



**CRI GROUP
ANNUAL RESEARCH
REPORT
2004**



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This annual research and technical report encompasses progress achieved within the CRI Group of research alliance partner organisations over the period January 2004 to December 2004. This was the fourth year of financing research through a levy on export fruit.

The overall structure of industry research and technical services was retained as initiated in 2001, with Research, Extension and the Citrus Improvement Programme making up the three CRI Divisions. The research portfolio was maintained in the form of four Programmes, namely Disease Management, Integrated Pest Management, Crop Load and Fruit Quality Management and Cultivar and Rootstock Development, with a set of projects under each Programme. The updating of research priorities was again coordinated by the Manager Extension Services, utilising a process of industry consultation that included inputs from growers, exporters, researchers, consultants and technical advisers. Research proposals were again vetted by Programme Committees with each Committee consisting of specialist advisers and chaired by a grower nominee member of the CRI Board of Directors.

Market Access remained the overarching priority directing the industry research and technical support actions. The majority of the input was required to maintain and enhance access to existing markets as a result of the current global fresh produce trade environment being dominated by phytosanitary issues. In terms of gaining access to new markets, it was again abundantly evident that this cannot be achieved without an industry's ability to address a myriad of scientific issues pertaining to the mitigation of phytosanitary risks. The citrus industry demonstrated its excellent positioning in this regard through its ability to successfully obtain access to the Chinese export market in an unprecedented short space of time.

The research levy was renewed towards the end of 2004. The increase in the levy rate included provision for addition of certain strategic research and technical capacity, namely an industry virologist, an industry cultivar manager, an additional horticulturist and an upgraded extension service. Since the increased levy was only approved towards the end of the 2004 export season, most of this capacity expansion will not occur before 2005. However, it was possible to proceed with the virologist post in 2004. The industry was fortunate to secure the services of Professor Gerhard Pietersen in this post, and in terms of the CRI Group Alliance partnership agreements, Prof Pietersen was seconded to the University of Pretoria. In strengthening of the industry's virology capacity, CRI also established its own citrus germplasm repository in Nelspruit and, in support of the CIP, has commenced providing Shoot Tip Grafting, Virus Indexing and CTV pre-immunisation services in Nelspruit.

The southern African citrus growers, the CRI and CGA Directors and the industry's research and technical service partners, are thanked for making it possible to continue progressing towards fulfilling the CRI Mission: "To maximise the long term global competitiveness of the southern African citrus growers through the development, support, coordination and provision of Research and Technical services by combining the strengths of all CRI Group partners".

INLEIDING

Hierdie navorsing en tegniese jaarverslag sluit vordering in deur die CRI-groep van verbonde navorsingsvennoot-organisasies vir die tydperk Januarie tot Desember 2004. Dit was die vierde jaar van finansiering van navorsing deur 'n heffing op uitvoervrugte.

Die algehele struktuur van nywerheidsnavorsing en tegniese dienste is behou soos dit ingestel is in 2001, met Navorsing, Voorligting en die Sitrusverbeteringsprogram wat die drie CRI-afdelings uitmaak. Die navorsingsportefolio is behou in die vorm van vier Programme, naamlik Siektebestuur, Geïntegreerde Pestbestuur, Oeslading en Vrugkwaliteitsbestuur en Kultivar- en Wortelstok-ontwikkeling, met verskeie projekte binne elke program. Opdatering van navorsingsprioriteite is weereens gekoördineer deur die Bestuurder Voorligtingsdienste deur gebruik te maak van 'n proses van bedryfskonsultasie wat insette van die produsente, uitvoerders, navorsers, konsultante en tegniese raadgewers insluit. Navorsingsvoorstelle is gekeur deur Programkomitees, met elke Komitee bestaande uit spesialis-raadgewers onder voorsitterskap van 'n produsent genomineerd lid van die CRI-direksie.

Marktoegang het die hoofprioriteit gebly wat die rigting van bedryfsnavorsing en tegniese ondersteuning aangedui het. Meeste van die insette is benodig om bestaande markte te behou en te verbeter, aangesien die huidige globale varspprodukte-handelsgewing oorheers word deur fitosanitêre aangelenthede. In terme van toegang tot nuwe markte, was dit weereens baie duidelik dat dit nie vermag kan word sonder die bedryf se vermoë om tallose wetenskaplike sake wat verband hou met die verligting van fitosanitêre risiko's

aan te spreek nie. Die sitrusbedryf het sy uitstekende posisie in hierdie verband gedemonstreer deur suksesvol toegang te verkry tot die Chinese uitvoermark in 'n ongekende kort tydperk.

Die navorsingsheffing is teen die einde van 2004 hernu. Die verhoging in heffings het voorsiening gemaak vir die toevoeging van sekere strategiese navorsing en tegniese kapasiteit, naamlik 'n bedryfsviroloog, 'n ekstra tuinboukundige en opgradering van die voorligtingsdiens. Aangesien die verhoogde heffing eers teen die einde van die 2004 uitvoerseisoen goedgekeur is, sal meeste van die kapasiteitsuitbreiding nie voor 2005 plaasvind nie. Dit was wel moontlik om voort te gaan met die viroloogpos in 2004. Die bedryf was bevoorreg om die dienste van Professor Gerhard Pietersen te bekom en in terme van die CRI-groep Alliansie-vernootskapsooreenkoms, is Prof. Pietersen na die Universiteit van Pretoria gesekeundeer. Om die bedryf se kapasiteit in virologie te verhoog, het die CRI ook sy eie sitrus kiemplasma-versameling in Nelspruit gevestig, en met ondersteuning van die Sitrus-verbeteringskema begin om enting, virus-indeksering en CTV pre-immuniseringsdienste in Nelspruit te voorsien.

Die suider-Afrikaanse sitrusprodusente, die CRI- en SKV-direkteure en die bedryf se navorsing en tegniese dienste vennote, word bedank vir hul bydrae tot die volgehoue vordering ter vervulling van die CRI missie: "Om die langtermyn globale mededingsvermoë van die suider-Afrikaanse sitruskwekers te verhoog deur die ontwikkeling, ondersteuning, koördinerende en voorsiening van Navorsing en Tegniese dienste deur die vermoëns van al die CRI-groep vennote te kombineer".

2 PROGRAMME: MARKET ACCESS TECHNICAL CO-ORDINATION

Co-ordinator: Vaughan Hattingh (CEO: CRI)

2.1 PROGRAMME SUMMARY

Sanitary and Phytosanitary (SPS) issues remain central considerations regulating access of fresh produce to an export market. It is an internationally accepted principle that such considerations must be based on sound scientific evidence. Industry Research and Technical services therefore form a critical and central component of any successful market access programme. Pursuing, maintaining and enhancing access to markets for southern African citrus exports is consequently an overarching priority for the entire CRI Group research portfolio. This programme covers specific aspects of research and technical support that have been applied to Market Access.

The year 2004 will be remembered as the year that the South African citrus industry gained access to the Chinese market. The opening of this market represents a huge opportunity for the citrus industry and was achieved through the execution of a coordinated, multi-faceted industry strategy under the CGA umbrella. As with the opening of all new markets, the process was centred on phytosanitary considerations, requiring the completion of a Pest Risk Analysis (PRA) in accordance with scientific principles. The wealth of specialist expertise within CRI staff, proved to be extremely valuable in the provision of large dossiers of information that were integrated into the PRA, enabling exceptionally rapid conclusion of the process.

Other key Market Access developments during this report period included the following. SA submitted final Citrus Black Spot (CBS) reports to the EU, in support of SA's appeal for amendments to the quarantine status of CBS. In Spain the Valencia provincial authorities impounded a large volume of SA citrus and subjected it to thorough scrutiny for the presence of phytosanitary pests and diseases. Although this was widely reported in the Spanish press, SA did not receive official notification of non-compliance with phytosanitary regulations pertaining to this impounded fruit. Negotiations with Japan, to gain access to this market for the export of SA Clementines, did not progress and appeared to be stalled at year-end. SA proceeded with the execution of trials to validate a revised cold treatment for all citrus types to be exported to Japan. The first phase of the trial was successfully completed and the execution of the final phase is scheduled for 2005, pending developments in discussions between SA and Japan. A framework procedure for the inclusion of additional SA production areas in the USA export programme, was finalised in SA and submitted to USA for consideration. The procedure is based on a model for recognition of "CBS-free places of production", within circumscribing areas of low CBS prevalence. Additional disease surveys were conducted in the Knysna, George and Mossel Bay Magisterial districts, as well as the Northern Cape and parts of Free State, in pursuit of potentially adding to the list of CBS-free production areas that could export to the USA. The commercialisation of a virus product for the control of FCM was initiated in 2004 and promises to greatly improve the industry's ability to regulate FCM population levels. The development of the Sterile Insect Release (SIR) technique for the control of False Codling Moth (FCM), continued to progress rapidly. A proposed trial protocol, aimed at establishing the efficacy of post harvest fruit irradiation as a disinfestation treatment, was submitted to USDA for consideration. Whereas the S Korean phytosanitary inspectors previously rejected consignments on the basis of a single FCM or red scale intercepted during

inspection, technical phytosanitary arguments were successful in attaining the implementation of a tolerance for interception of these organisms during inspection.

The industry's ability to manage pesticide residue matters was enhanced by the CGA's appointment of Paul Hardman in the position of Industry Affairs Manager. The Recommended Usage Restrictions document was periodically revised to ensure that growers' production practices cater for sustained compliance with changing residue tolerances in the markets. In response to ongoing concerns about the industry's compliance with the export residue tolerance for Imazalil, the standard industry recommendations on the use of the product were amended. The SA-EU Pesticide Initiative Programme (PIP) commenced operating during 2004 and the citrus industry has secured participation, both in terms of an adaptive research portfolio and the generation of residue data to support the establishment of import residue tolerances in the EU.

PROGRAMOPSOMMING

Sanitêre en Fitosanitêre (SFS) kwessies het die sentrale oorwegings gebly wat toegang van varsprodukte tot uitvoermarkte reguleer. Dit is 'n internasional-aanvaarbare beginsel dat sulke oorwegings op wetenskaplike bewyse gegrond moet wees. Bedryfsnavorsing en Tegniiese dienste vorm 'n kritieke en sentrale komponent in enige suksesvolle marktoegang-program. Nastrewing, onderhouding en verbetering van toegang tot markte vir uitvoere van suider-Afrika sitrus is gevolglik 'n oorkoepelende prioriteit vir die hele CRI-groep navorsingsportefeulje. Die program dek spesifieke aspekte van navorsing en tegniese ondersteuning wat gerig is op marktoegang.

Die jaar 2004 sal onthou word as die jaar wat Suid-Afrika sitrusbedryf toegang verkry het tot die Chinese mark. Die opening van hierdie mark verteenwoordig 'n groot geleentheid vir die sitrusbedryf en is bereik deur die uitvoering van 'n gekoördineerde, multi-faset bedryfstrategie onder die SKV-sambreel. Soos met die opening van alle nuwe markte, het die proses gefokus op fitosanitêre oorwegings wat die voltooiing van 'n Pes Risiko Analise (PRA) in ooreenstemming met wetenskaplike beginsels vereis het. Die magdom van spesialiskundigheid binne die CRI-personeel was baie waardevol met die verskaffing van groot inligtingsdossiere wat in die PRA integreer is om die buitengewoon vinnige afhandeling van die proses te bewerkstellig.

Ander belangrike marktoegang-ontwikkelings tydens die verslagtydperk sluit die volgende in: SA het finale verslae oor sitrusswartvlek (SSV) voorgelê aan die EU ter ondersteuning van SA se versoek vir wysigings aan die kwarantynstatus van SSV. In Spanje het die Valencia provinsiale owerheid op 'n groot volume SA sitrus beslag gelê en dit onderwee aan streng toetse vir fitosanitêre peste en siektes. Alhoewel daar wyd in die Spaanse pers hieroor verslag gedoen is, het SA nie amptelike kennis gekry rakende nie-nakoming van fitosanitêre regulasies soverre dit die vrugte waarop beslag gelê is aanbetref nie. Onderhandelings met Japan om marktoegang te verkry vir SA Clementines, het nie gevorder nie en blyk teen jaareinde om gestaak te wees. SA het voortgegaan met die uitvoer van proewe ter staving van 'n hersiene kouebehandeling van alle sitrustipes wat na Japan uitgevoer sal word. Die eerste fase van die proef is suksesvol voltooi en die uitvoering van die finale fase is geskeduleer vir 2005, ahangende van ontwikkelings met besprekings tussen SA en Japan. 'n Raamwerk-prosedure vir die insluiting van bykomende SA produksiegebiede in die VSA uitvoerprogram is in SA gefinaliseer en aan die VSA oorhandig. Die prosedure is baseer op 'n model vir die uitkenning van SSV-vrye plekke van produksie, binne afgebakende gebiede met lae voorkoms van SSV. Bykomende siekte-opnames is gedoen in die landdrosdistrikte Knysna, George en Mosselbaai, sowel as die Noord-Kaap en dele van die Vrystaat, om moontlik by die lys van SSV-vrye produksiegebiede te voeg wat na die VSA kan uitvoer. Die kommersialisering van 'n virusprodukt vir die beheer van valskoddingmot (VKM) is in 2004 begin en beloop om die bedryf se vermoë om VKM-populasies te reguleer aansienlik te verbeter. Die ontwikkeling van die Steriele Insek Vrylatingstegniese vir die beheer van VKM het weereens vinnig gevorder. 'n Voorgestelde proefprotokol om die doeltreffendheid van na-oesbestraling te toets as 'n ontsmettingsbehandeling, is aan die USDA voorgelê vir oorweging. Waar die Suid-Koreaanse fitosanitêre inspekteurs voorheen besendings afgekeur het op basis van die onderskepping van 'n enkele VMK of rooidopluis tydens inspeksie, was tegniese fitosanitêre argumente suksesvol om die implementering van toleransie met die onderskepping van hierdie organismes gedurende inspeksie te verkry.

