River Valley Citrus Study Group	Cape College, KAT River Valley	11	Melton	• 2014/15 Activity Plan
				Melton	• FF, FCM and BI
				Melton	• Leaf and Soil Sampling
12/02/2014	Patensie Citrus Study Group	Tobacco Office Patensie	7	Melton	• 2014/15 Activity Plan
				Melton	• FF, FCM and BI
				Melton	• Leaf and Soil Sampling
14/02/2014	Vhembe Citrus Study Group	Hlanganani Tribal Office, Vuwani	36	Andrew	• FF and FCM
				DAFF	• Responsible use of Chemicals
				Andrew	• Irrigation of young citrus trees
				Andrew	• Fertilization of young trees
				DAFF	• Monitoring and control of BI
26/02/2014	KZN Citrus Study Group	Thulwane Farm, Nkwaleni Valley	9	Melton	• 2014/15 Activity Plan
				Melton	• FF, FCM and BI
				Mzo	• Feedback on Chamber meeting
				Melton	• Leaf and Soil Sampling
06/03/2014	Waterberg Citrus Study Group	Ratanang Farm, Northam, Thabazimbi.	17	Andrew	• Caring of young citrus trees
				Andrew	• Fertilization of young trees
				Andrew	• Pest of young citrus trees
				Andrew	• Proper furrow irrigation system

The Citrus Growers Development Chamber (CGDC)

The Chamber held two meetings during the 2013/2014 financial year. The first meeting was held on 13 November 2013 and the second meeting was held on 19 March 2014 at the Birchwood hotel and the Willow Park View Centre in Johannesburg respectively. The focus of these meetings was on the formation of Strategic Partnership between Citrus Growers Association of Southern Africa (CGA) and Department of Rural Development and Land Reform (DRDLR), the Conducting of the Situational Analysis for the Small Citrus Growers, the Formation of the Chamber Task Team, Signing of the MoUs with Government Departments and the Formation of the Marketing company.

1. Strategic Partnership between CGA and DRDLR

The CGA was required to have a BEE Certificate to enter into such agreement with DRDLR. It is a requirement by the DRDLR and as such CGA could not enter into such agreement with DRDLR.

2. CGDC Situational Analysis

Survey was initiated by CGDC at the beginning of 2013 with the purpose of gathering accurate data on the estimated 130 emergent citrus enterprises in South Africa. The Citrus Academy was tasked with managing the project on behalf of the CGA. The project was funded by the AgriSETA, ADA in KwaZulu-Natal, and the CGA. A questionnaire for the survey was developed with the help of the CGDC executive committee, CRI extension staff and CGA transformation staff. An electronic data gathering tool was developed with the help of Paul Hardman of the CGA. Lima Rural Development was contracted as a service provider to assist with the data gathering process. The survey was undertaken in September and October 2013, and completed in February 2014.

3. MoU between CGA and Government Departments

The Citrus Growers Association of Southern Africa (CGA) signed the following Memorandum of Understandings (MoUs) with the different government departments during 2013/2014 financial year. CGA signed the MoU with the Department of Rural Development and Agrarian Reform (DRDAR) in the Eastern Cape, Department of Rural Development and Land Reform (National) in Pretoria and the Department of Agriculture Rural Development and Land Administration (DARDLA) in Mpumalanga.

Recapitalization and Development Programme

The citrus farms in the Eastern Cape and Mpumalanga provinces have benefited from the recapitalization funding. No citrus farms in Limpopo, Gauteng and North West Provinces benefited from the recapitalization funding.

1. Mpumalanga

Champagne farm located in the Bushbuckridge area has benefited from the recapitalization funding. The renovation of the packhouse was completed. The orchards are also looking much better than they were previously.

Sibonelo lemon farm located at Eilandshoek also benefited from the recapitalization funding. They have bought a small truck and they are on the process of planning to build an office. The process of building an office is now near completion and they have also planted 3ha of lemons

2. Eastern Cape

Four additional farms have received recap and the names of the farms that received recap are Orange Grange, Torties, Lovers Retreat and Naudeshoek.

Female Farmer of the Year Competition

Mrs Buyiswa Ndyenga from Willow Tree SRCC farm contested for female farmer of the year at the local municipality level, district level and provincial level and she won on all of the categories.

Challenges

- Lack of willingness by the senior government officials to implement the MoU between CGA and DRDAR in the Eastern Cape.
- Growers in some parts of the southern regions (i.e., KZN and Eastern Cape) and northern region (i.e., Limpopo and Mpumalanga) were hit by floods and hails storms.
- Lack of funds to finance the training of the Limpopo Department of Agriculture extension officers who are coordinating citrus in the province. They managed to attend a three days Safety Training Workshop.
- Nkwaleni Valley and Klein Hoewe Boer are faced with a challenge of citrus black spot
- Most of the upcoming citrus growers are faced with financial challenges.
- Poor communication between the government officials and citrus growers with regard to recapitalization funding.

Shortage of credible mentors to assist in the CGA mentorship programme

8.2 NAVORSINGSPRIORITEITE/RESEARCH PRIORITIES

The Citrus Research Priorities for 2014 were determined from July–October 2013 taking into account the inputs from the producers, the Exporters Technical Panel and the CRI Workshops. A communication was sent to all the citrus producers who are on CRI's Technology Transfer Group (TTG) list, listing all the research approved for 2013. This provided a means for growers to assess which of their previous requests were being addressed. Growers were requested to study these research projects and indicate any additional research required as a priority. They were advised to weight requirements from 1-3, with 3 the higher priority. Once the TTG's Technical Committee had received all the research requests from the area they compiled a summary and a meeting was held with the Area Extension Manager and the Technical committees. In a few cases the TTGs indicated that there were no changes to their research needs and cancelled the proposed meeting. The Area Extension Managers from the North and the South compiled the resultant research priorities and forwarded these to the Manager Research and Technical. Unfortunately many producers, including CRI directors, do not read their e-mails and are therefore not aware of the way in which the research priorities are determined and are therefore criticising the process. The respective TTGs are responsible themselves who they choose both as chairman and as technical committee for each of the areas.

The research priorities were also determined at the five CRI Post Harvest Workshops held in Limpopo, Mpumalanga, KwaZulu Natal, the Eastern Cape and the Western Cape in January/February 2013 as well as the CRI Production Workshops and the CRI Spring Pest Complex and Disease Management Workshops held in May/ June and September 2013 respectively. It was also determined for the Exporters Technical Panel and the growers involved in the Transformation process. The majority of the research priorities are the same as the previous years. The Research Priorities can be summarised as follows with the project numbers addressing each of these problems or the reason why a specific problem is not addressed.

1. DISEASE MANAGEMENT

1.1 CITRUS BLACK SPOT	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
The fact that South Africa would only be allowed 5 strikes in the EU, the fact that the EFSA report indicated that they do not accept South Africa's argument that the fruit do not pose a pathway to spread CBS and the fact that the disease cannot establish in a Mediterranean climate had a huge impact on the growers perception of what should be the citrus industry's highest priority. To the growers in the summer rainfall areas it was definitely CBS and the phytosanitary status of CBS. As in the latter part of 2012 the growers experienced enormous challenges to adhere to the latest DAFF regulations to be able to export into the EU.. It is thus of the utmost importance that the status of CBS should be changed from a phytosanitary to a cosmetic disease in order to get rid of these disruptions. This requires that all the research that was completed on CBS should be published in refereed journals. Something that were done over the last couple of years.	919 970 977 1012 1026 1088 Market Access			
A request by growers in CBS affected areas is still that the USA should be opened up for all citrus producing areas in South Africa, with the hope that the EU would then accept the fact that fruit do not pose a threat as a pathway to spread the disease.	1026,1088 Market Access			
Develop alternative spray programmes and application methods which is more effective than the current programmes. It would be even better if these programmes could control *Alternaria* as well.	970,750			
Complete the study to determine the critical period for CBS infection in the Eastern Cape.	919,1026			
Develop alternative strategies to interrupt the diseases life cycle. Protection of the leaves prior to falling should be re-investigated as well as the destruction of inoculum (dead leaves) during the rainy season. Genetic manipulation to build in resistance genes should also receive urgent attention..			X	
Convince the chemical companies selling strobilurens to change the labels in order to be able to spray these products without mancozeb as there is no threat of resistance of CBS against the strobulirines.	970			
Investigate the possibility to spray a third strobilurine late in February on old clone valencias and lemons to prevent a late infestation of CBS. Get the USDA to acknowledge the Weipe & Tshipise areas as areas of low pest prevalence.	970 Market Access			
Determine where does the third Phylosticta sp found on citrus fits in	1088			
Register the combination of the strobulirines with benomil without mancozeb.	970			
The MRL for citrus to Canada is 0,1ppm. However, in Canada itself the MRL for use on their produce is 0,5ppm. Paul Hardman to follow up.	Market Access			
CBS spray programmes for the EU cannot be statuary for everybody. There is groves in the areas of low pest prevalence that has never sprayed and has been CBS free to date because of other measures taken to prevent the introduction and spread of inoculum.	Market Access			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Lower Orange River must be declared CBS free in the EU	Market Access			
CBS pest free areas must be maintained according to international law.	Market Access			
Find postharvest options to prevent development of CBS symptoms.	UKZN project in PHD		X	
1.2 ALTERNARIA				
Alternative spray programmes to control *Alternaria* more effectively with fewer sprays and more effective spraying techniques to reduce the volume of water needed to apply the chemicals effectively	750,891 ,1089, 1096			
Establish an on-going project to screen all new cultivars for *Alternaria* tolerance.			X	
Determine the effect of shade netting on *Alternaria* on susceptible cultivars.	New ARC			
Confirm the possibility of *Alternaria* resistance against the strobilurins				X
1.3 *BOTRYTIS*				
Spray programmes to control *Botrytis* on lemons during flowering.	1015			
Determine the effect of *Botrytis* on lemons in the Sundays River Valley.	1015			
Identify fungal growth on blossoms of Satsumas and other varieties				DC
1.4 *PHYTOPHTHORA CITROPHTHORA*				
More effective control programmes.			X	X
Establish an ongoing project to screen all new cultivars against *P. citrophthora*			X	
Need effective control option for snails in W-Cape			X	
Current program only delays problem. Need effective control option			X	
1.5 POST–HARVEST DISEASES				
Optimisation of the flooder to allow commercial use in packhouses	1050, 1x new CRI@USPP project			
Optimisation of fungicide treatments in the packhouse to be the most effective to protect the fruit and to prevent resistance developing.	936,1050, 3x new CRI@USPP projects			
Optimising the GRAS chemicals such as sodium bicarbonate, especially in an imazilil protection programme. (pH correlations, concentration, temperature, exposure times etc.)	936, 1x new CRI@USPP project			
Develop techniques to use the quaternary ammonium products safely for exports to Japan. Assist with the re-introduction of Sporekill	Market Access		X	
Development of wax standards			X	
Alternatives for Guazatine	123			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Determine the rate of residue breakdown of all products used in post-harvest treatments			X	X
Determine the viability to pack citrus under certain conditions as chem.-free without causing decay problems	UKZN			
Control options for *Rhizopus*			X	
Control options for sour rot and brown rot	123			
Need options to do away with warm water bath	1050, 1x new CRI@USPP project			
Supermarkets requests third of EU MRL – need alternative control products that will be effective at low concentrations	936, 1050, 2x new CRI@USPP project			
Need tool for early detection of decay in packhouses	1073		X	
Investigate guazatine burn	123			
Evaluate the use of Orosorb in the warm bath to get better run-off and drying in the tunnel		X		
Control and prevention of fungal growth on wooden pallets			X	
Investigate "stripping" of mancozeb to prevent residue issues to Canada			X	
1.6 *PHYTOPHTHORA* ROOT AND COLLAR ROT				
Alternative control options	1030			
Screening of new rootstocks against *Phytophthora*	UP/CRI 01/09			
More effective and safer phosphonate treatments			X	X
Determine the effect of compost teas and commercially applied microbial applications against *Phytophthora* root rot			X	
1.7 CITRUS NEMATODE				
Evaluation of pre-plant fumigation products on replant soils.	762			
Alternative control options (E.g. Imidacloprid?)	1030			
Control options for sheeth nematode			X	
1.8 *ARMILARIA* ROOT AND COLLAR ROT				
Develop control options for *Armillaria.*	1068			
Test/screen all commercial and experimental citrus rootstocks against *Armillaria*.			X	
1.9 ROOT HEALTH				
Develop a more holistic approach to root health in general.	910		X	
1.10 CITRUS TRISTEZA VIRUS				
Optimising cross protection. There are TSR orchards in Hoedspruit with heavy stem pitting but the fruit is large and the tonnage 100 tons/ha.	738,739, 742,789, 968,885 885B, 1056, new CRI@US project			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Evaluation of different cultivars in either the selection or suppression of different CTV strains.	1056, new CRI@US project			
1.11 CITRUS GREENING (HUANGLONGBING)				
Development of greening resistant cultivars. Challenging the greening tolerant cultivars developed by Fanie van Vuuren must be accelerated. The genes must be identified and built into other varieties as well	815			
Monitoring the spread of African greening towards the Eastern Cape citrus producing areas.	CIS-Biosecu-rity			
Monitoring KwaZulu-Natal for a possible introduction of Asian HLB.	CIS-Biosecu-rity			
Develop methods to cure greening-infested trees			X	
Study the role of alternative hosts in the epidemiology and spread of greening				
Search for greening resistance through embryo rescuing	815			
Search for greening protection using mild strain CTV.			X	
Investigate the transmission and infection of Candidatus Liberibacter africanus at different times of the season.	988			
Confirm if there are any alternative hosts for Asian HLB in South Africa	886B			X
Confidor NB for control of psylla. Find alternative systemic products to control psylla	IPM priority			
1.12 VIROIDS				
Ensure that the CFB is free of all graft transmissible pathogens including the viroids.	CIS790, 796			
Study the effect of viroids on the horticultural characteristics of different cultivars eg. Fruit set, maturity, colour.	1074			
1.13 BIO-SECURITY				
Make industry aware of the Act on the Distribution of Plant Material	CIS-Biosecu-rity			X
2 INTEGRATED PEST MANAGEMENT				
2.1 FALSE CODLING MOTH				
Fine tuning the systems approach to ensure that the industry will be able to send citrus into the EU without cold steri.	1039 & 1085			
Develop more effective control methods. This includes the optimization of the SIT programme, more effective use of the granuloviruses, commercializing the entomopathogenic nematodes and the entomopathogenic fungi.	1024, 1042, 1049, 1065, 1079 & 1083			
Develop alternative mating disruption systems	955, 1063 &1080			
Develop amelioration techniques to control FCM in fewer days during cold sterilization.	965 & IBB02/12			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Develop techniques to detect FCM on the pack line.	976, 1022, 1066, 1071 & 1090			
Develop techniques to enhance the release of parasites late in the season.	1021			
Find alternative pheromones to attract FCM		X		
Need mating disruption to be effective on areas smaller than 10ha		X		
Need information to establish bats in the orchards		X		
Need information to establish bats in the orchards				
Evaluate EPN's later (Apr/May) to control FCM	1042			
Start to asses' cold treatment at 2 degrees on semi commercial level.	1039			
Categorize lemons as low risk variety at commercial level.	1087			
Determine mating potential of FCM survivors after 2 degree treatment.	1039			
Carob moth increase in citrus. Need ID to distinguish between carob moth and FCM	US/ENT-08-02, US/ENT-11-A3 & 1051			
Quantify the effect of carob moth on citrus	US/ENT-11-A3 & 1051			
2.2 FRUIT FLY				
2.2.1 *Bactrocera invadens*				
Monitoring of the spread of *Bactrocera invadens* in South Africa, Zimbabwe, Botswana and Mozambique.	966, 1075 & RU(Timm)			
Investigating a M3 and MAT block barrier in the valleys between Vhembe and Tshipise to protect Tshipise from re-infestations with *Bactrocera.*	(1075 test feasibility)			X
Develop a risk mitigation strategy to be able to export citrus from areas where *Bactrocera* was detected. (on an orchard to orchard basis)				X(DAFF)
Get all SADEC countries together to deal with *Bactrocera* together.				X (DAFF)
Registration of Hymlure/Prolure + Spinosad both as aerial and ground sprays.	Contract trial			
RB is requested to sell methyl eugenol directly to the growers to recharge their MAT blocks themselves. Especially for Zimbabwe.	RB			
Put pressure on DAFF so that more MAT blocks and M3s are distributed amongst the rural villages				X (BiSC)
GF-120 not as effective as Malathion – need a shorter PHI for Malathion				X (BiSC)
Develop yeast to attract females (technique from Australia)	1093			
2.2.2 Mediteranean and Natal fruit fly				
Develop a more attractive attract and kill option than the M3 that will last as long but will reduce the number of traps /ha.	915			
Develop a M3 with both a male and a female attractant.		X		