Die bedryf se vermoë om die residu-effek van plaagdoders te beheer is verbeter deur die SKV-aanstelling van Paul Hardman as Bedryfsakebestuurder. Die aanbevole gebruikbeperkings-dokument is van tyd tot tyd opdateer om te verseker dat die produsente se produksiepraktyke voorsiening mak vir die veranderde residu-toleransie in die markte. In reaksie op voortdurende besorgdheid oor die bedryf se nakoming van die uitvoer residu-toleransie vir imazalil, is die standard bedryfsaanbevelings vir die gebruik van die produk aangepas. Die SA-EU Plaagdoder Inisiatiefprogram (PIP) het gedurende 2004 begin en die sitrusbedryf het

deelname verseker in terme van beide 'n aanpasbare navorsingportefeulje en die daarstelling van residu-data om die instellings van invoer residu-toleransies in die EU te ondersteun.

2.2 CHINA

Opening of the Chinese market for export of southern African citrus was identified by citrus growers as a top priority. The process of gaining access was initiated in December 2001, when SA supplied China with data on the presence of citrus pests and diseases, together with an application to open the Chinese market for exports of citrus from SA. The CEO CGA accompanied the Minister of Agriculture to meet with the Chinese Minister of Agriculture in 2003. The Chairman of the CGA Board visited the Chinese citrus industry and initiated discussions regarding research and technical collaboration between the Chinese and SA citrus industries. CRI (CFB) sold large quantities of seed to China in 2002, 2003 and 2004. Two delegations of high-ranking Chinese officials visited SA in 2003 and were hosted by the citrus industry for part of the time.

On advice from the Chinese delegation that visited SA in December 2003, an SA delegation went to China in April 2004, to provide impetus to SA's application for access to the market. This trip was very successful and included a meeting between high-ranking SA and Chinese officials. SA fruit industry representatives provided technical support to the SA officials at this meeting. Although it was obviously valuable to have inputs made by many parties to provide a multi-faceted approach to SA's pursuit of access to this market, it was again obvious that no market access can be gained without going through the science-based, phytosanitary Pest Risk Analysis (PRA) process.

The delegation's visit to China was followed by the frenzied generation of scientific data packages that were supplied to China to assist in the execution of the Pest Risk Analysis. This was followed by the further generation of science-based arguments relating to specific details of the Pest Risk Analysis. Such processes usually take approximately five years to complete. However, the availability and commitment of CRI scientists, who demonstrated the requisite depth of knowledge and expertise, combined with the urgency created by SA's multi-faceted approach to advancing its market access application, made it possible to successfully conclude the PRA process in the space of a few months after the delegation's visit to China. Access for SA citrus to the Chinese market was officially granted on 29 June 2004 during a visit to SA by the Chinese Vice President.

Although it was a breakthrough for the citrus industry to gain official access to the Chinese market, there remains a critically urgent need for further refinement of the export protocol, before material realisation of the market potential can take place. Whereas, fruit may be supplied to the Chinese market from production sites that can demonstrate FCM-freedom, it is essential that the protocol also cater for the option to apply post-harvest cold sterilization as an FCM disinfestation treatment. A scientific data package was compiled and submitted to China in September 2004, in support of a proposal to include this cold treatment into the official export protocol. China responded in December 2004, by indicating that it wished to first send scientists and plant health regulators to SA, to clarify various technical details associated with the treatment, before potentially adopting it in the export protocol. The citrus industry has advised that it welcomes such a visit, that the industry's scientists will be available to assist and requested that the visit take place early in 2005.

2.3 EUROPE

SA's objection to the EU phytosanitary restrictions relating to CBS, on the basis that these regulations are more restrictive to trade than can be justified on the basis of scientific evidence, remained outstanding. The remaining study called for by the EU was aimed at establishing whether *Guignardia citricarpa* pycnidiospores from CBS infected fruit, may be able to colonise fallen citrus leaves and produce ascospores. This study was completed in 2004 and submitted to the EU in July 2004. The EU has acknowledged receipt and given an undertaking to revert after evaluation.

Exports of citrus from Argentina and Brazil, to Spain and the EU, were temporarily suspended in 2003, due to interceptions of Argentinian and Brazilian citrus infected with *Xanthomonas campestris* (*axonopodis*) *pv citri* (citrus canker), *Elsinoe* spp. (scab) and *Guignardia citricarpa* (CBS). After implementation of additional risk management systems, exports of citrus from these countries to EU commenced again in 2004. Exports from Argentina were again threatened in the 2004 season due to a recurrence of interceptions, but were not suspended as in the previous year.

A large quantity (approximately 70 tons) of SA citrus was impounded in Spain by the Valencia provincial authorities during the 2004 export season and the fruit was subjected to thorough scrutiny for the presence of phytosanitary pests and diseases. These events were widely reported in the Spanish press, but SA has to date not received any official notification of non-compliance with phytosanitary regulations pertaining to this

impounded fruit. It seems that the incident was an endeavour by the Valencian authorities, to demonstrate to the national Spanish authorities, that more diligent enforcement of phytosanitary regulations is required to provide the Spanish citrus producers with a higher level of protection from the potential introduction of exotic pests and diseases. Systems have subsequently been implemented in Spain to re-inspect citrus entering the Valencia province, regardless of whether it was previously inspected by national authorities at the port of entry. These developments serve to highlight the particular sensitivity of the Valencia province to the presence of imported citrus in this region. These matters have been brought to the attention of SA exporters, who need to take careful and critical consideration of this in their future operational decisions.

2.4 JAPAN

Large-scale validation of a fruit fly cold sterilization treatment had previously been conducted by A.B. Ware (1999, 2000, 2001 and 2002) and submitted to Japan in support of a request to commence exporting Clementines to Japan. Japan was supplied with additional explanation of technical details in 2003. Japan indicated that it accepts the explanations provided. However, SA and Japan failed to reach agreement on further requirements for commencement of Clementine exports to Japan. Japanese authorities were scheduled to visit SA in 2004 to inspect the Clementine industry, but this did not transpire in light of the failure to reach an agreement on the programme for the Japanese visit. This matter remained unresolved at year-end.

In a parallel process, SA requested Japan to accept a less stringent cold sterilization treatment for citrus types that already have access to the Japanese market. A technical motivation, based on a call for equivalency with Japanese import requirements for citrus from other countries, was submitted to Japan in 2002. SA was advised by Japan in 2003 that Japan would require SA to conduct a large scale validation trial for such revised treatment conditions and that this trial would need to be conducted in the presence of Japanese scientists. A trial designed to validate a less stringent cold treatment for all citrus types, including Clementines, was designed and the proposed trial protocol was submitted to Japan for consideration prior to commencement with the trials in SA. By year-end, the SA authorities had received no response from the Japanese authorities. Nonetheless, the first phase of this trial was successfully executed in the second half of 2004.

Further actions aimed at potentially gaining inclusion of lemons and limes from Swaziland into the Japan export programme, were put on hold in light of the lack of progress with Japan on the cold treatment issues.

2.5 USA

The 21 day running average, maximum permissible phytosanitary rejection rate, imposed by USDA was decreased from 25% in 2003 to 20% in 2004. The rejection levels were maintained below this threshold during the 2004 season, but rejections for unidentified mealybugs was cause for concern at one stage in the season when it was responsible for elevating the rejection rate to a level dangerously close to the 20% threshold. The continued high mealybug rejection rate, was attributable to exporters and growers having declined to make use of the molecular (PCR based) identification technique, as accepted by USDA in 2003 for implementation in the pre-clearance phytosanitary inspection procedures. As a consequence, the ARC-ITSC laboratory authorised to conduct the identifications, was understandably no longer positioned to offer the service when it was requested mid-way through the 2004 season. Mealybug rejection rates can be expected to remain high until use of this technique is taken up by exporters and growers sending fruit to the USA. The Department of Agriculture diagnostic laboratory in Stellenbosch has been requested to commence providing this identification service as a priority.

On completion of the 2004 export season, SA was notified by USDA that it required the implementation of additional risk mitigation procedures to reduce the level of phytosanitary rejections for FCM, mealybugs and grain chinch bug. Agreement was reached between the SA Agriculture Food Quarantine Inspection Service (SAAFQIS), formerly the Directorate Plant Health and Quality (DPHQ), and representatives from the citrus industry, that SA would implement formal Good Agricultural Practice (GAP) Standard Operating Procedures (SOP) for these three pests. The decision was based on the principle that, the various relevant Good Agricultural Practices that are currently standard practice among producers supplying the USA market, would be packaged into formal GAP SOPs. A telephone conference was held between USDA APHIS and SAAFQIS, with SA citrus industry representation, and this was accordingly agreed between the parties. It was further agreed that a delegation of USDA APHIS officials would visit SA in 2005 in connection with these added risk mitigation procedures. However, SA advised USDA APHIS that, in the absence of any appropriate additional technical data from USDA, SA was not prepared to accept further changes to the tolerances for the level of phytosanitary interceptions during pre-shipping inspections.

Citrus producers from several regions where Citrus Black Spot is not known to occur, requested that disease surveys be conducted to determine whether these regions could potentially be recognised as being CBS-free, as a first step in gaining access to the USA market. CBS surveys were conducted in parts of the Limpopo Province, Eastern Cape and Northern Cape in 2002. Additional surveys were conducted in parts of the Limpopo Province, Eastern Cape and eastern regions of the Western Cape in 2003. Further surveys were conducted in parts of the Eastern Cape, eastern parts of Western Cape and parts of Free State Province in 2004. A final report detailing the results of the survey conducted in the Northern Cape was submitted to SAAFQIS with a request to proceed with applying for official recognition of the Northern Cape Province as a CBS-free area. Conclusive identification of some of the samples collected from the Knysna region in December 2004 was problematic and had not been concluded by year-end.

An alternative mechanism for potentially expanding the USA supply base has been under development since 2002. A framework document, based on the model of “disease-free-production-sites” within circumscribing areas of low pest prevalence, was finalised in consultation with growers in 2004. SAAFQIS submitted this document to USDA APHIS in 2004 for consideration. No response had been received by year-end, but a discussion of the model has been scheduled for inclusion in the USDA APHIS visit to SA in 2005.

Evaluation of irradiation as a potential post-harvest FCM disinfestation treatment was initiated in 2003. In 2004, a proposed trial protocol for validating the phytosanitary efficacy of the treatment was agreed to by CRI and USDA ARS scientists. SAAFQIS submitted this trial protocol to USDA APHIS in 2004 for official acceptance, before the final efficacy trials commence in 2005. Experiments aimed at establishing the limits of citrus fruit’s exposure to irradiation are planned for execution in 2005. The sterile insect release (SIR) technique is another application of irradiation technology that is being evaluated as a means of providing improved control of FCM. The commercialisation of a granulovirus for the pre-harvest control of FCM was initiated in 2004. The successful utilisation of these treatments promises to materially improve the industry’s ability to regulate FCM population levels.

2.6 SOUTH KOREA

The technical justification for not implementing a tolerance for interceptions of Red Scale, FCM and Fruit Flies during pre-shipping inspection was contested by the industry, on the basis that such inspections are followed by a validated disinfestation treatment for these organisms. The S Korean authorities acknowledged the validity of the objection and an inspection tolerance was introduced as of 2004, significantly enhancing the value of this export programme.

SA previously requested S Korean agreement to commence applying the molecular (PCR-based) mealybug identification technique into the phytosanitary inspection process for exports to this market. A joint SA – S Korean validation trial was conducted in 2003 to demonstrate the reliability of this identification technique. S Korea reverted to SA in 2004 proposing certain modifications to the procedure, in order to make it acceptable to S Korea as a phytosanitary diagnostic procedure. By year-end, provision had been made for incorporation of the S Korean proposals into the diagnostic procedure. Final approval from S Korean officials is awaited before commencing with implementation of the technique in the export programme, potentially in 2005.

Whereas a market opportunity exists for inclusion of lemons and grapefruit in the South Korean export programme, S Korea requires cold sterilization as a disinfestation treatment for FCM. This treatment entails exposing fruit to temperatures < -0.3C for 22 days. Both lemons and grapefruit regularly display heavy chilling injury when exposed to this treatment. Progress with potentially including lemons into the S Korean export programme will be determined by the outcome of FCM host suitability trials, to be conducted on lemons in 2005.

2.7 THAILAND

A revised draft protocol for citrus exports from SA to Thailand was compiled in December 2003 and submitted to Thailand by SA authorities in June 2004. Thailand acknowledged receipt in August 2004 and in September 2004 provided assurance that they would revert with a response, but nothing had been received by SA at year-end.

2.8 OTHER MARKET ACCESS ISSUES

SA’s application to open the Israeli market for the export of lemons remains outstanding, pending SA providing scientific data on several pests and diseases in support of the PRA. Likewise, the provision of

technical and scientific data by SA is required to advance SA's application to gain access to the Australian market. Endeavours to re-instate access to the Iran market is still at an early stage of development.

India also represents a large potential market for citrus exports. During 2004, the Indian government indicated that it would forthwith enforce compliance with a comprehensive set of phytosanitary requirements. It seems that SA citrus will be able to comply with these requirements and the implementation of the necessary formal processes and procedures will be pursued in 2005.

An international standard for the treatment of wood packaging material was finalised in 2004 and will be internationally enforced in the near future.

2.9 RESIDUES AND FOOD SAFETY

The southern African citrus industry has been taking steps since 1992 to pro-actively adjust pesticide usage practices in the industry and thereby remain compliant with changes to the residue requirements of its export markets. A document entitled "Recommended Usage Restrictions for Plant Protection Products on Southern African Export Citrus" is periodically updated as a guideline to the industry. Prior to commencement of each export season, the Department of Agriculture, in collaboration with the industry, issues a list of updated MRLs in market countries. Whereas 2003 was marked by the 2,4D residue crisis, 2004 was free from residue issues of great significance.

It was previously reported that discussions had been initiated between the SA Citrus Industry, COLEACP and the European Commission, to investigate the possibility of procuring financial support for SA fresh produce industries, in their endeavours to overcome the challenges posed by changing EU residue legislation. The consequent SA-EU Pesticide Initiative Programme (PIP) was accepted by the EC in 2003, with a budget of approximately R40m over four years. The programme commenced operations in SA during 2004. The first PIP-funded citrus residue trials, aimed at supporting EU import MRLs, were initiated in the 2004/5 production season.

Whereas SA food safety legislation has been under protracted revision, in 2004 this developed to the point of finalising relevant legislation for implementation during 2005. Throughout the process the citrus industry made inputs into the drafting of the legislation and continues to make inputs regarding the future implementation of the legislation. The implications of the new legislation were communicated to the industry through a series of workshops presented by the Department of Agriculture.