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Study the role of entomopathogenic nematodes and – fungi on fruit fly larvae in the soil.	930, 980 & 1042			
Investigate EGO (enriched ginger oil) as attractant for males	Contract trial			
2.3 MEALYBUG				
Develop alternative control methods against mealybug to replace products such as Dursban, Applaud, Ultracide and Tokuthion.	985, 1017, 1029 & 1048			
Find biocontrol agents that can be used against mealybug.	As above			
Determine the possible role of entomopathogenic nematodes against mealybugs.	985			
Investigate the importation of parasites from Australia.		X		
Determine effect of Anagyrus as alternative predator	1017			
2.4 CAROB MOTH				
Study the morphology and control of carob moth in citrus.	US/ENT-08-02			
Study the effect of EPNs on carob moth.		X		
Monitoring of carob moth.	US/ENT-11-A3,1051			
Investigate the presence of carob moth in Tshipise				X
Carob moth increase in citrus. Need ID to distinguish between carob moth and FCM	US/ENT-08-02			
2.5 FRUIT PIERCING MOTHS				
Monitoring and control of fruit piercing moths.	1058			
2.6 LEAFHOPPERS				
Develop methods to control leafhopper.	942 & 1061			
Also need biological control option		X		
2.7 LEPIDOPTERAN PESTS				
Determine the effect of imidacloprid on lepidopteran pests in citrus.	954			
2.8 ANTS				
Develop and commercialize ant baits for both pugnacious and brown house ants.	857			
Find methods to keep ants out of trees	857			
2.9 THRIPS				
Develop alternative control options for thrips (Abamectin is overused but a registration @ 30ml/100l is needed) especially for November – January control.	1029 & 1061			
Investigate the importation of a predatory thrip from Koppert		X		
Investigate the effect of Cryptonem on the soilborne phase of thrips.	1042			
Thrips trials must be conducted in Letsitele.	1029 (elsewhere in Limpopo)			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Find alternative to Abamectin	1029 & 1061			
Find attractant for use during Dec/Jan that is acceptable to all markets	(done years ago)			
2.10 MITES				
Registration of a generic for Mitigate to reduce its price.	NA			
Alternative control options to replace Acarol which is affordable and effective. Especially budmite is becoming an increasing problem even on Star Rubies.		X		
Revise registration of Mitigate in Canada (Paul Hardman)				CGA
2.11 RED SCALE				
Alternative control options for imidacloprid, especially on heavier soils.	1076			
2.12 SNAILS				
Control options more affordable than Moloxide.	Contract trial			
Moloxide not registered – need registered options	Na			
Need effective control for whole spectrum snails in W-Cape			X	
2.13 SLUGMOTH				
Epidemiology and control		X		
2.14 WOOLLY WHITEFLY				
Alternative control options	1061 & 1082			
Movento not registered – needs registered products				
2.15 PSYLLA				
Find alternative systemic insecticides to control psylla	1061			
Investigate the possible accelerated degradation of imidacloprid		X		
2.16 BOLLWORM				
Bolldex registered on stone fruit – Helicovir to be resistered asap	RB			
2.17 LEMON BORER MOTH				
Increasingly problematic – find control options	715 & 933			

3. CROP AND FRUIT QUALITY MANAGEMENT

3.1 Rind condition	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
3.1.1 Peteca				
Effective control measures.	833			
3.1.2 Creasing and splitting				
Effective control measures.			X	
Better understanding of the physiology of creasing.			X	
Test the product Oenosan.		X		
Investigate the effect of silicon and boron on the uptake of calcium		X		
Splitting on navels, Midknights, soft citrus	1027			
3.1.3 Chilling injury				
Develop post-harvest treatments to control chilling injury.	832			
Determine step-down temperature for all varieties to prevent CI	832			
3.1.4 Rind breakdown/pitting				
Develop better methods to predict and control rind breakdown especially on Bennys and Turkeys	958,1031			
Need alternatives for TBZ. And find out why does TBZ help.	958			
3.1.5 Blossom end clearing				
Develop a better understanding of the problem and ways to prevent it. The problem is on the increase.			X	
3.1.6 Cold damage				
Develop techniques to protect fruit and trees against frost damage			X	
3.1.7 Shelf life				
Develop techniques to extend the shelf life of citrus fruit.			X	
Method to quantify over ripeness and puffiness.		X		
Monitor the trial which is conducted at Letaba with ozone.		X		
3.1.8 Tear staining				
Determine the cause of tear staining on Nadorcotts and how to prevent it.			X	
3.1.9 Silicon				
Determine the role silicon can play to reduce all of the above damages	974, 1057			
Determine the effect of silicon on fruit set, size, sunburn, etc.	974, 1057			

3.2 FRUIT PRODUCTION & QUALITY	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
3.2.1 Flowering				
Alternating flower on Mors, Orrs and Nadorcotts a problem.	981			
Effect of netting on flowering			X	
3.2.2 Fruit set				
Fruit set a huge problem on Eureka Seedless! But also on Navels in the Eastern Cape and Deltas and Midknights in many citrus areas. Also with TSR in Nkwaleni.			X	
Effect of products such as Reflecto, Silica, Kaolin and Shade cloth on fruit set.			X	
Effect of gibb applications on the western side of the tree only.			X	
Sort out alternate bearing on Mor and Nadorcott	981			
Increase production on oranges and soft citrus in the south, without compromising on fruit size			X	
Investigate effect of higher concentrations Gibb on fruit set of mandaryns			X	
Investigate effect of netting, and different colour netting, on set			X	
Evaluate girdling of framework branches vs trunk and timing for better set on mandarins			X	
Fine tune irrigation scheduling to minimise stress and improve set	986			
3.2.3 Regrowth				
A major problem especially on lemons and late mandarins especially when fruit set was poor. Need to test and register products such as Sunny, Cultar or Regalis			X	
Determine the MRLs for plant growth stimulants.			X	
3.2.4 Pruning				
Pruning techniques on late mandarins and lemons need to be developed.				X
Compare winter pruning with late pruning and summer pruning.				X
Do trials to manipulate fruit set, alternate bearing, fruit size and regrowth on soft citrus	981			
3.2.5 Fertilisation				
Recommendations needed to increase carbohydrate levels quicker after harvesting a large crop.	1028			
Manipulation of fertilization to decrease rind problems.	958			
Role of humic and fulvic acids. Does carbon fertilizers act as a carrier for the absorbsion of fertilisers or is it merely slowing the rate of N leaching from the soil?	1028			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Role of silicon.	974,1057			
Timing of first N applications on different cultivars.			X	
The influence of different formulations on the foliar uptake of elements	1037			
Prove that liquid carbon fertilzers does have an effect on the microbial life in the soil. (Not talking of normal organic material).			X	
Evaluate different foliar applications available in industry – too many snake oils		X		
Evaluate effect of silica on rind defects, fruit size, etc-		X		
3.2.6 Internal quality				
Ways to drop the acid levels		X		
Ways to increase the acid levels		X		
Optimization of internal quality under OHS systems			X	
Effect of shade nets on internal quality			X	
3.2.7 Sheepnose				
Climate effect on sheep nose of grapefruit			X	
Effect of shade netting on sheep nose			X	
3.2.8 Cold damage				
How to prevent frost damage (frost bite, Copper Silica, etc.)		X		
Influence of rootstocks on frost damage		X		
Effect of netting on cold damage			X	
3.2.9 Sunburn				
Methods to reduce sun burn				X
Alternatives for oil to reduce sun burn on grapefruit			X	
Effect of netting on sunburn				X
3.2.10 Fruit colour				
Methods to improve fruit colour, especially in the north			X	
Methods to initiate earlier colour			X	
Methods to intensify fruit colour to prevent cold damage during cold steri			X	
Effect of netting on fruit colour			X	
3.2.11 Fruit size				
Methods to increase fruit size on Deltas, Clementines and Rustenburg navels.			X	
3.2.12 Blossom end clearing on grapefruit				
Causes and control			X	
3.2.13 Evaluation of biostimulants				
Biostimulants such as Alexin, Citrox, CropBiolife, Cilic, Messanger, Mannitol, Sorbitol and GA14 should be evaluated.		X		

3.2.14 Water usage	YES	NO		
	Project no.	Low rating	Lack of capacity	Extension function
The Water Research Council is looking at the water usage of the different fruit crops. They gave CRI the opportunity to be involved. This is important as this could affect water quotas in the future.	986			
3.2.15 Fish lips				
Producers are harassed in Letsitele by PPECB for what they call fish lips at the button end side of fruit. No mealy bug is involved.		X		
3.2.16 Weed control				
Investigate resistance to chemicals		X		
Find softer products for weed control		X		
Find solutions for control of 'motvanger' and 'cat's claw creeper'		X		
3.3 COLD CHAIN & PACKAGING				
3.3.1 Cold Chain Management				
Investigate optimum shipping temperature and RH to control waste.				X
Updated manual annually for decay control (Production Guidelines & Booklet).				X
Set time and temperature protocols for new varieties		X		
Investigate the correlation between variation in temperature on vessels and decay		X		
Determine effect of forced air cooling on rind disorders	832			
Determine influence of loading at room temperature on decay and shelf life	932			
Determine optimum pre-cooling temperature to prevent excessive condensation during handling in port and loading of vessels		X		
Determine optimum rate of cooling to restrict rind disorders		X		
Investigate the variation and influence of temperature and humidity during transport with Tautliners vs flat bed trucks.			X	
Do trials on ambient loading of soft citrus	832			
3.3.2 Packaging and Palletizing				
Evaluate new pallets and set minimum specifications for pallets, including fungal en pest treatments. Ongoing project.				X
Find alternative material to wood for manufacturing of pallets. Ongoing.				X
Investigate stronger board combinations to replace end pieces. Ongoing.				X
Set handling guidelines for all aspects of the cold chain. Ongoing.				X
Set guidelines and specifications with photos for palletizing of all cartons (strapping, securing sheets, corner pieces, etc.)				X

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Evaluate different sizes and types of corner pieces. Ongoing for new corner pieces				X
Accreditation process for packaging manufacturers and service providers in the cold chain should be implemented				X
Use of short corner pieces instead of end pieces in open tops should be investigated.				X
Set handling guidelines for all aspects of the cold chain. Cooling Working Group to finalize.				X
Evaluation of fruit in Supervent cartons under cold sterilization. Ongoing	832			X
Develop control options for wood rotting fungi on pallets.			X	

4. CULTIVAR EVALUATION

Cultivar Evaluation	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Expand and update the Cultivar Fact Sheets	Yes, CFS			
4.1 Rootstocks				
More suitable rootstocks for high pH soils in general	922, 923			
More suitable replant rootstocks for high pH soils	922, 923			
More suitable dwarfing rootstocks on high pH soils	922, 923			
Lemon cultivar/rootstock trial for the Sundays River Valley	1010A			
Reintroduction of Flying Dragon from San Miguel to be tested on heavier soils	Yes, New			
Evaluation of Argentinean rootstocks	Yes, New			
Get clarity on incompatibility of Fukumoto and Mor with trifoliate rootstocks	Fukumoto, 1007	Mor, No		
4.2 Cultivars				
Earlier and later Satsumas with a better internal quality	57A, 57 B, 57C, 57D			
Earlier and later Clementines	1000A 1000B 1000C 1000D			
Late mandarins of which the plantings are not restricted	964D			
Late mandarins for the hotter areas	812E, 75C,899B			
Navels that yield better with acceptable fruit size	812A, 899C, 963B,964C			
Early navel with round fruit with good yields	899C, 941A, 963B			
Earlier and later Star Ruby selections	Yes, 812G			
Early grapefruit which is not prone to sheepnose		No		
A better tasting red grapefruit	Yes, 812G			

	YES	NO		
	Project no.	**Low rating**	**Lack of capacity**	**Extension function**
Earlier and later Valencia selections	812D 75A&B 740A 899A 963A 964B			
Olinda Valencia to be reintroduced to the Foundation Block	Yes			
Need cross pollination chart / table for soft citrus		No		

8.3 **STUDY GROUP CHAIRMEN FOR 2013-14.**

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8.4 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PORTFOLIOS AND PROGRAMMES FOR 2013-14

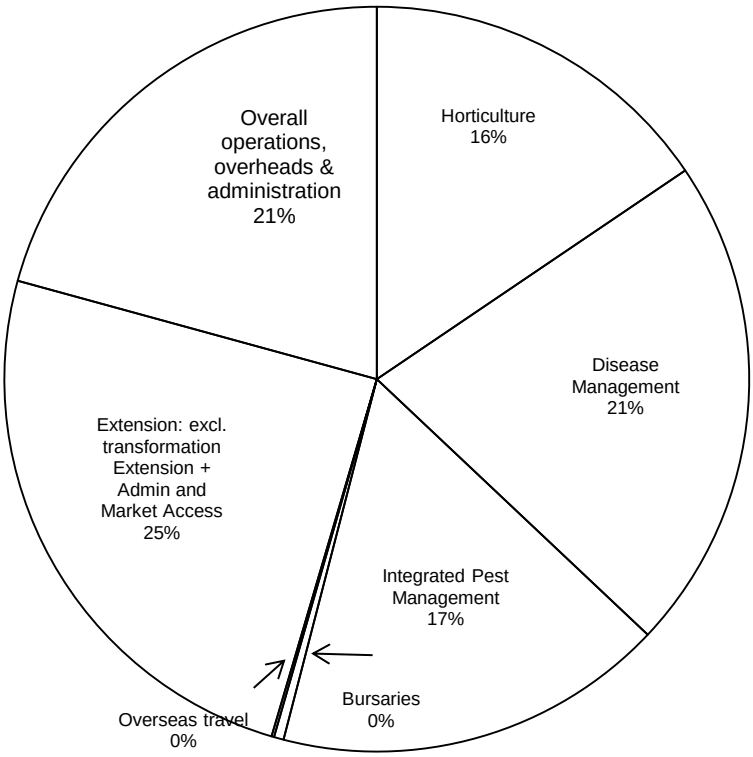

Fig. 8.4.1. Percentage funding in each CRI Portfolio and the rest of the budget for 2013-14.