2.10 SURVEY OF CITRUS BLACK SPOT IN THE NORTHERN CAPE AND FREE STATE PROVINCES

J. Coetzee, H.F. le Roux¹ & V. Hattingh²

J. Coetzee, Plant Health Inspector, Directorate SAAFQIS, Private Bag X20580, Bloemfontein, 9300

¹ H.F. le Roux, Extension Manager, Citrus Research International, P O Box 28, Nelspruit, 1200

² V. Hattingh, CEO, Citrus Research International, P O Box 2201, Stellenbosch, 7602

Summary

Citrus Black Spot (CBS) is a fungal disease of citrus caused by *Guignardia citricarpa*. CBS is clarified as a quarantine organism by some countries to which South Africa does or could export citrus fruit. One means of ensuring that whichever importing country is provided with adequate phytosanitary security is to export fruit from areas that are declared free of the quarantine organism. The South West winter rainfall region of the W. Cape Province and some magisterial districts in the eastern portion of the N. Cape are already recognised as CBS-free. The absence of recorded occurrence of CBS infected fruit in pre-export inspection of citrus from other production areas in the N. Cape and Free State Provinces, suggested that these regions may also be suitable for recognition as CBS-free areas. Consequently, several South African citrus producing areas were surveyed to establish the presence of *Guignardia* spp. At least two surveys were conducted in each area, firstly to inspect fruit for CBS symptoms and, secondly, to monitor dead leaves for the presence of perithecia and ascospores. Both backyard citrus trees and commercial citrus plantings were inspected from across the regions surveyed. In the Northern Cape the Gariep River was followed from the town of Douglas through to Alexander Bay. This included the magisterial districts of Herbert, Prieska, Hay, Gordonia, Kenhardt and Namaqualand. In the Free State the magisterial districts adjacent to the Northern Cape viz. Boshof, Jacobsdal, Fauresmith and Philippolis were surveyed. No lesions could be found on any fruit, neither could *Guignardia* spp be isolated from the leaves.

The survey results correlate closely with the results of earlier climatological studies which showed that both the Northern Cape Province and the south-western Free State are unsuitable for the establishment of CBS. A request for CBS-free status should therefore be made for the magisterial districts surveyed in the Northern Cape and the Free State Provinces. The magisterial districts of Hartswater and Warrenton, also in the Northern Cape Province, already have CBS-free status, and the districts not surveyed are unsuitable for citrus production.

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Introduction

Citrus Black Spot (CBS) is an economically important disease in many of the citrus producing areas of southern Africa. The distribution of the disease in South Africa is restricted to the northern and eastern regions. There are, however, areas in which the disease has never occurred on fruit and where no rejections of fruit have ever been found as a result of this disease. This is most probably due to synchrony between the phenology of the fruit and the climatological infection requirements of the fungus. It is unlikely that the pathogen *Guignardia citricarpa* Kiely, has never been introduced into all these areas at some stage or another through the movement of planting material before such movement was prohibited by law.

The regions from which no rejections as a result of CBS have been documented to date are the Western Cape, the Northern Cape (Vaalharts and the Lower Orange /Gariiep River), Tshipise, Weipe and a few other minor citrus producing areas in the Eastern Cape. The Western Cape has a Mediterranean climate and it is known that CBS does not occur under these conditions. The other areas mentioned have extremely dry conditions, with low humidity and almost no dew. As a result of this, the CRI, in collaboration with the Citrus Growers Association of Southern Africa and SAAFRTS, investigated the apparent absence of the black spot fungus, *G. citricarpa*, in these areas by conducting surveys across the region. This report only covers surveys conducted in the Northern Cape and Free State Province.

Materials and methods

Fruit lesions:

The first inspection in each area was a visual inspection for CBS lesions on ripe fruit. The oldest plantings of sensitive cultivars was selected in each of the areas. This included both orchards and backyard trees as old lemon trees could be found in most of the magisterial districts in home gardens. If any fruit with suspect lesions were found, these lesions would be cultured for *Guignardia* and PCR-ed to determine if *G. citricarpa* was present. If available, 500 fruit were inspected per site. Some of the sites in home gardens had less fruit.

Leaf samples:

Approximately 500 dry leaves were selected from each site where possible. A GPS reading was taken at each of the sites. The leaves were placed in aerated bags with a data card containing the following information: Production unit, orchard, variety and tree age. The samples were forwarded to Du Roi laboratories in the case of the samples collected in the Northern Cape, and to Plant Pathological Laboratories at the University of Pretoria in the case of samples taken in the Free State Province. At these laboratories the leaves were processed as follows:

- Placed leaves between two plastic mesh grids to cover approximately 700 cm² and secured with cable ties.
- Submerged each grid in hot water (40°C ± 2°C) for 5 minutes, removed and allowed to drain for 5 minutes.
- Placed each grid in an inoculum monitor/collector (developed by Quest Developments CC), to establish if perithecia with asci developed, and turned the power supply on for 1 hour.
- Trap released spores on a standard microscope slide coated with Vaseline.
- Removed slide after the running time, stained with lactophenol cotton blue and covered with a cover slip.
- Examined slides under a light microscope at 400X magnification.
- Determined the number of ascospores resembling the morphology of *Guignardia citricarpa* by counting three lanes, covering the width of the microscope field (these lanes stretch the length of the microscope slide, from the starting point to where the trapping process was stopped, and represent a surface of approximately 5 mm²).

If any *Guignardia* spores were found the leaf sample had to be PCR-ed to determine the presence of *G. citricarpa*.

Northern Cape Province:

The surveys were conducted from Douglas in the Northern Cape where the Vaal and the Gariep Rivers join. The Gariep River was followed till the point of entry into the Atlantic Ocean. This includes the magisterial districts of Herbert, Prieska, Hay, Gordonias, Kenhardt and Namaqualand, and included the towns of Douglas, Prieska, Groblershoop, Upington, Keimoes, Kakamas, Augrabies, Pofadder, Springbok and Alexanderbaai. The areas surveyed are extremely dry and the only cultivation that takes place is in a narrow strip along the river. Grapes and lucern are mostly the only crops grown in these areas.

Two surveys were conducted and 32 sites were sampled, both commercial orchards and backyard trees. During the first survey in July 2002, the trees were surveyed for fruit with CBS or related symptoms. During October 2002 dead leaves were collected from the sites visited previously. The leaf samples were then sent to the plant pathology laboratories of Du Roi Quality Management Systems (QMS) to determine the presence of *Guignardia* in the leaves using the inoculum trap developed for that purpose (see Addendum A).

Free State Province:

Two surveys were conducted in the Boshof, Jacobsdal, Fauresmith, and Philippolis magisterial districts. Boshof borders the Vaal River and is adjacent to the Northern Cape provinces that have previously been recognised as CBS-free and approved by the USDA for citrus exports to the USA. The magisterial district of Jacobsdal has the Modder- and the Rietrivier running through the district, also bordering the Northern Cape, whereas the Gariepriver forms the border between the Free State districts of Fauresmith and Philippolis and the Northern Province. Irrigation is used next to the rivers with lucern and maize being the most common crops. The rest of the area is arid and used either for sheep farming or for game farms.

During the first survey that was conducted in July 2004, 20 sites were inspected. Several of these sites consisted of back yard trees as there are only a few commercial orchards currently established in these districts. During this survey, trees were surveyed for fruit with CBS or related symptoms. Dead leaves were collected from these sites in December 2004. The samples were sent to Plant Pathological Laboratories at the University of Pretoria to determine the presence of *Guignardia* spp. in the leaves using the inoculum trap. (See Addendum B).

Results

Northern Cape Province:

Table 2.10.1 shows the co-ordinates of the sample sites. No fruit was found with CBS-like symptoms during the July 2002 survey throughout the area surveyed. The October 2002 survey confirmed the absence of *Guignardia* spp. in all of the dead leaf samples taken along the Gariep River.

Free State Province:

As was the case in the Northern Cape no CBS fruit lesions could be found in magisterial districts surveyed in the Free State Province in July 2004. The dead leaves sampled in December 2004 confirmed the absence of *Guignardia* spp. in these districts. (Table 2.10.2).

Discussion

The Northern Cape and the Free State Provinces should be added to the Western Cape as CBS free areas. No CBS lesions have ever been found in these areas and the inoculum trap confirmed the absence of *Guignardia* spp. spores in all the samples tested. The area is as dry as the Vaalharts area (Hartswater & Warrenton magisterial districts) which are also in the Northern Cape and which are recommended as being CBS-free. As a result of the climatological conditions being unsuitable, it is unlikely that the disease will ever establish in this area even in the event of the pathogen being introduced into the area (Paul, *et al.*, 2003).

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TABLE 2.10.1. BLACK SPOT SURVEY - NORTHERN CAPE - JULY TO OCTOBER 2002

Magisterial District	Town	Farm & Street address	Owner	Contact no.	Cultivar	Age (yrs)	Home garden/ Comm.	°S	°E	Altitude (m)	CBS lesions (fruit)	Guignardia spores from leaf samples
Herbert	Douglas	Cnr. D'arcy Loch/Ropes	Mev. Lahoud	082 897 0644	Navel & Valencias	60+	HG	29°03.417	23°46.762	1010	None	0
Herbert	Douglas	Reynoldstraat 1	L. Wilken	082 578 5576	Navels	10+	HG	497	019	1009	None	0
Prieska	Prieska	Du Toitstraat 18	A.T. van Heerden	053 3533504	Navels, Valencias & Minneolas	5+	HG	2940.198	22°45.214	965	None	0
Prieska	Prieska	Kom maar nader Kwekery Lang straat	A. du Toit	053 31417	Grapefruit (GF)	5+	Nursery	29°39.624	22°44.926	954	None	0
Prieska	Prieska	Kameelfontein	J. Beukes	053 3540829	Navels	40	Orchard	29°23.551	22°38.130	1008	None	0
Prieska	Prieska	Kameelfontein	J. Beukes	053 3540829	Minneolas	40	Orchard	29°23.551	22°38.130	1008	None	0
Prieska	Prieska	Kameelfontein	J. Beukes	053 3540829	Navels	20+	Orchard	29°23.551	22°38.130	1008	None	0
Prieska	Groblershoop	Sandpunt	B. v/d Westhuizen	082 825 2711	Navels	20+	Orchard	28°53.436	22°10.190	889	None	0
Prieska	Groblershoop	Sandpunt	B. v/d Westhuizen	082 825 2711	Navels	20+	Orchard	28°53.436	22°10.190	889	None	0
Prieska	Groblershoop	Salveris Boerdery	P.S.V. Venter	054 8330210	Eureka lemon	10	Orchard	28°54.002	21°59.733	890	None	0
Hay	Groblershoop	Winstead	S. van Zyl	082 898 6245	Eureka lemon	3	Orchard	28°55.155	22°09.393	883	None	0
Prieska	Groblershoop	Bakpoort	Hanekom		Navels	20	Orchard	28°48.875	21°55.831	885	None	0
Gordonia	Upington	Econos	K. de Wet	054 3311084	Delta Valencia	6	Orchard	28°23.580	21°24.218	853	None	0
Gordonia	Upington	V/d Wath Landgoed	H. van der Wath	054 3322970	Eureka lemon	6	Orchard	28°25.028	21°26.153	845	None	0
Gordonia	Upington	Dyasons Klip Farm	H.O. Davis	054 4911326	Eureka lemon	20	HG	28°36.822	21°06.688	769	None	0

Gordonia	Keimoes	Von Weillich Straat 4	S. Baart	082 4925160	Eureka lemon Star Ruby g/fruit	5+	HG	20°42.569	20°58.932	841	None	0
Gordonia	Keimoes	Loxtonvale Lot 5	F. Loxton	054 4630094	Valencias	10+	HG	28°43.318	20°51.246	834	None	0
Gordonia	Keimoes	Warmzand	A. Valentin	054 4630077	Eureka lemon	4	Orchard	28°44.656	20°48.832	728	None	0
Gordonia	Kakamas	Zwartbosberg	J. Spannenberg	054 4316100	Eureka lemon	7	Orchard	28°45.163	20°42.403	728	None	0
Gordonia	Kakamas	Krantzberg	J. Nel	082 5326469	Eureka lemon	3	Orchard	28°45.448	20°42.056	707	None	0
Gordonia	Kakamas	Kromhout Boerdery	D. Janse v Rensburg	082 7716758	Star Ruby g/fruit	15	Orchard	28°44.553	20°39.107	737	None	0
Gordonia	Kakamas	Kromhout Boerdery	D. Janse v Rensburg	082 7716758	Eureka lemon	4	Orchard	28°47.829	20°40.473	722	None	0
Gordonia	Kakamas	Nuwepos	L. Viljoen	0823747972	Delta Valencia	3	Orchard	28°38.796	20°18.246	495	None	0
Gordonia	Kakamas	J.H. Retief Boord	J. Koch Brand	082 5503802	Eureka lemon	6	Orchard	28°38.796	20°18.219	571	None	0
Gordonia	Kakamas	Rooikruin	J.M. de Kock	082 925 2120	Eureka lemon	3	Orchard	28°42.150	20°27.810	671	None	0
Gordonia	Kakamas	Augrabies	J. Spannenberg	054 4316100	Delta Valencias	15	Orchard	28°39.884	20°27.256	641	None	0
Gordonia	Blouputz	Zeekoeisteeek	A. Valentin	(H. Visser) 054 4510023	Star Ruby g/fruit	12	Orchard	28°27.948	20°05.611	498	None	0
Kenhardt	Onseepkoms Kenhardt	Perseel 61	F.N. Potgieter	Niekie Tidion 054 9510005	Navels	8	Orchard	28°46.446	19°14.969	640	None	0
Kenhardt	Pofadder	Klein Pella	P. Carstens		Var. blok	10	Orchard	28°46.446	19°14.969	640	None	0
Namaqualand	Alexanderbaai	Brandkaros	Alexcor	027 8311856	Eureka lemon	2	Orchard	28°28.587	16°40.871	43	None	0

Magisterial District	Town	Farm & Street address	Owner	Contact no.	Cultivar	Age (yrs)	Home garden/ Comm.	°S	°E	Altitude (m)	CBS lesions (fruit)	<i>Guignardia</i> spores from leaf samples
Namaqualand	Alexanderbaai	Brandkaros	Alexcor	027 8311856	Valencias	10	Orchard	28°28.573	16°40.958	44	None	0
Brits*	Brits	Buffelsfontein	H. le Roux	(013) 7598000	Eureka lemon	20	Orchard	25°48.333	27°38.597	744	None	0
Brits*	Brits	Buffelsfontein	H. le Roux	(013) 7598000	Eureka lemon	20	Orchard	25°48.414	27°38.585	744	None	22

*Positive control samples from the North West Province

TABLE 2.10.2. BLACK SPOT SURVEY – FREE STATE – JULY & DECEMBER 2004

Description of site	Surname & initials	Phone / Fax no.	Culti-var	Tree Age (yrs)	Home garden/ Comm.	°S	°E	Altitude (m)	CBS lesions (fruit)	Guignardia spores (leaves)
BOSHOF										
Gould Street	M.J. Vorster	053-5410415	Val.	50+	HG	28° 32.660 ¹	25° 13.947	1256	None	0
Hoof Street	?	?	Val.	20+	HG	28° 32.469 ¹	25° 14.319	1252	None	0
Oranje Street	J.A. Vosloo	072 141 4532	Val. Eur.	20+ 20+	HG HG	28° 32.401 ¹ as above	25° 14.427 as above	1263 as above	None None	0 0
Orange Street	Vosloo's neighbour	-	GF Nav.	20+ 20+	HG HG	as above as above	as above as above	as above as above	None None	0 0
JACOBSDAL										
Andries Pretorius Begrafnisdienste	H.A. Crous	053-5910191	Nav.	10	HG	29° 07.765 ¹	24° 46.462 ¹	1139	None	0
21 Kerk Street	H.J. Herholdt	053-5910204	Mand	25+	HG	29° 07.683	24° 46.381 ¹	1138	None	0
Heuningneskloof Road plots	P. Wilker	053-5917040	Nav. Val. Mand	40+ 40+ 30+	Comm. Comm. Comm.	29° 07.683 as above as above	24° 43.162 as above as above	1158 as above as above	None None None	0 0 0
FAURESMITH										
Harrismith Street	F.G. Coetzee	051-7230495	Val.	20+	HG	29° 45.021 ¹	25° 19.075 ¹	1384	None	0
Agterkerk Street	H.I. Scheepers	051-7230212	Val.	15	HG	29° 45.063 ¹	25° 19.000 ¹	1382	None	0
Oos Burger Street	Wilma Smit	051-7230087	Val. Eur. Nav.	8 8 8	HG HG HG	29° 45.080 ¹ as above as above	25° 19.005 ¹ as above as above	1377 as above as above	None None None	0 0 0
Kerk Street	Leandra	051-7230495	RL	20	HG	29° 44.940	25° 19.095 ¹	1362	None	0

Rust										
Glen da Loch	I.A. Malherbe	082 371 8890	Nav.	4-9	Comm.	29° 57.288 ¹	24° 40.847 ¹	1139	None	0
Uitsig	J. Botha	082 855 4833	Nav.	10	Comm.	29° 58.220 ¹	24° 41.702	1161	None	0
			Eur.	10	HG	as above	as above	as above	None	0
			Min.	10	HG	as above	as above	as above	None	0
			SR	10	HG	as above	as above	as above	None	0
Van der Kloof / Luckhoff road										
Dundee	J. Lombard	082 787 7181	Nav.	50+	Comm.	29° 55.683	24° 43.101	1186	None	0
			GF	50+	HG	as above	as above	as above	None	0
			Eur.	50+	HG	as above	as above	as above	None	0
			Nav.	10	HG	29° 55.654	24° 42.998	1177	None	0
Luckhoff										
Cnr Fowler & Voortrekker Streets	S. Rabie	-	Eur.	10	HG	29° 45.139 ¹	24° 47.302 ¹	1279	None	0
			Nav.	10	HG	as above	as above	as above	None	0
Rabie Street	J.D. Theron	082 771 2036	Val.	15+	HG	29° 44.867	24° 47.393	1283	None	0
			Nav.	15+	HG	as above	as above	as above	None	0
PHILIPPOLIS										
Voortrekker Street	J.A. Celliers	051-7730051	Val.	30+	HG	30° 15.820 ¹	25° 16.428 ¹	1387	None	0
			Nav.	30+	HG	as above	as above	as above	None	0
Tobie Muller Street	K. du Plessis	051-7730022	Val.	10	HG	30° 16.108 ¹	25° 16.465	1383	None	0
			Nav.	10	HG	as above	as above	as above	None	0
			Mand	10	HG	as above	as above	as above	None	0

ADDENDUM A



DU ROI QMS & PATHOLOGICAL SERVICES

PO BOX 66, LETSITELE, 0885
Vat 4930117363
Tel / Fax : (015) 3451227
email: duroi@telkomsa.net

<u>Client</u> :	CRI	<u>Ref.N^o</u> :	-
<u>Contact person</u> :	Dr Hennie Le Roux	<u>Client/Order N^o</u> :	-
<u>Postal address</u> :	-	<u>Date received</u> :	November 2002
<u>Fax N^o</u> :	-	<u>Date completed</u> :	6 January 2003
<u>Sample submitted by</u> :	Dr Hennie Le Roux		

Results of citrus leaf samples obtained from different geographical production areas in South Africa

Material and Methods

Well-decomposed citrus leaves were gathered from the orchard floor in several different citrus production areas. Leaves were placed between two plastic mesh grids (supplied by Quest Developments CC) to cover approximately 700 cm² and secured with cable ties. Each grid was submerged in hot water (40°C ± 2°C) for 5 minutes, removed and allowed to drain for 5 minutes. It was then placed in the inoculum monitor (developed by Quest Developments CC) and the power supply turned on for 1 hour.

Spores were trapped on a standard microscope slide coated with vaseline. The slide was removed after the running time, stained with lactophenol cotton blue and covered with a cover slip. Slides were examined under a light microscope at 400x magnification. The number of ascospores, resembling the morphology of *Guignardia citricarpa*, was determined by counting three lanes, covering the width of the microscope field. These lanes stretched in the length of the microscope slide, from the starting point to where the trapping process was stopped and represented a surface of approximately 5 mm².

Samples where *Guignardia*-type ascospores were found will be sent to the University of Pretoria to confirm the identity of *Guignardia citricarpa* using PCR techniques.

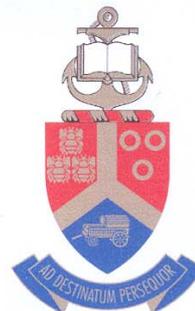
Results

Sample No.	Sample collected from	<i>Guignardia</i> spores
1	Northern Cape	0
2	Northern Cape	0
3	Northern Cape	0
4	Northern Cape	0
5	Northern Cape	0
6	Northern Cape	0
7	Northern Cape	0
8	Northern Cape	0
9	Northern Cape	0
10	Northern Cape	0
11	Northern Cape	0
12	Northern Cape	0
13	Northern Cape	0
14	Northern Cape	0
15	Northern Cape	0
16	Northern Cape	0
17	Northern Cape	0
18	Northern Cape	0
19	Northern Cape	0
20	Northern Cape	0
21	Northern Cape	0
22	Northern Cape	0
23	Northern Cape	0
24	Northern Cape	0
25	Northern Cape	0
26	Northern Cape	0
27	Northern Cape	0
28	Northern Cape	0
29	Northern Cape	0
30	Northern Cape	0
31	Northern Cape	0
32	Brits	0
33	Brits	22

Thank you for your support.

W. van Broekhuizen (Plant Pathologist)
Cell: 083 559 2018

ADDENDUM B



PLANT PATHOLOGY LABORATORIES

Department of Microbiology and Plant Pathology

University of Pretoria

Hillcrest, Pretoria 0001

Tel: 012-420 3297 Fax: 012-420 4588

Test Report

TR 05/02/04 A

***Guignardia* ascospore capturing with Kotzé Inoculum Monitor**

18 March 2005

Dr. Hennie le Roux
Citrus Research International
PO Box 28
Nelspruit
1200

Test Report:

Test report 05/02/04 dated 10 March 2005 was split into three reports (A, B and C), reporting on samples collected in the Free State, Eastern Cape and Western Cape, respectively.

Material:

Twenty samples (1 to 16 and 45 to 48) collected in the Free State were received on 10 January 2005. The first samples were processed on 13 January 2005.

Processing of leaf litter samples:

Leaves were randomly selected from the sample and secured between two circular plastic grids (350 mm diameter, 10 mm mesh size) with cable ties. Grids with leaves were submerged in water at 40 °C for 5 min, followed by draining on paper towels to remove

excess water. A grid with leaves was placed in the Kotzé Inoculum Monitor and a microscope slide coated with Vaseline was used to collect spores.

Counting of ascospores:

After the 2-h operation at room temperature, the slide was removed, stained with lactofuchsin and examined with a compound microscope at 400x magnification. Each slide was divided into 5 mm sections along the longitudinal transect and ascospores resembling those of *Guignardia* spp. were counted along four lanes, covering the width of the microscope field within the centre longitudinal transect. These lanes stretched in the length of the microscope slide from the starting point to where the trapping process was stopped.

Results:

No ascospores were captured from the samples collected in the Free State (Table 1).

Table 1. *Guignardia* spp. ascospores collected with the Kotzé Inoculum Monitor from citrus leaf litter collected in the Free State

Sample no	Ascospore count ^a
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0

Table 1. Continued

Sample no	Ascospore count ^a
16	0
45	0
46	0
47	0
48	0

^aTotal ascospore counts from four microscope field lanes.

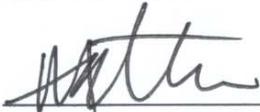
We do not accept responsibility for deviations in test results if samples were not collected/taken by us.

The results given relate only to the samples tested.

Only the original copy of this report may be accepted as correct. We do not accept responsibility for content or confidentiality of documents transmitted electronically.

This test report may not be reproduced, except with full and written approval of the Head of the Division of the PLANT PATHOLOGY LABORATORIES.

Analysed by: Mrs. M. Truter

Signature:  _____

Date: 18 March 2005

Technical manager: Mrs. A. Lombard

Signature:  _____

Date: 18 March 2005

3 PROGRAMME: INTEGRATED PEST MANAGEMENT

3.1 PROGRAMME SUMMARY

By Tim G. Grout (Research & Technical Manager)

Research within the Integrated Pest Management programme centred around the improvement of biocontrol and microbial control of pests, developing biorational control techniques such as the Sterile Insect Technique for false codling moth, and the evaluation of possible alternatives to commonly used pesticides that may not be available in the future. In addition, further labour intensive, cold sterilisation research was conducted on Medfly with a view to gaining Japanese acceptance of a treatment regime that is less detrimental to citrus than the current one. The non-target effects of commonly used pesticides on a predator of the key pest citrus thrips were determined and an evaluation of possible alternatives to chemicals commonly used for citrus thrips control showed that kaolin sprays were probably not efficacious enough for control early in the season. The efficacy of a commercial Australian nucleopolyhedrovirus against bollworm was disappointing and not as good as the non-commercialised South African strain evaluated a few years ago. Alternative toxicants evaluated to date in fruit fly baits were not very promising but research continues. Some progress was made on the control of citrus psylla with new IPM-compatible treatments but unfortunately spinosad and spiroticlofen were ineffective. No further knowledge was gained on the biocontrol of oleander mealybug as infested plants placed in orchards were not parasitised. However, meaningful progress was made in various aspects of reducing dependence on chemicals for the control of false codling moth. Many of the breakthroughs involved the development of techniques required for the use of SIT for the control of this phytosanitary pest.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

Navorsing in die geïntegreerde plaagbestuur (IPB) program het gekonsentreer op die verbetering van biologiese en mikrobiële beheer van peste, ontwikkeling van biorasionele beheertegniese soos die Steriele Insek Tegniek (SIT) vir valskodlingmot en die evaluasie van moontlike alternatiewe vir algemeen-gebruikte insekdoders wat dalk nie meer in die toekoms beskikbaar mag wees nie. Verdere arbeidsintensiewe navorsing is gedoen op koue-sterilisering vir die beheer van Medvlieg vir aanvaarding in Japan van 'n behandeling wat minder skadelik vir sitrus is as die huidige een. Die nie-teiken effekte van algemeen-gebruikte insekdoders op die predatore van die sleutelpes sitrusblaaspootjie is ondersoek. Evaluasie van moontlike alternatiewe vir algemeen-gebruikte chemiese middels om sitrusblaaspootjie te beheer het getoon dat kaolien-bespuitinge nie doeltreffend genoeg is vir beheer vroeg in die seisoen nie. Die effektiwiteit van 'n kommersiële Australiese nukleêre polihedrovirus teen bolwurm was teleurstellend en nie so goed soos dié van 'n nie-komersiële Suid-Afrikaanse ras wat 'n paar jaar gelede getoets is nie. Alternatiewe gifstowwe wat tot op datum geëvalueer is in vrugtevlieg-lokase was nie baie belowend nie, maar navorsing gaan voort. 'n Mate van vordering is gemaak met die beheer van sitruspsilla met nuwe IPB-verenigbare behandelings, maar ongelukkig was spinosad en spiroticlofen nie effektief nie. Geen verdere kennis is ingewin oor die biobeheer van oleander wiluis nie aangesien besmette plante wat in boorde geplaas is, nie gearasiteer is nie. Betekenisvolle vordering is nietemin gemaak t.o.v. verskeie aspekte gemik daarop om die afhanklikheid van chemikalieë vir die beheer van valskodlingmot te verminder. Verskeie van die deurbrake het die ontwikkeling van tegniese benodig vir gebruik in SIT vir die beheer van hierdie fitosanitêre pes behels.

3.2 PROJECT: BIOCONTROL DISRUPTION

Project Co-ordinator: Tim G. Grout (CRI)

3.2.1 Project summary

During 2004, most biocontrol disruption research was conducted on non-target bioassays with the soil predator of citrus thrips, *Androlaelaps* sp. Several bioassays had to be repeated due to unusual behaviour or inconsistency but the final results showed some differences from the arboreal predatory mites. These mainly involved the carbamates to which *Androlaelaps* was more tolerant than *Euseius* mites (3.2.2). Non-target effects of a range of chemicals could not be tested on the predatory thrips *Franklinothrips megalops* as planned due to it not being possible to establish a culture of this predator. There is therefore no report on this research.