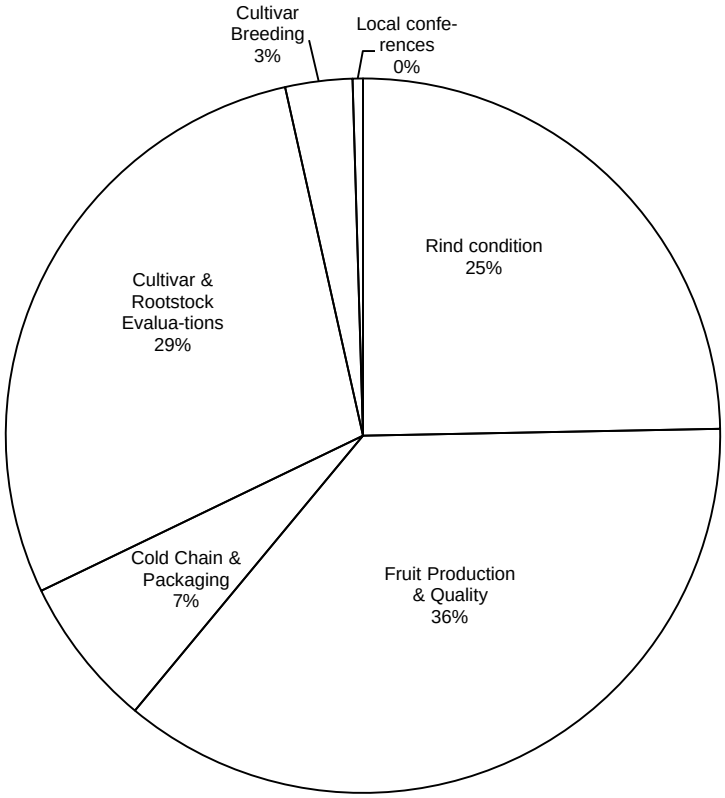

Fig. 8.4.2. Percentage funding to programmes in the CRI Research Portfolio: Horticulture for 2013-14.

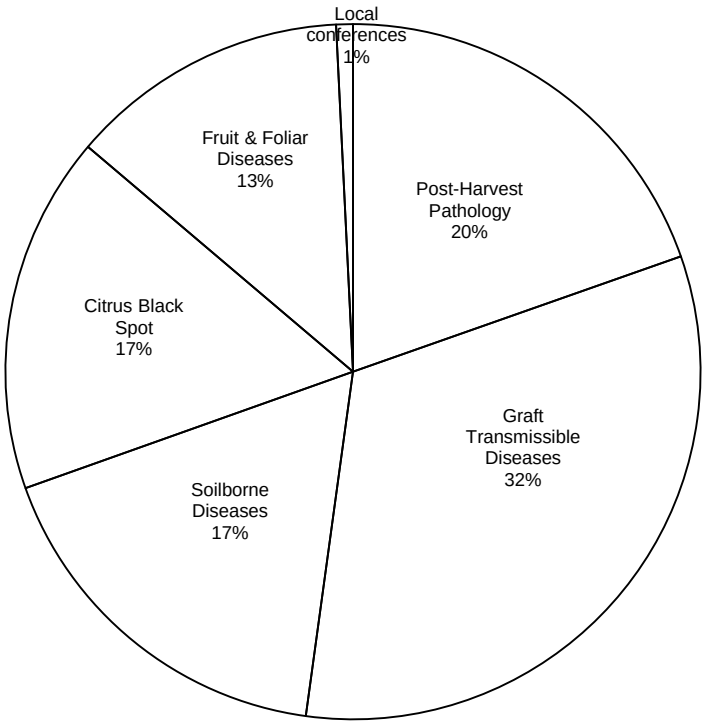

Fig. 8.4.3. Percentage funding to programmes in the CRI Research Portfolio: Disease Management for 2013-14.

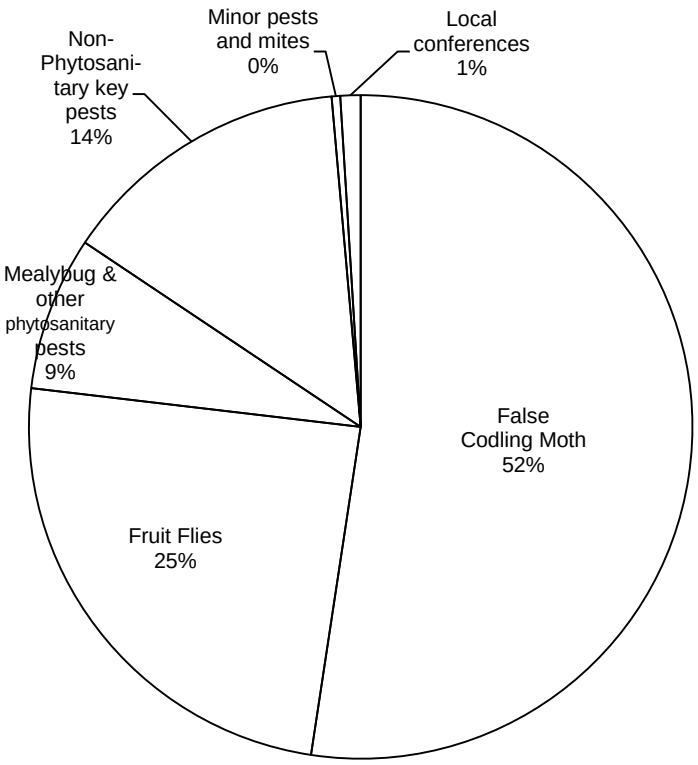

Fig. 8.4.4. Percentage funding to programmes in the CRI Research Portfolio: Integrated Pest Management for 2013-14.