Projekopsomming

Gedurende 2004 is navorsing oor biobeheer-ontwrigting grootendeels gerig op nie-teiken biotoetse met die grondpredator van sitrusblaaspootjie, *Androlaelaps* sp. Verskeie biotoetse moes herhaal word as gevolg van ongewone optrede of teenstrydighede, maar die finale resultate het gedui op 'n aantal verskille met

boomwonende roofmyte. Hierdie verskille het hoofsaaklik die karbamiede ingesluit, waarteen *Androlaelaps* meer verdraagsaam was as *Euseius* myte (3.2.2). Nie-teiken effekte van 'n reeks insekdoders kon nie teen die roofblaaspootjie, *Franklinothrips megalops*, getoets word soos beplan nie, omdat dit nie moontlik was om 'n populasie van die predator te vestig nie. Daar is dus geen verslag oor hierdie navorsing nie.

3.2.2 Determine the non-target effects of key pesticides used in citrus to the soil predator of citrus thrips, *Androlaelaps* sp.

Experiment 723 by Tim G Grout, Kim Stoltz, Bruce Tate and Peter Stephen (CRI)

Opsomming

Aangesien sitrusblaaspootjie, *Scirtothrips aurantii*, 'n inheemse pes is, het dit 'n kompleks van natuurlike vyande eerder as een of twee hoogs-effektiewe vyande. Hierdie kompleks dra by tot mortaliteit op verskillende lewenstadiums. Een van die natuurlike vyande is die grondroofmyt, *Androlaelaps* sp., wat op sitrusblaaspootjies roof wat op die grond verpop. Om behoud van hierdie roofmyt te verbeter is 'n reeks bioetse uitgevoer om die nadelige nawerking van 'n wye reeks insekdoders wat in sitrusboorde gebruik kan word, vas te stel. In teenstelling met die boomwonende roofmyt, *Euseius* spp., wat baie vatbaar is vir karbamaat lokase, was *Androlaelaps* heelwat minder sensitief vir Mesurol en Dicarzol lokase, maar gevoelig vir voldekkingbespuiting met Lannate. Organofosfaat insekdoders het gewissel van effens tot baie skadelik, maar die nuwe blaaspootjiedoders, Calypso en Tracer, was soos abamectin-, braakwynsteen- en oliebespuitings, skadeloos. Indien voldoende beheer van sitrusblaaspootjie verkry kan word met laasgenoemde behandelings, kan predasie van papies deur *Androlaelaps* in die grond verhoog word. Verdere navorsing met *Androlaelaps* word nie beplan nie.

Introduction

In most citrus production regions of South Africa, citrus thrips, *Scirtothrips aurantii*, is a key cosmetic pest requiring chemical intervention after petal fall. Typically, two to four pesticide applications are required depending on the population density of the pest. These treatments are variably detrimental to natural enemies of citrus thrips and other citrus pests and are therefore detrimental to IPM. Most important pesticides used in citrus have now been tested against 5 key arboreal natural enemies and standard bioassay techniques have been developed (Hattingh et al. 2000). Research on soil predators that are known to prey on citrus thrips can now be conducted. Run-off from film-wet sprays and direct spray from low spray nozzles may be detrimental to these natural enemies and reduce the degree of biocontrol in IPM orchards. It is not known whether the soil predators are more or less susceptible than *Euseius* predatory mites found in the trees and included in previous bioassays (Grout & Richards 1992; Grout et al. 1997). This knowledge is required to maximise biological suppression of citrus thrips that is becoming increasingly important with the loss of some thripicides and other pesticides due to residue restrictions in Europe.

Materials and methods

Androlaelaps sp. (Laelapidae) predatory mites were obtained from a laboratory culture used previously for mass releases in orchards (Grout & Stephen 2003) and established from mites collected from commercial citrus orchards in 1999. The culture was reared on damp ceramic tiles using *Tyrophagus putrescentiae* as a food source that was in turn fed with ground cat food and Pro-Nutro.

Potted citrus trees of approximately 0.5 - 1 m high were sprayed with the pesticides under investigation to the point of run-off using a knap-sack sprayer. After spraying, the plants were exposed to sun and wind for 24 hours before leaves were picked for the bioassays. Five plants were sprayed per pesticide to provide sufficient flat leaves.

Pesticides evaluated were chosen from a broad range of chemical groups and are shown in Table 3.2.2.1. The most commonly used, registered dosage rate for use on citrus was evaluated using commercial pesticide formulations. A maximum of six pesticides were compared with an untreated control in each bioassay. One of the pesticide treatments in each bioassay was Lannate (methomyl) at 100 g/hl to serve as a standard and to test for consistency between bioassays.

Ten replicate test cells were used per pesticide evaluated. Each cell consisted of a small petri dish lid, which when placed on a leaf provided a test arena of 34 mm diameter and 10 mm high. Ventilation holes (6 mm diameter) were made on opposite sides of the lid. A third hole (6.8 mm diameter) was made on top to insert the mites and later closed by inserting half a dental roll. The dental rolls were used to provide a water source for the mites and were wrapped with masking tape over 50% of the surface to slow evaporation of water. A short length (10 cm) of polyvinyl chloride tubing (3 mm diameter) was covered at one end with fine

polyester mesh material and forced into each ventilation hole. One of these tubes was connected to an air-supply plenum that ensured that the air in each cell was exchanged once per minute to prevent fumigative effects. Residue-bearing leaves were placed on top of glass plates (100 mm x 65 mm x 3 mm) with their adaxial surface uppermost. A small scoop (6 mg) of *Tyrophagus putrescentiae* mites and their diet was placed in each cell using a small spatula to serve as food for the predators. Each petri dish was tightly clamped over the leaf surface using two spring-loaded clamps.

Gravid, adult female *Androlaelaps* mites were found to be phoretic and immediately climbed onto filter paper disks (5.9 mm diameter) that were held with forceps just above areas of high activity in the culture. Once more than 20 mites had climbed onto a disk it was used to transfer the mites into the cells via the hole in the top of the dish. One disk was placed per cell and the dental roll inserted to prevent mites escaping. A few drops of water were placed on each dental roll immediately after transfer. Water was given a further 3-4 times during the next 48 hours before results were determined.

After 48 hours exposure, the numbers of live adult *Androlaelaps* relative to the total number of mites per cell, were determined. The treatment mortalities were corrected for control mortality using Abbott's (1925) formula. Bioassays were started on 25 August and were conducted when convenient until 30 November 2004.

Results and discussion

During the development of the bioassay technique it was found that the presence of both water and food was essential to maintain low control mortalities. Once these requirements were provided, control mortality remained below 7% in all bioassays. Three bioassays were scrapped and repeated due to inexplicable results. In two of these bioassays, all mortalities were close to zero except for the Lannate treatment that was unusually low in one case and appeared normal in the other. In the third bioassay that was scrapped, all mortalities were unusually high, except for the untreated control. Very little is known about the biology of these mites and whether there is variable susceptibility to pesticides with age. The bioassay that was scrapped due to high mortality was one where insufficient numbers of phoretic mites were present and mites were taken from the culture with a brush that may have not been gravid females. In one of the scrapped bioassays with low mortality it was noted that the mites were unusually active and ate more food than normal. Perhaps the heightened activity also meant that they detoxified the chemicals more rapidly.

The results from the remaining bioassays are presented in Table 3.2.2.1 and show similar trends to results found with *Euseius* spp. except that the carbamate baits Mesurool plus sugar and Dicarzol plus sugar were much less toxic to *Androlaelaps* than to *Euseius* mites and less toxic than the 20 g/hl dosage rate of Lannate. Perhaps *Androlaelaps* is not attracted to sugar as most other arboreal natural enemies are. The new thripicides Tracer and Calypso were both harmless to *Androlaelaps*, as were tartar emetic plus sugar, abamectin and spray oil residues. Mancozeb also appeared harmless in this bioassay but the design did not take into account the effect on immature stages that may be more acute. The three organophosphates tested (Dursban, Phosdrin and Ultracide) ranged in toxicity from Slightly harmful to Very harmful.

Spray programmes that include full cover sprays of organophosphates and Lannate for mealybug control may reduce populations of *Androlaelaps* mites in the soil and their contribution towards biological suppression of citrus thrips. On the other hand, if adequate citrus thrips control can be obtained by using Tracer or Calypso followed by abamectin or tartar emetic plus sugar, biocontrol of citrus thrips pupae in the soil will be maximised.

Table 3.2.2.1. Toxicity of various pesticides used in citrus against the soil predatory mite *Androlaelaps* sp.

Pesticide	Dosage used	Corrected mortality (%)	Toxicity
Abamectin EC + hort. oil (medium)	20 ml + 300 ml	1.6	Harmless
Calypso SC + hort. oil (heavy)	30 ml + 250 ml	0.0	Harmless
Dicarzol SP + sugar	25 g + 200 g/hl	31.9	Slightly harmful
Dursban WG	64 g/hl	49.8	Slightly harmful
Endosulfan WP	113 g/hl	79.4	Very harmful
Erador EC	75 ml/hl	26.4	Slightly harmful
Hunter SC	30 ml	57.1	Harmful
Hunter SC + sugar	15 ml + 200 g/hl	45.2	Slightly harmful
Lannate SP	20 g/hl	99.2	Very harmful
Lannate SP	100 g/hl	99.2*	Very harmful

Mancozeb WP	200 g/hl	7.5	Harmless
Mesurool WP + sugar	10 g + 200 g/hl	9.5*	Harmless
Orchex mineral oil	1.25 l/hl	2.0	Harmless
Phosdrin 500 SL	30 ml	67.6	Harmful
Tartar emetic SP + sugar	200 g + 200 g/hl	2.2*	Harmless
Torque SC	55 ml/hl	33.8	Slightly harmful
Tracer SC + hort. oil (medium)	15 ml + 300 ml	7.7	Harmless
Ultracide EC	100 ml	100.0	Very harmful

* Mean result from more than one series.

Conclusion

Although some bioassay series had to be scrapped due to erratic results, the remaining bioassays showed results that were a little different to those found previously for *Euseius* mites. Carbamate baits such as Mesurool plus sugar and Dicarzol plus sugar appeared to be less toxic than organophosphate sprays. The new thripicides Calypso and Tracer were harmless to *Androlaelaps*, as were residues of tartar emetic plus sugar, spray oil and abamectin plus oil.

Future research

No further research on *Androlaelaps* sp. is planned, although non-target bioassays on other less-known natural enemies of citrus thrips will be attempted.

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3.3 PROJECT: COSMETIC PESTS

Project coordinator: Tim G Grout (CRI)

3.3.1 Project summary

Some conclusive results were obtained in the search for OP-alternative thripicides because citrus thrips population densities were higher at the sites used than for a number of years. It was found that when the amount of oil combined with abamectin was reduced from 300 ml to 150 ml/hl the efficacy against citrus thrips improved but at the expense of reducing suppression of red scale (3.3.4). Kaolin sprays could not provide sufficient control of citrus thrips but did offer some suppression. Experiments involving the developmental thresholds of citrus thrips (3.3.2) and the switching of citrus thrips from one host plant to another (3.3.3) were not as conclusive. A culture of *Bryophyllum*-adapted citrus thrips such as has become established in Australia could not be started here due to insufficient numbers of these thrips in Pretoria. No evidence could be obtained on the molecular level to indicate that citrus thrips collected from different host plants was not always *Scirtothrips aurantii* (3.3.6). Recent damage to lemons by moths was found to be caused by *Prays citri* and numbers were suppressed by *Bacillus thuringiensis* or mevinphos (3.3.5). Good numbers of the predatory thrips *Franklinothrips megalops* were collected from *Rhus pendulina* growing along the Orange River near Kakamas but a satisfactory rearing technique could not be developed to further evaluate this predator (3.3.7). An Australian commercial formulation of *Helicoverpa armigera* nuclearpolyhedrovirus was found to be less efficacious than the South African strain evaluated a few years ago (3.3.8). Further research will continue on cosmetic pests with a view to finding less disruptive means of reducing their pest status.

Projekopsomming

Etlieke oortuigende resultate is verkry in die soektog na OP-alternatiewe blaaspootjiedoders, aangesien die digtheid van sitrusblaaspootjiepopulasies hoër was in die toetsgebiede as die afgelope aantal jare. Dit is gevind dat die effektiwiteit teen sitrusblaaspootjie verbeter het wanneer die hoeveelheid olie gekombineer met abamectin verminder is vanaf 300 ml na 150 ml/ha, maar ten koste van die onderdrukking van rooidopluis (3.3.4). Kaolienbespuitings het nie voldoende beheer van sitrusblaaspootjie gegee nie, alhoewel dit 'n mate van onderdrukking gebied het. Eksperimente met betrekking tot die ontwikkelingsdrumpel van sitrusblaaspootjie (3.3.2) en die verwisseling van gashere deur sitrusblaaspootjie (3.3.3) was minder oortuigend. 'n Kultuur van *Bryophyllum*-aangepaste sitrusblaaspootjie kon nie gevestig word soos in Australië nie, aangesien daar onvoldoende getalle van hierdie blaaspootjie in Pretoria was. Geen bewys kon op molekulêre vlak verkry word dat sitrusblaaspootjies van verskillende gashere nie noodwendig *Scirtothrips aurantii* is nie (3.3.6). Onlangse metskade aan suurlemoene is gevind om veroorsaak te wees deur *Prays citri* en die getalle is onderdruk met *Bacillus thuringiensis* of mevinphos (3.3.5). Goeie getalle roofblaaspootjie, *Franklinothrips megalops*, is versamel vanaf *Rhus pendulina* langs die Oranjerivier naby Kakamas, alhoewel 'n geskikte telingstegniek nie ontwikkel kon word om die predator verder te evalueer nie (3.3.7). 'n Australiese kommersiële formulering van *Helicoverpa armigera* nukleêre polihedrovirus was minder effektief as die Suid-Afrikaanse ras wat 'n paar jaar gelede getoets is (3.3.8). Verdere navorsing oor kosmetiese peste sal voortgaan ten einde 'n minder skadelike metode te vind om die status van die peste te verlaag.

3.3.2 Determining the developmental thresholds for citrus thrips Experiment 697 by Khakhathi David Matshaya (UP)

Opsomming

Die doel van die studie is om die effek van temperatuur op die ontwikkeling en oorlewing van *S. aurantii* te bepaal. Ten einde die onderste en boonste ontwikkelingsdrumpels vas te stel word tans eksperimente by vyf konstante temperature (18, 21, 25, 30 en 35°C) uitgevoer. Resultate is nog nie beskikbaar nie.

Summary

The objective of the study is to examine the effect of temperature on the development and survival of *S. aurantii*. To determine lower and upper developmental thresholds on citrus, experiments are currently being carried out at five different constant temperatures (18, 21, 25, 30 and 35°C). No results are available at this time.

3.3.3 Determine the ability of citrus thrips to switch hosts and development of a rearing technique on *Bryophyllum delagoense* Experiment 709 by Tim Grout, Kim Stoltz and Peter Stephen (CRI)

Opsomming

Samewerking met Australiese entomoloë en ons eie voorlopige navorsing dui daarop dat sitrusblaaspootjies aangepas is by sekere gashere en nie maklik op onverwante plante vestig nie. Verskeie *Bryophyllum* spesies word in potte gekweek in Nelspruit, maar 'n *Bryophyllum*-aangepaste ras van sitrusblaaspootjie kon nog nie van Pretoria verkry word nie, vanweë lae getalle. Verdere navorsing oor gasheerverwisseling en vergelyking van rasse sal vroeg in 2005 gedoen word.

Introduction

Scirtothrips aurantii was accidentally introduced into Queensland, Australia, on the succulent weed *Bryophyllum delagoense*. Unpublished experiments there by Chris Freebairn indicate that citrus thrips on *Bryophyllum* do not readily move onto citrus, suggesting strain differences in host susceptibility.

In South Africa, the current understanding is that citrus thrips will move from one host plant to another when foliage hardens off and that citrus thrips readily moves between citrus and its many alternative host plants. How readily this occurs and whether there is initially poor survival on the new host, needs to be determined to better understand the threat of citrus thrips from alternative hosts around citrus orchards.

In addition, citrus thrips is difficult to rear on citrus seedlings in the laboratory, whereas phyllodes from *Bryophyllum* would be very convenient for bioassays or other studies with thrips because a vial could be placed over a detached phyllode that would remain suitable for citrus thrips for more than two weeks.

Materials and methods

The research comprised two parts. One involved the collaboration with Laurence Mound at CSIRO in Australia for whom citrus thrips were collected from citrus and other hosts at various locations in Mpumalanga and Limpopo provinces. The other part involved our own work in growing different plants and transferring citrus thrips onto them.

Citrus thrips were collected from citrus at locations in Letsitele, Hoedspruit, Tzaneen and Nelspruit and sent to Australia in 99% ethanol. Laurence Mound confirmed their morphological identification before DNA was extracted. Techniques for PCR amplification and DNA sequencing are provided in Morris & Mound 2004.

In Nelspruit, some seedlings of Pride of Barbados (*Caesalpinia pulcherrima*) have been grown but germination was poor and further plants are required before transfers of citrus thrips onto this host can be tested. Transfers of citrus thrips were made onto Rough Lemon seedlings from mango plants and from Pride of Barbados plants. In both cases, multiple cages containing Rough Lemon seedlings were used. Each cage was constructed from a 2-litre Coca Cola. The bottle was cut in half, approximately 10 cm above the base and three Rough Lemon seedlings were transplanted into a bark and sand mix in the base of the bottle. The top half of the bottle could be refitted over the lower part of the bottle to form a cage. A screen window was placed in the top half of the bottle to provide ventilation and the lid was retained.

Citrus thrips larvae were collected in November from potted mango plants at the Institute for Tropical and Subtropical Crops using an aspirator. Fifteen larvae were transferred onto the Rough Lemon seedlings in each of 10 cages (150 thrips in total). The join between the top and bottom half of each bottle was sealed with masking tape. The plants were observed every few days for signs of infestation and the cages were not opened for a month.

Citrus thrips were collected from Pride of Barbados plants by cutting branches with new growth and beating them over a grid in the laboratory. Fifty larvae were transferred to Rough Lemon seedlings in each of five cages and 20 adults were transferred to Rough Lemon seedlings in each of another five cages. The plants were closely observed for a period of one month.

Four species of *Bryophyllum* have been obtained from PPRI, Pretoria to rear the strain of citrus thrips adapted to this plant but no citrus thrips were present on the plants at the time.

Results and discussion

The conclusion from the Australian work was that all citrus thrips collected belonged to *Scirtothrips aurantii*, although two apparent strains may be present. The strains were not associated with certain host plants and the strain collected from *Bryophyllum diagremontianum* in Pretoria was the same as the strain collected from citrus in Nelspruit, Hoedspruit and Letsitele (Morris & Mound 2004). However, some people have suggested that the techniques used may not have been correct. Although the citrus thrips strain in Australia has not yet become a pest of citrus or mangoes, no assurance can be given that it will not in the future.

Our own transfers of citrus thrips larvae from mango to citrus resulted in no establishment on any citrus seedlings. Similarly, the transfer of citrus thrips larvae and adults from Pride of Barbados resulted in no survivors on citrus. Unless nutrients used to grow the citrus seedlings are making the citrus unpalatable, it appears that citrus thrips does not readily switch hosts. Further experiments will be conducted using citrus seedlings grown with different fertilizers and using both *Bryophyllum* and Pride of Barbados plants.

Conclusion

Both our own provisional experiments and those conducted by collaborators in Australia suggest that citrus thrips becomes adapted to certain host plants and does not readily transfer to unrelated host plants. However, further research is required.

Future research

The establishment of a culture of citrus thrips that feed on *Bryophyllum* will allow for further comparisons between this population and one from citrus. More transfers between different plant species will also be conducted.

Reference cited

Morris, D.C. & Mound, L.A. 2004. Molecular relationships between populations of South Africa citrus thrips (*Scirtothrips aurantii* Faure) in South Africa and Queensland, Australia. Australian J. Entomol. 43: 353-358.

3.3.4 OP alternatives for citrus thrips, compatible with bio-intensive IPM or organic production Experiment 713 by Tim Grout, Peter Stephen, Bruce Tate and John-Henry Daneel (CRI)

Opsomming

Sitrusblaaspootjieberbestuur is moeilik in 'n IPB program aangesien blaaspootjiedoders met 'n lang nawerking die natuurlike vyande ontwig en daar minder doders bestaan met aanvaarbare maksimum oorblywende vlakke vir uitvoermarkte. Hierdie navorsing het verskeie, en moontlik goedkoper, metodes as die huidige geregistreerde doders ondersoek, asook nuwe lokaas-kombinasies. Abamectin teen 20 ml/ha saam met Orchex teen 150 ml/ha was meer effektief teen sitrusblaaspootjie as dieselfde dosis van abamectin met Orchex teen 300 ml/ha, maar het nie rooidopluis so effektief onderdruk nie. Dieselfde dosis abamectin saam met NUFilm17 teen 20 ml/ha was net so doeltreffend as abamectin saam met Orchex 300 ml/ha. Die kaolien-produkte Fruitcote en Mangocote teen 3 kg/ha het sitrusblaaspootjie tot 'n mate onderdruk, maar kon nie ernstige skade voorkom nie. Dit het ook brand veroorsaak op jong blare. Braakwynsteen met witsuiker was meer doeltreffend as met bruinsuiker by Burgersfort, maar die blaaspootjie-getalle by Barberton was te laag om dit te bevestig. Mesurool lokaasbespuiting het sitrusrooimytpopulasies betekenisvol verhoog. Hunter teen 15 of 20 ml/ha plus witsuiker teen 200 g/ha was 'n doeltreffende lokaas maar het rooimyt populasies selfs meer as Mesurool verhoog. Verdere ondersoek na OP alternatiewe sal voortgaan.

Introduction

The need for alternatives to organophosphates (OPs) continues to be given a high priority by citrus growers due to their MRLs being reduced to the limit of determination. There is also growing interest in organic production for which thrips management is a challenge. Erador (pyrethrum & azadirachtin) was evaluated in 2003 but was inadequately efficacious and the distributor is no longer selling the product. Spinosad (Tracer) has been registered on citrus in South Africa and may now be used in organic production. Although it is at least as efficacious as abamectin plus oil, it is too expensive for most citrus spray programmes. The botanical product rotenone also works out to be an expensive spray, as it does not provide much residual control. Kaolin sprays do seem to have some effect on thrips and require further investigation but multiple sprays are likely to cause pest repercussions. Chlorfenapyr should be evaluated at lower dosages and volumes with sugar to see whether it can be used as an effective thripicide without causing the pest repercussions it has become known for. Other promising alternatives to abamectin urgently need to be evaluated as the selective pressure for resistance to abamectin is immense. The following research was conducted in order to address some of these needs.

Materials and methods

The experiment was divided into two parts. One half comprised outside cover film sprays to investigate alternatives to the rate of 0.3% oil used with abamectin and different local formulations of kaolin. The other half comprised various bait sprays to investigate the use of less expensive brown sugar with tartar emetic and to determine whether Hunter baits could be a practical option. Both parts of each experiment were replicated at two sites. One site was a Valencia orchard at PLM Boerdery near Burgersfort and the other was a Palmer navel orchard at Hopewell farm north of Barberton. The experimental design used was a nested block with each site divided into two regions. Within each region the trees were divided into treatment blocks that were at least six trees long and were four rows wide. Treatments were randomly assigned to each block and all sprays were applied by hand using high-pressure (30 bar) handguns. Evaluations were conducted on eight data trees in the central two rows in each block. Twenty outside fruit were inspected per tree and rated as infested or not with immatures or adults (separate counts). For some evaluations, any fruit scarring caused by citrus thrips was also recorded for each fruit inspected as scarred or not. Results were analysed by two-way ANOVA after an arc-sine square root transformation. Means were compared further using Student-Newman-Keul's test at $P=0.05$.

Film sprays

Citrus thrips population density was highest at Burgersfort and the first treatments were applied on 18 October 2004. Approximately 9 l spray mix were applied per tree and treatments are listed in Table 3.3.4.1. The first evaluation of citrus thrips infestation was conducted on 1 November and the treatments were

reapplied on 2 and 3 November 2004. A second evaluation of infestation was conducted on 16 November and fruit were rated for citrus thrips scarring at the same time. Pest management then reverted to the grower who sprayed out the trial site. In January 2005, there were signs of red scale infestation increasing on the fruit so an evaluation of red scale was conducted on 8 February using 20 fruit per tree on each of eight data trees per replicate. Results were analysed by two-way ANOVA after an arc-sine square root transformation. Means were compared further using Student-Newman-Keul's test at $P=0.05$.

At the site near Barberton, the first spray was applied on 26 October using approximately 3 l spray mix per tree (Table 3.3.4.2) and an evaluation of both infestation and scarring was conducted on 10 November. The treatments were reapplied on 18 November and a further evaluation of both infestation and scarring was conducted on 7 December. When this second evaluation was conducted, there were signs of citrus red mite and low levels of red scale in the orchard so a further rating for infestation by these pests was conducted on the control, the standard abamectin plus Orchex 300 ml/hl and the abamectin plus EXP 1500 adjuvant. These ratings for red mite and red scale were done on 15 December by examining two sides of each of eight data trees per treatment block. The main focus of examination was the light green but expanded new leaves where mites were easy to see. However, some older leaves and fruit were examined, the latter being used for red scale infestation ratings. Ratings for red mite were assigned as follows.

- 1 = Average of 0 to 1 mite seen per terminal and no damage visible.
- 2 = 2 to 10 mites seen per terminal. No damage visible.
- 3 = 10-20 mites seen per terminal. Some damage seen.
- 4 = 20-50 mites per terminal with damage on many fruit and older leaves.
- 5 = 50+ mites per terminal with damage very noticeable.

For red scale, the following infestation ratings based on fruit were used.

- 1 = 0-1 infested fruit seen per tree.
- 2 = 2 infested fruit seen per tree.
- 3 = 3 infested fruit seen per tree.
- 4 = 4 infested fruit seen per tree.
- 5 = 5+ infested fruit seen per tree.

A two-way ANOVA was also used to analyse the red mite and red scale ratings but the data were not transformed before analysis.

Bait sprays

At Burgersfort, bait sprays were applied and evaluated on the same dates and in the same manner as the cover sprays described above with the exception of the first evaluation being one day later than the cover sprays on 2 November 2004. Treatment details are supplied in Tables 3.3.4.3 and 3.3.4.4. A red scale evaluation was also conducted as described above on 8 February 2005.

Near Barberton, the levels of citrus thrips infestation in the portion of the orchard assigned to the bait sprays were lower than where the cover sprays were applied and in addition, inclement weather delayed the first bait applications. These were finally applied on 17 November at the same time as the second cover sprays at this site. Only one evaluation of citrus thrips infestation of fruit was conducted on 7 December and due to the low numbers of thrips and the presence of some scars from feeding before treatments were applied, no scarring rating was conducted. However, whole tree ratings for red mite and red scale infestation were conducted at this site on 15 December.

Results and discussion

Film sprays

One very interesting result that was supported at both sites was that the lower dosage of oil with abamectin gave significantly less thrips scarring ($P<0.05$) than the registered 300 ml/hl oil rate (Tables 3.3.4.1 & 2). At Burgersfort this significant difference was also found when larval and adult citrus thrips infestations were compared in the evaluation on 16 November (Table 3.3.4.1). This contradicts rumours that suggest that abamectin is more effective when sprayed with high concentrations of oil. If it is more effective when combined with 1% oil sprays it may just be due to the volumes being higher and the foliar absorption better than when sprayed for citrus thrips. This result means that growers who are concerned about using oil with abamectin at petal fall could safely reduce the concentration to 150 ml/hl and may even get better results at a lower price. Oil concentrations below 0.3% were not used during the initial registration work for abamectin against citrus thrips in South Africa (Grout et al. 1996) as the oil concentrations being used with abamectin in the USA at the time ranged between 0.25 and 0.5%.

Replacing oil with NuFilm 17 at 20 ml/hl in combination with abamectin was as effective as the registered combination with Orchex 300 ml/hl. This combination is not currently registered but registration could be pursued if there was enough demand to move away from oil completely. The alternative adjuvant EXP 1500 was not as effective as oil or NuFilm 17 when used at 20 ml/hl, but it did not appear to cause any phytotoxicity. The non-target effects of some of the treatments against red scale at Burgersfort showed that there was a significant reduction in suppression of red scale when the concentration of oil with abamectin was reduced from 300 to 150 ml/hl (Table 3.3.4.3). Both kaolin formulations at 3 kg/hl increased red scale slightly, but not significantly so. At Barberton, abamectin with oil at 300 ml/hl, or with EXP 1500, did not reduce red mite or red scale populations significantly (data not presented).