8.5 EXTENSION PRESENTATIONS BY CRI RESEARCHERS IN 2013-14

Name	Date	Place	Topic
Cronjé, P.J.R.	28-31 Jan 2014	Polokwane, Loskopdam	Packhouse Workshops
	12-14 Feb 2014	Port Elizabeth, Durban	Packhouse Workshops
	18-19 Feb 2014	Stellenbosch	Packhouse Workshops
Erasmus, A.	Jan - Feb 2014	Packhouse Workshops in Limpopo, Mpumalanga, KZN, E. Cape & W. Cape	The fungicide dip tank
			Harvest to Packhouse Risk-management: Picking, handling & decay
			The heated flooder
			Drench research feedback
			Fungicide resistance management
Grout, T.G.	23 Apr 2013	ITSC, Nelspruit	Talk on fruit flies of citrus to West Africans and USDA.
	21 Jun 2013	CRI, Nelspruit	Talk to Argentinian visitors about citrus research.
	28 Jun 2013	CRI, Nelspruit	Discussion with visitors from Florida about citrus cultivars.
	25 Jul 2013	Larten Estate, Karino	Talk to growers on the latest options for pest management.
	31 Jul 2013	CRI, Nelspruit	Talk to IOCV delegates about citrus research in South Africa.
	15 Aug 2013	CRI, Nelspruit	Discussions with Nulandis and consultants about pest management.
	3-5 Sep 2013	Groblersdal, Letsitele	To growers on managing the spring pest complex.
	10 Sep 2013	CRI, Nelspruit	To growers on managing the

			spring pest complex.
	10 Oct 2013	Thohoyandou, Vhembe	Talk to growers and DAFF on *B. invadens*
	13 Mar 2014	CRI, Nelspruit	Talk on spraying citrus to international BASF visitors.
Joubert, J.	28-31 May 2013	Letsitele & Nelspruit	Cultivars
	04-07 June 2013	Durban & Addo	Cultivars
	11 June 2013	Citrusdal	Cultivars
Lesar, K.H.	Jan-Feb 2014	Packhouse Workshops in Limpopo, Mpumalanga, KZN, E. Cape & W. Cape	Exporters Technical Panel – Market Feedback for 2013 season
			Progress in citrus packhouse practices
			Pre-packhouse, degreening and packhouse treatment recommendations for 2014 season
Manrakhan, A.	17 May 2013	Laerskool Sandrift, Brits	CRI *B.i.* FF meeting. The invasive FF, *Bactrocera invadens*: background on the pest, incursions and eradication actions in SA.
	28 Aug 2013	Hortgro Crop Protection Seminar	The battle against *Bactrocera invadens* in SA.
	3-13 Sept 2013	CRI Pest & Disease Management Workshops	Fruit Fly
	11 Feb 2014	Letsitele Study Group	*Bactrocera invadens*: new fruit fly pest.
Moore, S.D.	31 May 2013	Stellenbosch	Role player workshop -EPNs for FCM
	4-5 Sep 2013	Groblersdal	Grower meeting - FCM
	6-7 Sep 2013	Tshipise	Grower meeting - FCM
	26 Sep 2013	Nelspruit	Grower meeting - FCM
	2-3 Sep 2013	Addo	Grower meeting - FCM and spring pests
	28-29 Jan 2014	Polokwane	Packhouse workshop - FCM systems approach for EU
	30-31 Jan 2014	Loskop	Packhouse workshop - FCM systems approach for EU
	11-12 Feb 2012	Durban	Packhouse workshop - FCM systems approach for EU
	31 May 2013	Port Elizabeth	Packhouse workshop - FCM systems approach for EU
	18-19 Feb 2012	Stellenbosch	PPECB Meeting - FCM systems approach for EU
	14 March 2014	Port Elizabeth	FCM systems approach for EU
Schutte, G.C.	20 Jun 2013	Argentinian visitors	Citrus Black Spot
	28 Jun 2013	American visitors	Citrus Black Spot
	23-25 Jun 2013	Sundays River workshop	Citrus Black Spot
	3 – 4 Sep 2013	Groblersdal	Citrus Black Spot
	5 – 6 Sep 2013	Letsitele	Citrus Black Spot
	11 Sep 2013	Nelspruit	Citrus Black Spot
	12-13 Sep 2013	Addo	Citrus Black Spot
	25 – 26 Sep 2013	Citrusdal	Citrus Black Spot
Vahrmeijer, J.T.	28-31 May 2013	Letsitele & Neslpruit	Conventional fertilization and irrigation
	04-07 June 2013	Durban & Addo	Conventonal fertilization and irrigation
	11 June 2013	Citrusdal	Conventional fertilization and irrigation

8.6 OTHER MEANS OF TECHNOLOGY TRANSFER

8.6.1 **SA Fruit Journal** by Tim G Grout (CRI)

Every exporting citrus grower receives the SA Fruit Journal so it is one of the best means of transferring technology on technical issues. Bimonthly Extension Briefs are edited by Hennie le Roux and Hannes Bester and provide reminders of practices that need to be implemented at that time. All researchers contribute to these on a regular basis. In-depth, semi-scientific research articles are also provided that are usually of a practical nature and other topical or news articles are sometimes included. The citrus articles published in the SA Fruit Journal during 2013/4 are listed in Table 8.6.1.1. Due to the lag time of two months between submission of the articles and circulation of the journal, urgent information is circulated to growers as Cutting Edge or Snykant articles via CRInet and emails to the technology transfer groups.

Table 8.6.1.1. SA Fruit Journal articles in 2013-14

Issue	Article	Author
April/May	Na-oes Vriesskade van Sitrusvrugte	P. Cronjé & J. Mouton
	CRI training day for DAFF inspectors	T.G. Grout
	Efficacy of M3 bait stations and GF-120 for control of fruit flies in a citrus orchard in Mpumalanga	A. Manrakhan & J-H. Daneel
Jun/July	CRI Na-Oes Werkswinkels uiters gewild	J.J. Bester & H.F. le Roux
Aug/Sept	Controlling *Bactrocera invadens* by using protein bait and male annihilation	T.G. Grout & P.R. Stephen
Oct/Nov	Extension Briefs	H.F. le Roux & J.J. Bester
Dec/Jan	Another CRI award for young scientists	T.G. Grout
Feb/Mar	19th Conference of the International Organisation of Citrus Virologists (IOCV)	G. Cook & H.F. le Roux
	Citrus Viroids …. Friend or Foe?	G. Cook, J. Joubert, S.P. van Vuuren & P.H. Fourie
	The lemon borer moth = the citrus flower moth, *Prays citri*: Its biology and control on citrus.	Moore, S.D. & Kirkman, W.

8.6.2 **CRI website** by Tim G Grout (CRI)

The average number of hits per month on the CRI website has increased significantly compared with 2012/3 with the most activity being in March 2014. Most visits were from South African IP addresses, followed by unknown domains, dot-net domains and dot-com. Of countries that could be identified, the most bandwidth was used in decreasing order by China, Turkey, Ukraine, Germany, Poland and Sweden. Statistics on usage are given in Table 8.6.2.1.

Table 8.6.2.1. Visits and page requests on www.cri.co.za since April 2013.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2012/3	10785	17806	116764	362577	4.44 GB
Apr 2013	1529	2693	13707	40209	447.37 MB
May 2013	1717	3068	15842	43513	470.02 MB
Jun 2013	1613	2606	14676	41108	503.41 MB
Jul 2013	1093	1677	10936	34099	379.12 MB
Aug 2013	1495	2817	16934	40477	464.88 MB
Sep 2013	1314	2369	11680	38118	619.31 MB
Oct 2013	1776	3612	22134	53076	978.17 MB
Nov 2013	1721	3580	16912	41045	660.09 MB
Dec 2013	2414	4441	19880	37447	548.27 MB
Jan 2014	2531	4538	19265	43476	705.86 MB
Feb 2014	2793	4469	16587	41692	688.14 MB
Mar 2014	5772	10578	33284	60118	781.96 MB
Total 2013/4	25768	46448	211837	514378	7.25 GB

8.6.3 **CRInet** by Tim G Grout (CRI)

Usage of CRInet during the report period was close to the average of around 53 messages per annum for the last 7 years (Table 8.6.3.1). It provides a good opportunity for growers to share opinions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. Membership has now passed 480.

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
2014	4	3	4										
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)

In March 2014 the Cutting Edge reached the 177th issue. Some growers consider it 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 2013/4 are given in Table 8.6.4.1.

Table 8.6.4.1. Cutting Edge issues during 2013-14.