Table 3.3.4.1. Results of cover sprays applied for control of citrus thrips near Burgersfort in October and November 2004.

Treatments applied 18 Oct and 3 Nov 2004	Evaluation on 1 Nov 2004		Evaluation on 16 Nov 2004		
	Fruit with larvae (%)	Fruit with adults (%)	Fruit with larvae (%)	Fruit with adults (%)	Fruit scarred by thrips (%)
Untreated control	25.6 a	20.0 a	41.3 a	31.3 a	62.2 a
Abamectin 20 ml plus Orchex 150 ml/hl	3.4 d	2.2 b	0.0 e	2.2 d	9.4 e
Abamectin 20 ml plus Orchex 300 ml/hl	2.2 d	6.6 b	10.0 d	8.8 c	19.4 d
Abamectin 20 ml plus NuFilm17 20 ml/hl	4.1 d	4.7 b	11.6 d	8.4 c	18.8 d
Abamectin 20 ml plus EXP 1500 20 ml/hl	14.7 b	18.8 a	16.3 cd	13.1 bc	33.4 c
Fruitcote 1.5 kg/hl	13.1 bc	21.3 a	24.7 bc	15.6 bc	51.9 b
Fruitcote 3 kg/hl	5.6 cd	7.5 b	18.8 c	19.1 b	36.9 c
Mangocote 3 kg/hl	6.6 cd	19.1 a	30.0 b	19.1 b	59.7 ab

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

Applications of kaolin in the form of either Fruitcote or Mangocote (with copper oxychloride) did not give effective control of citrus thrips at either site (Tables 3.3.4.1 & 2) and some burn of semi-expanded leaves and shoot tips was also observed at Barberton with both products (Fig. 3.3.4.1). Scarring in the Mangocote treatment at Burgersfort was not significantly different from the untreated control and was significantly worse than the registered abamectin plus 0.3% oil. However, the same treatment at Barberton where citrus thrips numbers were not quite as high resulted in scarring that was worse than the registered abamectin but not significantly so (P>0.05). There was a noticeable dosage response between the 1.5 kg and 3 kg/hl rates of Fruitcote at Burgersfort, but this was not as obvious at Barberton. The 3 kg rate of Fruitcote was more effective than Mangocote at Burgersfort (Table 3.3.4.1) but less effective at Barberton (Table 3.3.4.2). In 2003, a different kaolin formulation at 2.5 kg/hl could not reduce adult citrus thrips populations significantly less than in the control, although the larval infestation was significantly lower. Efficacy was similar to that provided by sulphur and significantly inferior to abamectin 20 ml plus Orchex 300 ml/hl (Grout & Stephen 2003). It therefore appears that kaolin at a dosage rate of 2.5 or 3 kg/hl does give some suppression of citrus thrips and could perhaps be mentioned as an additional benefit when used for another purpose. However, its registration as a thripicide cannot be justified unless it is specifically for organic production or low citrus thrips populations where sulphur may be considered.

Table 3.3.4.2. Results of cover sprays applied for the control of citrus thrips near Barberton between October and December 2004.

Treatments applied 26 Oct and 17 Nov 2004	Evaluation on 10 Nov 2004			Evaluation on 7 Dec 2004		
	Fruit with larvae (%)	Fruit with adults (%)	Fruit scarred by thrips (%)	Fruit with larvae (%)	Fruit with adults (%)	Fruit scarred by thrips (%)
Untreated control	25.9 a	16.6 a	31.6 a	19.7 a	8.8 a	64.1 a
Abamectin 20 ml plus Orchex 150 ml/hl	1.6 c	1.6 b	5.6 e	0.3 c	0.9 b	12.5 e
Abamectin 20 ml plus Orchex 300 ml/hl	1.9 c	4.4 b	10.0 de	0.3 c	0.6 b	26.3 d
Abamectin 20 ml plus NuFilm17 20 ml/hl	5.0 c	4.1 b	12.5 cd	2.5 c	0.9 b	23.4 d
Fruitcote 1.5 kg/hl	14.1 b	14.1 a	27.8 ab	10.0 b	3.1 b	43.8 bc

Fruitcote 3 kg/hl	13.8 b	11.6 a	20.6 bc	5.9 bc	3.8 b	50.9 b
Mangocote 3 kg/hl	9.7 b	6.3 b	19.1 bc	5.3 bc	1.3 b	35.0 cd

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

Table 3.3.4.3. Impact of certain film spray treatments on red scale infestation of fruit near Burgersfort on 8 February 2005.

Treatments applied 26 Oct and 17 Nov 2004	Evaluation on 8 Feb 2005	
	Fruit with red scale (%)	Fruit with >5 red scale (%)
Untreated control	21.9 a	7.5 a
Abamectin 20 ml plus Orchex 150 ml/hl	23.1 a	6.9 a
Abamectin 20 ml plus Orchex 300 ml/hl	5.6 b	0.9 b
Fruitcote 3 kg/hl	34.7 a	13.1 a
Mangocote 3 kg/hl	29.1 a	11.3 a

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



Figure 3.3.4.1. Burn of new growth caused by Mangocote at 3 kg/hl

Bait sprays

Results at Burgersfort were good (Table 3.3.4.4) and for the first time we could show that the use of brown sugar with tartar emetic was significantly inferior to the use of white sugar. Unfortunately this could not be confirmed at the Barberton site because the numbers of citrus thrips were too low (Table 3.3.4.5). The only results that could be shown against citrus thrips at this site were that all bait treatments reduced citrus thrips populations relative to the control (Table 3.3.4.5). At Burgersfort the addition of NuFilm 17 to tartar emetic plus white sugar did not decrease efficacy but it also did not increase efficacy relative to tartar emetic plus white sugar alone. Moore and Pittaway (1997) showed that NuFilm 17 did improve rain fastness of tartar emetic so this may be useful when frequent drizzle occurs. However, the conditions in Burgersfort were hot and dry with occasional thunderstorms so not really suitable to show any benefit from rain fastness. There were few significant differences between the other baits except that the higher rate of Hunter resulted in significantly less scarring than Mesurol plus white sugar (Table 3.3.4.4).

Table 3.3.4.4. Results of bait sprays applied for control of citrus thrips near Burgersfort in October and November 2004.

Treatments applied 18 Oct and 3 Nov 2004	Evaluation on 2 Nov 2004		Evaluation on 16 Nov 2004		
	Fruit with larvae (%)	Fruit with adults (%)	Fruit with larvae (%)	Fruit with adults (%)	Fruit scarred by thrips (%)
Untreated control	16.9 a	15.6 a	35.3 a	25.3 a	47.5 a
Tartar emetic 200 g + white sugar 200 g/hl	1.3 b	0.9 b	3.8 cd	4.4 c	9.7 bcde
Tartar emetic 200 g + brown sugar 200 g/hl	6.3 b	3.8 b	14.4 b	12.2 b	21.6 b
Tartar emetic 200 g + white sugar 200 g + NuFilm 17 20 ml/hl	1.3 b	0.9 b	9.7 bc	3.8 c	12.5 bcd
Dicarzol 25 g + white sugar 200 g/hl	0.0 b	3.1 b	0.6 d	2.8 c	6.9 de
Mesurol 10 g + white sugar 200 g/hl	3.1 b	2.8 b	5.6 cd	6.6 c	17.8 bc
Hunter 15 ml + white sugar 200 g/hl	0.0 b	0.3 b	3.8 cd	2.2 c	8.4 cde
Hunter 20 ml + white sugar 200 g/hl	0.0 b	0.0 b	0.0 d	0.9 c	2.2 e

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

Evaluation of non-target effects at the Barberton site showed repercussions in citrus red mite populations induced by Mesurol and Hunter (Table 3.3.4.5). The difference between Hunter dosages was not significant but both Hunter bait treatments had significantly more red mite than Mesurol bait treatments that in turn had more red mite than Dicarzol bait, tartar emetic bait or the untreated control. Only one treatment showed a significant increase in red scale and that was the lower rate of Hunter (Table 3.3.4.5). Hunter was at one stage investigated as a scalcicide and did cause some mortality so perhaps the 20 ml/hl dosage rate is suppressing some red scale while the 15 ml/hl rate is only disrupting natural enemies. Similar results were found at Burgersfort with red scale infestation being similar for all treatments except for the 15ml/hl Hunter that showed a higher (but not significant at P=0.05) level of infestation (Table 3.3.4.6).

Table 3.3.4.5. Results of bait sprays applied for the control of citrus thrips near Barberton in November and December 2004.

Treatments applied 17 Nov 2004	Evaluation on 7 Dec 2004		Ratings on 15 Dec 2004	
	Fruit with larvae (%)	Fruit with adults (%)	Citrus red mite	Red scale
Untreated control	15.0 a	7.8 a	1.3 a	1.1 a
Tartar emetic 200 g + white sugar 200 g/hl	2.2 b	0.6 b	1.6 a	1.5 a
Tartar emetic 200 g + brown sugar 200 g/hl	0.9 b	0.9 b	-	-
Tartar emetic 200 g + white sugar 200 g + NuFilm 17 20 ml/hl	0.9 b	2.5 b	-	-
Dicarzol 25 g + white sugar 200 g/hl	0.6 b	0.6 b	1.8 a	1.3 a
Mesurol 10 g + white sugar 200 g/hl	0.6 b	0.3 b	2.4 b	1.5 a
Hunter 15 ml + white sugar 200 g/hl	0.3 b	0.0 b	4.0 c	2.3 b
Hunter 20 ml + white sugar 200 g/hl	0.3 b	0.0 b	3.6 c	1.4 a

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

Table 3.3.4.6. Non-target effects of bait sprays applied for control of citrus thrips near Burgersfort on red scale in February 2005.

Treatments applied 18 Oct and 3 Nov 2004	Evaluation on 8 Feb 2005*	
	Fruit with red scale (%)	Fruit with >5 red scale (%)
Untreated control	38.4	16.3
Tartar emetic 200 g + white sugar 200 g/hl	30.9	10.9
Dicarzol 25 g + white sugar 200 g/hl	31.3	9.7
Mesurol 10 g + white sugar 200 g/hl	36.3	11.3

Hunter 15 ml + white sugar 200 g/hl	48.1	22.5
Hunter 20 ml + white sugar 200 g/hl	38.8	12.8

*No treatments were significantly different at P=0.05 (SNK)

Conclusion

Abamectin at 20 ml/hl plus Orchex at 150 ml/hl was more effective against citrus thrips than the same dosage of abamectin with Orchex at 300 ml/hl, although less effective at suppressing red scale. The same rate of abamectin plus NuFilm17 at 20 ml/hl was as effective against citrus thrips as abamectin plus Orchex 300 ml/hl. The kaolin products Fruitcote and Mangocote at 3 kg/hl gave slight suppression of citrus thrips but this was inadequate to prevent severe damage. They also showed a tendency to increase red scale, although this was not significant ($P>0.05$). Tartar emetic with white sugar was shown to be more effective than tartar emetic with brown sugar at Burgersfort but the thrips numbers were too low at Barberton to confirm this. Mesurol bait spray significantly increased populations of citrus red mite. Hunter at either 15 or 20 ml/hl plus white sugar at 200 g/hl was an effective bait but increased red mite populations even more than Mesurol.

Future research

The comparison of brown and white sugar for use with baits will be repeated. Other possible treatments for citrus thrips control that are compatible with IPM will be investigated.

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3.3.5 The status and control of new moth pests on lemons

Experiment 715 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Gedurende die 2001/02 seisoen is suurlemoenmotskade op suurlemoene in die Oos-Kaap aan *Cryptoblabes gnidiella* en moontlik nog twee Lepidoptera spesies, *Lobesia stericta* en *Pyroderces tripola*, toegeskryf. Gedurende die 2002/03 seisoen is vergelykbare skade op suurlemoene in die Wes-Kaap met *Prays citri* geassocieër. Die skade wat opgemerk is, is nie tipies van enige van hierdie spesies nie. *C. gnidiella* feromoonlokvalle is in boorde in die Oos-Kaap gehang wat opsigtelik besmet was, maar geen motte van hierdie spesie is gevang nie. *P. citri* feromoonlokvalle is daarom in die lente van 2004 in suurlemoenboorde in die Oos-Kaap gehang. Tot 300 *P. citri* motte is per lokval per week gevang. Hierdie vangste is met lae tot matige vlakke van besmetting van larwes and papies op blomme en vruggies geassocieër. Beide Phosdrin en Dipel het eier- en larwegetalle, asook vrugskade, verminder maar die vermindering was nie bevredigend nie. Dit is moontlik dat vroeër behandeling, groter behandelingsblokke en 'n produk met 'n langer nabywende werking, beter sou gewerk het. Dit is ook moontlik dat larwes tot 'n mate deur saamgespinde blare en blomme teen bespuitings beskerm was.

Introduction

During the 2000/01 season conspicuous brown scabs, not dissimilar to leafhopper damage, were noticed on lemons in the Eastern and Western Cape Provinces. During the 2001/02 season this damage reoccurred and was associated with the honeydew moth, *Cryptoblabes gnidiella*, and possibly with two other lepidopteran species, *Lobesia stericta* and *Pyroderces tripola* (Moore, 2003). This was the first report of these species on citrus in South Africa. However, *C. gnidiella* is well known as a citrus pest in other parts of the world (Anshelevich *et al.*, 1993; Avidov & Gothliff, 1960). What is peculiar is that elsewhere in the world *C. gnidiella* is associated with honeydew and therefore the pest species which excrete honeydew (Wysoki *et al.*, 1975; Wysoki, 1989; Swirski *et al.*, 1980). In none of the orchards in which *C. gnidiella* nor its presumed damage were recorded, was any conspicuous level of honeydew producing insects present. As *C. gnidiella* was identified by a reputable lepidopteran taxonomist from the Transvaal museum and as it is morphologically distinct from *Prays citri*, there is no reason to doubt its identification. However, during the 2002/03 and 2003/04 seasons *C. gnidiella* traps hung in Eastern Cape lemon orchards caught no moths of this species (Moore *et al.*, 2003). Therefore, its association (and that of the other species identified) with

damage on lemons in the Eastern Cape Province must be confirmed. Damage to lemons in the Western Cape was associated with *Prays citri* (the citrus flower moth), a well known sporadic citrus pest. However, the type of damage recorded had not previously been associated with this pest. Chemical control trials targeted against the egg stage on lemon fruitlets showed that all products used, reduced infestation. However, it was apparent that sprays should have been applied earlier, against the larvae of the previous generation which probably infested the lemon blossoms. During this, the 2004/05 season, *Prays citri* traps were hung in two lemon orchards in the Eastern Cape and another spray trial was conducted to determine whether earlier application was more effective. This report details the results of these trials.