No.	Title	Issue	Author
154	Update on the status of *Bactrocera invadens* (*B.i.*) in South Africa and grower's guide to relevant actions in different regions.	Mar	A..Manrakhan & V. Hattingh, on behalf of *Bactrocera invadens* Steering Committee
155	Packhouse inspections for False Codling Moth	April	S.D. Moore & V. Hattingh
156	Consumer Assurance Update	April	P. Hardman
157	Packing material specifications for the 2013 season	April	D. Groenewald
158	Food Safety Update	May	P. Hardman
159	Current status of the Neonicotinoid insecticides (Clothianidin, Imidacloprid and Thiamethoxam) within the EU	May	P. Hardman
160	Proposed practises to reduce risk of chilling injury on 'Nova' mandarin and late mandarins to cold sterilization markets	May	P. Cronjé & J.J. Bester
161	Selection of new citrus cultivars: handling of mutations	July	Thys du Toit, Johan Joubert, Hennie le Roux, Hannes Bester, Fanie van Vuuren and Paul Fourie
162	Certified nurseries in Southern African Citrus Improvement Scheme, and regulated movement of citrus propagation material	Aug	P. Fourie, M.M.N. du Toit & M. le Roux
163	Diagnostic Centre – request for analysis Document	Aug	M.C. Pretorius & E. Basson
164	QUICK GUIDE *Bactrocera invadens:* Your region's status and what you should do	Sept	A. Manrakhan & V. Hattingh
165	Consumer Assurance Update	Oct	P. Hardman
166	spray application in citrus – sprayer calibration and checklist	Oct	J.G. van Zyl, P. Fourie, G.C. Schutte, T. Grout (CRI) and Johan van Zyl (Nexus^{ag})
167	Areas of low CBS prevalence in the far northern Limpopo province	Oct	E. Carstens, V. Hattingh & H.F. le Roux

168	Systems approach to FCM management: Pre-harvest protocols and practices	Nov	S.D. Moore & V. Hattingh
169	Update on status of *Bactrocera invadens (B. i)*	Dec	A. Manrakhan & V. Hattingh, (CRI) D. Donkin & E. Kleynhans, Wilna Stones, (Subtrop)
170	Gesertifiseerde kwekerye in die Suider Afrikaanse sitrus verbeteringskema en gereguleerde beweging van sitrusvoortplantingsmateriaal	Dec	P. Fourie, M.N.N. du Toit & M. le Roux
171	Fungicide sprays for the control of citrus black spot	Jan	G.C. Schutte
172	Export to the European Union	Feb	V. Hattingh & E. Carstens
173	Accreditation of carton manufacturers and testing of cartons	Feb	D. Groenewald, H.F. le Roux & J.J. Bester
174	Narrow distillation range Horticultural Mineral Oils available for use on citrus in 2014	Feb	T.G. Grout
175	Fruit Piercing Moth	Mar	S.D. Moore
176	Phytophthora warning	Mar	M.C. Pretorius & J.M. van Niekerk
177	POST-HARVEST DISEASES: Pre-Packhouse and Packhouse Treatment Recommendations for 2014	Mar	K.H. Lesar & A. Erasmus

9 PUBLICATIONS IN 2013-14

9.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

Coombes, C.A., Hill, M.P., Moore, S.D., Dames, J.F. and Fullard, T. 2013. Persistence and virulence of promising entomopathogenic fungal isolates for use in citrus orchards in South Africa. Biocontrol Science and Technology, 23(9): 1053-1066.

CBS Expert Panel, 2013. Response to EFSA Panel on Plant Health, 2013 - Draft Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. Panel members: Vaughan Hattingh, Paul H Fourie, Gerhardus C Schutte, Hendrik F le Roux, Elma Carstens, Mariette Truter, Christiaan R Kellerman, Stephanus H Swart, Jacobus J Serfontein, Alice P Baxter, Mashudu Silimela, Michael A Holtzhausen, Johannes M Kotze, Ida Paul, Lise Korsten, Tim R Gottwald, James H Graham, Megan M Dewdney, Timothy Schubert, Michael Irey, Edwin L Civerolo, Timothy D Riley, Stephen M Garnsey, Geraldo Jose Silva Junior, Renato Beozzo Bassanezi, Eduardo Feichtenberger, Marcel Bellato Sposito, Armando Bergamin Filho, Andrew K Miles, Pat Barkley, Nerida J Donovan, Tania Yonow, David Daniels, Daniel Ploper, Gabriela M Fogliata, Fernando Carrera and Hernan Salas. http://www.citrusres.com/market-access

G. Cook, V.Z. Maqutu & S.P. van Vuuren. 2014. Population dynamics and seasonal fluctuation in the percentage infection of *Trioza erytreae* with '*Candidatus*' Liberibacter africanus, the African citrus greening pathogen, in an orchard severely infected with African greening and transmission by field-collected *Trioza erytreae*. African Entomology 22(1): 127–135.

Cronje, P.J.R., Stander, O.P.J., Theron, K.I., 2013. Fruit Splitting in Citrus. Horticultural Review. Vol.41: 177-200.
Cronje, P.J.R., Graham H. Barry, Marius Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of 'Nules Clementine' mandarin fruit, thereby affecting rind condition. J. Amer. Soc. Hort. Sci. 138(3):217–224.

Defraeye Thijs, Rutger Lambrecht, Alemayehu Ambaw Tsige, Mulugeta Admasu Delele, Umezuruike Linus Opara, Paul Cronjé, Pieter Verboven, Bart Nicola. 2013 Forced-convective cooling of citrus fruit: package design. Journal of Food Engineering. 118(1): 8-18.

Defraeye Thijs, Rutger Lambrecht, Mulugeta Admasu Delele, Alemayehu Ambaw Tsige, Umezuruike Linus Opara, Paul Cronjé, Pieter Verboven, Bart Nicola. 2014. Forced-convective cooling of citrus fruit: Cooling conditions and energy consumption in relation to package design. Journal of Food Engineering 121: 118–127.

De Villiers, M., A. Manrakhan, P. Addison & V. Hattingh. 2013. The distribution, relative abundance and seasonal phenology of *Ceratitis capitata, Ceratitis rosa* and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. Environmental Entomology 42 (4): 831-840.

Fourie, P.H., Tian Schutte, Suzel Serfontein and Fanus Swart. 2013. Modeling the effect of temperature and wetness on *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards. Phytopathology 103: 281-292.

Jukes, M.D., Knox, C.M., Hill, M.P. and Moore, S.D. 2014. The isolation and genetic characterisation of a South African strain of *Phthorimaea operculella* granulovirus, PhopGV-SA. Virus Research. 183, 85-88.

Magwaza, L.S., Ford, H.D., Cronje, P.J.R., Opara, U.L., S. Landahl, Tatam, R.P., Terry, L.T. 2013. Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins. Postharvest Biology and Technology. 84: 16–21.

Magwaza, L.S., Opara, U.L., Cronje, P.J.R,. Landahl, S., Terry, L.T. 2013. Canopy position affects rind biochemical profile of 'Nules Clementine' mandarin fruit during postharvest storage. Postharvest Biology and Technology. 86: 300–308.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., Hanssens, A., Saeys, W., Nicolaï, B.M., 2013. Evaluation of Fourier transform-NIR spectroscopy for integrated external and internal quality assessment of Valencia oranges. Journal of Food Composition and Analysis 31, 144-154.

Magwaza, L.S., Opara. L.U, Cronje, PJ.R., L. A. Terry, S. Landahl, and B. M. Nicolai. 2013. Non-chilling Physiological Rind Disorders in Citrus Fruit. Horticultural Reviews. Vol 41: 131-176.

Magwaza, L.S., Opara, U.L., Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A. 2014. Assessment of rind quality of 'Nules Clementine' mandarin during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. Scientia Horticulturae 165 : 410 – 420.

Magwaza, L.S., Opara, U.L., Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A. 2014 . Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. Scientia Horticulturae 165:421–432.

Manrakhan, A., Daneel, J-H. and Moore, S.D. 2014. The impact of naturally occurring entomopathogenic nematodes on false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), in citrus orchards. Biocontrol Science and Technology, 24(2): 241–245.

Manrakhan, A. & P. Addison. 2014. Assessment of fruit fly management practices in deciduous fruit growing areas in South Africa. Pest Management Science 70: 651-660.

Moore, S.D., Richards, G.I., Chambers, C. & Hendry, D. 2014. An improved larval diet for commercial mass rearing of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). African Entomology, 22(1): 216-219.

Moore, S.D., Coombes, C.A., Manrakhan, A., Kirkman, W., Hill, M.P., Ehlers, R-U., Daneel, J-H., de Waal, J., Dames, J. and Malan, A.P. 2013. Subterranean control of an arboreal pest: EPNs and EPFs for FCM. Insect pathogens and entomoparasitic nematodes IOBC-WPRS Bulletin Vol. 90, pp. 247-250.

Njombolwana, Ncumisa S., Arno Erasmus, J. Gideon van Zyl, Wilma du Plooy, Paul J.R. Cronje and Paul H. Fourie. 2013. Effects of citrus wax coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges. Postharvest Biology and Technology 86: 362-371.

Opoku-Debrah, J.K., Moore, S.D., Hill, M.P. & Knox, C. 2013. Characterisation of novel CrleGV isolates for false codling moth control - lessons learnt from codling moth resistance to CpGV. Insect pathogens and entomoparasitic nematodes IOBC-WPRS Bulletin Vol. 90, pp. 155-159.

Stammler, G., G.C. SChutte, J. Speakman, S. Miessner, P.W. Crous. 2013. *Phyllosticta* species on citrus: Risk estimation of resistance to QoI fungicides and identification of species with cytochrome *b* gene sequences. Crop Protection, Vol. 48, pp. 6-12.

Van Zyl, J.G., P.H. Fourie, G.C. Schutte. 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride. Crop Protection 46: 80-87.

Wessels, Bernard A., Sandra C. Lamprecht, Celeste C. Linde, Paul H. Fourie, and Lizel Mostert. 2013. Characterization of the genetic variation and fungicide resistance in *Botrytis cinerea* populations on rooibos seedlings in the Western Cape of South Africa. European Journal of Plant Pathology 136: 407-417.

9.2 SEMI-SCIENTIFIC PUBLICATIONS

Taylor, N.J., N. Ibraimo, J.G. Annandale, C. Everson, M. Gush, J.T. Vahrmeijer. 2013. Are sap flow measurements useful for determining water use of fruit orchards, when absolute values are important. Acta Horticulturae 991. Proceedings: 9th International Workshop on Sap Flow.

10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Coombes, C., Dames, J., Hill, M., Moore, S. & Fullard, T. 2013. Fungi do it in the dirt. Entomological Society of southern Africa.

Chambers, C.B., Moore, S.D., Hill, M.P. & Knox, C. 2013. Production of the *Cydia pomonella* granulovirus (CypoGV) in a heterologous host. Entomological Society of southern Africa.

Erasmus, A., Lennox, C.L., Jordaan, H., Smilanick, J.L., Lesar, K., Fourie, P.H. 2013. Optimising imazalil application in citrus packhouses. Post-harvest Innovation Programme: Symposium, Spier Estate, South Africa, 19 November, p.27.

Fourie, P.H., van Zyl, J.G., Schutte, C.G., Grout, T.G. Optimisation of spray application in South African citrus orchards: challenges and progress. Oral presentation at 12th Workshop on spray application techniques in fruit growing (SuproFruit 2013), 26-28 June 2013, Universitat Politècnica De València, Valencia, Spain.

Goddard, M., Hill, M.P. & Moore, S.D. 2013. The use of banana odour as an attractant for monitoring fruit-feeding moth, *Serrodes partita* (Fabricius, 1775) (Lepidoptera: Noctuidae) and *Achaea lienardi* (Boiduval, 1833) (Lepidoptera: Noctuidae) in citrus orchards in the Kat River Valley, Eastern Cape, South Africa. Entomological Society of southern Africa.

Grout, T.G. and Stoltz, K.C. 2013. Developing a bait for ants in citrus trees. p. 56. XVIII Congress of the Entomological Society of Southern Africa. 30 June – 3 July 2013, North-West University, Potchefstroom.

Hattingh, V, Van Vuuren, F, Le Roux, HF, Fourie PH, Carstens, E. 2013. The Southern African Citrus Industry: Status, Diagnosis & Prognosis. Keynote address at 19th Conference of the IOCV (International Organisation of Citrus Virologists), 28 July - 2 August 2013, Kruger National Park, Mpumalanga, South Africa.

Kellerman, M., Erasmus, A., McLeod, A., Beukes, I., Rose, L., Viljoen, A., De Wever, G. & Fourie, P.H. 2013. Genotyping imazalil resistance in an international collection of *Pencillium digitatum* isolates. Poster presentation at the American Phytopathological Society (APS) and The Mycological Society of America (MSA) 2013 APS-MSA Joint Meeting in Austin, Texas (10–14 August 2013).

Love, C.N., Hill, M.P. & Moore, S.D. 2013. Some oranges are sweeter than others: False Codling moth ovipositional preferences and the differences in host susceptibility. Entomological Society of southern Africa.

Manrakhan, A. J-H. Venter & V. Hattingh 2013. The battle against an invasion: the case of the African invader fly, *Bactrocera invadens*. Entomological Society of southern Africa (ESSA) XVIII, Potchefstroom.

Marsberg, T., Hill, M.P., Moore, S.D. & Knox, C. 2013. The potential biological control of Diamondback Moth, *Plutella xylostella* (Linnaeus) (1758) (Lepidoptera: Plutellidae), using a host specific virus. Entomological Society of southern Africa.

Moore, S., van der Walt, R., Kirkman, W. & Du Preez, D. 2013. Can imidacloprid cause lepidopteran pest repercussions? Entomological Society of southern Africa.

Moore, S., Kirkman, W., Stotter, R., Cohen, M., Steinberg, S. & Friedman, R. 2013. Augmentation of *Anagyrus* sp. near *pseudococci* for control of mealybug on citrus. Entomological Society of southern Africa.

Mwanza, P., Dealtry, G.B., Lee, M.E. and Moore, S.D. 2013. The effect of ultraviolet radiation on the plant biopesticide, *Cryptophlebia leucotreta* granulovirus. Microscopy Society of South Africa.

Ncumisa S. Njombolwana, Arno Erasmus, Paul Cronje, Wilma du Plooy and Paul H. Fourie. 2013. The effect of wax coating and brush type on imazalil residue loading and citrus green mould control. Poster presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.

Opoku-Debrah, J.K., Knox, C., Hill, M.P. & Moore, S.D. 2013. Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) populations from a range of geographic regions in South Africa. Entomological Society of southern Africa.

Stander O.P.J., K.I. Theron and P.J.R. Cronje. 2013. Foliar 2,4-Dichlorophenoxy Acetic Acid (2,4-D) Application after Physiological Fruit Drop Reduces Fruit Splitting and Increases Fruit Size in Mandarin. 12th International Symposium on Plant Bio-regulators in Fruit Production. Orlando, Florida, USA (28 - 31 July 2013).

Van Zyl, J.G., Schutte, C.G., Fourie, P.H. Improvement of spray deposition and control of Alternaria brown spot on mandarin leaves following sprays with copper oxychloride and selected adjuvants. Poster presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.

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