Materials and methods

Monitoring and surveying

During spring of the 2004/05 season, two lemon orchards in Sundays River Valley (SRV) which had a history of lemon moth infestation were identified. On 1 October 2004 one bucket (IPS) trap was hung in each of the two orchards. A *P. citri* pheromone dispenser was inserted into the lid of each trap. Dispensers were obtained from Insect Science. Traps were erected in the third tree of the third row on the southern side of each orchard. As the prevailing wind direction was south-easterly or south-westerly, the pheromone could be carried by the wind into the orchard, and detected by any male moths in the orchard. Traps were checked weekly on the same day for a period of 7 weeks (i.e. from 8 October to 17 November 2004). Simultaneously, inspections of fruit and blossoms were conducted for the presence of lepidopteran larvae and eggs.

Chemical control

A chemical control trial was conducted in one of the orchards which was being monitored. This was a 5-year old lemon orchard (orchard 16, Carden Farm, SRV) in which there was a conspicuous level of infestation. Due to the mobility of the pest, the trial was laid out in a block format. Each treatment was applied in one block of 112 trees i.e. four rows of 28 trees. Two treatments were used: Phosdrin 500 SL (30 ml/100 l) + Agral 90 (18 ml/100 l) and Dipel 2X WP (12.5 g/100 l) + Agral 90 (18 ml/100 l). Sprays were applied on 9 November 2004, more than two weeks earlier than treatments had been applied in the trial during the previous year. Sprays were applied at 8.9 l of spray mix per tree using a mist-blower. The efficacy of the treatments was evaluated on 17 November 2004 by scouting each of 10 trees in the middle of each treatment block (including a block of untreated control trees) for larvae and pupae. Each tree was scrutinised for exactly 3 minutes. Evaluation of fruit damage was conducted on 1 December 2004 by selectively picking 100 spring-set fruit from each treatment (including the untreated control). Fruit were then carefully inspected with the aid of a magnifying head-loop and all eggs and penetration marks recorded. As all fruit were grouped together it was not possible to statistically analyse the results.

Results and discussion

Monitoring and surveying

Initially, lemon moth damage to fruit was associated with *C. gnidiella* (Moore *et al.*, 2003; Moore, 2003). However, *C. gnidiella* pheromone traps failed to catch any moths of this species, even in orchards conspicuously infested with lepidoptera which were attacking blossoms and fruitlets. During October and November of 2004, high numbers of *P. citri* moths were caught in *P. citri* pheromone traps (Table 3.3.5.1) hung in orchards which had experienced high levels of lemon moth infestation during the previous season. The numbers of moths caught seemed out of proportion with the low numbers of larvae and pupae recorded on the trees. Low numbers of larvae and pupae were observed for the first time on 29 October, when moth numbers were already very high. Numbers of larvae and pupae were substantially higher during the following week, at which time it was decided to conduct a spray trial. There therefore appears to be a notable lag-time between the first high catches of moths and conspicuous larval infestation of fruitlets.

Table 3.3.5.1. Moth catches in *P. citri* pheromone traps hung in two lemon orchards on Carden Farm in Sundays River Valley.

Date	Moths caught per trap per week	
	Orchard 16	Orchard 15
8 October	51	11
15 October	114	51
22 October	182	118
29 October	255	101

4 November	187	45
11 November	301	119
17 November	96	18

As the original identification of the moths associated with lemon borer type damage in the Eastern Cape was conducted by a recognised lepidopteran taxonomist from the Transvaal Museum (Moore, 2003), there is no reason to doubt this identification. It is therefore possible that *C. gnidiella* can cause similar damage to that observed for *Prays citri*, but that the latter species is the more common pest of the two. Another explanation is difficult to come by, as the identification of *C. gnidiella* was obtained from individuals reared from both eggs and larvae obtained from damaged lemon fruitlets.

Chemical control

Larval infestation of fruitlets was markedly lower on both Phosdrin and Dipel treated trees than on untreated trees (Table 3.3.5.2). However, infestation was not particularly high, even in the control.

Table 3.3.5.2. Total numbers of larvae and pupae recorded per ten trees per treatment. Each tree was inspected for a total of 3 minutes.

Treatment	Number of larvae	Number of pupae
Untreated control	19	4
Phosdrin	1	0
Dipel	4	1

Surprisingly, reduction in eggs and penetration marks per fruit was not dramatic (Table 3.3.5.3). During the previous year's trial, Mevinphos and Dipel sprays reduced egg numbers by 39% and 22%, respectively. Numbers of penetration marks were reduced by 38% and 40%, respectively. During this trial in 2004, Mevinphos (Phosdrin) and Dipel reduced egg numbers by 36% and 35%, respectively. Numbers of penetration marks were reduced by 36% and 48%, respectively. Therefore, even though sprays were applied earlier than during the previous trial (i.e. targeted against larvae of the first generation rather than eggs of the second generation) and treatments were applied to larger blocks than during the previous trial, results were not better.

Table 3.3.5.3. Lepidopteran eggs and damage on lemon fruit (1 December 2004) subjected to different treatments (9 November 2004).

Treatment	Eggs/fruit*	Penetration marks/fruit*
Untreated control	1.90	0.89
Phosdrin SL	1.21	0.57
Dipel	1.23	0.46

*Values in the same column followed by the same letter are not significantly different ($P < 0.05$; Bonferroni LSD multiple range test).

It has been noted that lemon moth problems rarely, if ever, occur in orchards which receive an organophosphate or pyrethroid spray in spring. It is therefore possible that sprays should have been applied even earlier than was the case in this trial (even though larvae were not yet evident). This would imply that pheromone traps might be of use in the earlier timing of sprays against peaks in moth catches. It is also possible that sprays should have been applied to even bigger blocks (i.e. wider than four rows) in order to experience the full potential impact of the spray.

It would be difficult to accept that both Phosdrin and Dipel are not sufficiently effective against such a small lepidopteran larva. Although both of these products have a short residue and it is possible that larvae could acquire some protection from flowers and foliage, particularly by webbing them together. Results might therefore be better with longer residual products. Good penetration of sprays might also be important in countering the described protection which larvae experience within the tree.

Conclusion

From the limited surveys conducted, it appears that *Prays citri* is responsible for most of the lemon moth damage observed in the Eastern and Western Cape. Low to moderate levels of infestation were associated with pheromone trap catches of up to 300 moths per trap per week. Both Phosdrin and Dipel successfully reduced numbers of eggs and larvae and damage levels to fruit. However, this reduction was not

satisfactory. It is possible that sprays should have been applied sooner, treatment blocks were not wide enough, residual efficacy of products used was not long enough, or larvae acquired protection against sprays by webbing themselves into foliage and flowers.

Future research

No further work is planned on this topic.

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3.3.6 Developing a molecular database for *Scirtothrips aurantii* from different host plants in southern Africa

Experiment 721 by Tim Grout and Peter Stephen (CRI)

Opsomming

Hierdie navorsing is begin deur Prof. G. Moritz van Duitsland wat monsters van pes blaaspootjies via Bayer Cropscience vanaf regoor die wêreld aangevra het. Ons het sitrusblaaspootjies versamel van sitrus in verskeie lokaliteite in Suid Afrika met die ondersteuning van verskeie entomoloë. Ons het ook sitrusblaaspootjie versamel vanaf mango en poueblom, *Caesalpinia pulcherrima*. Resultate tot dusver toon dat al ons sitrusblaaspootjies *Scirtothrips aurantii* is en dat daar onvoldoende genetiese variasie tussen populasies voorkom om 'n ander spesie in te sluit. Verwante navorsing is ook gedoen deur L.A. Mound in Australië en verslag daarvoor word gedoen in 3.3.3. Geen verdere navorsing word beplan nie.

Summary

This research was initiated by Prof. G. Moritz in Germany who requested samples of pest thrips species via Bayer Cropscience from crops all over the world. We collected citrus thrips from citrus in various locations in South Africa with some assistance from various entomologists. We also collected some citrus thrips from mango and pride of Barbados, *Caesalpinia pulcherrima*. Results to date have shown that all our citrus thrips are *Scirtothrips aurantii* and there is insufficient genetic variation between populations or host plants to consider another species. Related research was also conducted by L.A. Mound in Australia and is reported on in 3.3.3. No further research is planned.

3.3.7 Development of a rearing technique for the thrips predator *Franklinothrips megalops*

Experiment 756 by Tim Grout and Peter Stephen (CRI)

Opsomming

Franklinothrips megalops is 'n predator in die kompleks van natuurlike vyande van sitrusblaaspootjie, maar word slegs waargeneem in hoë getalle in boorde waar min insekdoders gebruik word, soos in die Benede-Oranjerivier. Om meer uit te vind oor nie-teiken effekte, is 'n poging aangewend om 'n populasie van die predator te vestig aangesien *F. orizabensis* suksesvol geteel word in Kalifornië. Twee versameltogte is na Kakamas onderneem om *F. megalops* te versamel. Met die eerste tog, in April 2004 is slegs 15 predatore versamel vanaf sitrus en oorlewing was laag. In November 2004 is 130 van die predatore versamel vanaf

Rhus pendulina aangrensend aan citrus langs die Oranjerivier, maar kon nie suksesvol na die derde generasie geteel word nie. Geen verdere pogings sal aangewend word om hierdie roofblaaspoetjie te teel nie.

Introduction

Franklinothrips megalops (Aeolothripidae) was noticed on citrus in 2003 in the vicinity of Kakamas where citrus thrips is not generally considered to be a pest (Grout 2004). As this thrips species is a known predator of other thrips it was possible that it may be playing an important role in suppressing citrus thrips in the Lower Orange River. In order to find out more about its susceptibility to pesticides commonly used in commercial citrus orchards and possibly investigate augmentative releases, plans were made to develop a rearing technique for the insect.

Materials and methods

In late April, 15 *F. megalops* were collected by beating untreated citrus foliage in a farm on the Orange River for a day and a half. The predator was clearly less abundant than at the same time the previous year. Six adults and three larvae were placed on green bean plants in honey jars and were provided with *Ephestia cautella* eggs for food. Six larvae were kept in a vial as a backup and these were fed with previously frozen *Ephestia* eggs and fresh fruit fly eggs. The thrips formed cocoons (typical for this family) and pupae and when the adults eclosed they were provided with a green bean and a *Bryophyllum delagoense* phyllode for substrate and oviposition.

We considered that the use of frozen *Ephestia* eggs may not have been optimal and as no insectaries or universities could be found that were rearing an *Ephestia* species, we started a new culture from flour contaminants. Once the *Ephestia* culture was going well, another collecting trip was made to Kakamas late in November 2004 to try to establish a culture of *F. megalops*. After beating citrus for three hours, only two specimens were found so attention was then focused on *Rhus pendulina* that grows along the Orange River and is known to host some citrus pests. Within a short time, several specimens of *F. megalops* were collected in fruit clusters. Collecting continued on *Rhus* the whole of the next day and in total, approximately 130 larvae and adults of both sexes were collected. Green beans, *Bryophyllum* phyllodes and *Rhus* fruit were provided as oviposition substrates and both *Ephestia* eggs and fruit fly eggs were supplied in excess for food.

Results and discussion

With the first culturing attempt, the thrips on green bean plants in honey jars did not survive, possibly due to insecticide drift on the bean plants. In the backup vial the adults emerged two to three weeks after collecting the larvae. It was noticed that three were more slender than the rest and were probably males. The slender thrips died within three days while the heavier individuals lasted for longer than a week. At least three second-generation larvae developed from this colony with only one reaching the adult stage. Although this rearing attempt ended unsuccessfully, it showed that there was some potential for the rearing of *F. megalops* and for this reason the second attempt was made.

With the higher numbers of the second collection, some were placed in plastic cake boxes and others were placed in large coffee jars to provide more space than in the past. Numbers of *F. megalops* steadily declined in all types of containers but it could not be determined whether the diet or the ovipositional substrate was at fault. Only a few third-generation larvae appeared and the rearing process was considered a failure. This meant that the non-target effect testing of this species was not possible (CRI 728).

Conclusion

Although relatively large numbers of *F. megalops* were collected to start a culture and fresh *Ephestia* eggs were supplied as food, a viable culture could not be started.

Future research

No further work on *F. megalops* is planned.

3.3.8 Evaluation of the *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) for control of bollworm on citrus

Experiment 782 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Gedurende 1996-1998 is 'n Suid-Afrikaanse isolaat van 'n nukleêre polihedrovirus (HearNPV) teen sitrusbolwurm (*Helicoverpa armigera*) getoets met uitstekende resultate. Desondanks is navorsing daarop beëindig aangesien die virus nie kommersieel beskikbaar was nie. 'n Australiese maatskappy het onlangs 'n HearNPV-produk, bekend as Vivus, vir die handel begin vervaardig. In die huidige eksperiment is Vivus in boordproewe met HearNPV-SA, Mevinphos en Dipel vergelyk. Resultate met Vivus op Valencias in Mpumalanga was teleurstellend. Dit is waarskynlik omdat die larwes te groot was ten tye van die bespuiting en ook as gevolg van die relatiewe lae hoeveelheid virus wat per hektaar of relatief tot boomgrootte, toegedien is. Nietemin het die beste Vivus-behandeling twee weke na toediening bolwurmbesmetting met 77% verminder in vergelyking met die onbehandelde kontrole. In twee proewe op nawellemoene in die Oos-Kaap, het HearNPV-SA 'n groter afname in besmetting as die Vivus-behandelings tot gevolg gehad, alhoewel nie statisties betekenisvol nie. Die twee standaardbehandelings, Mevinphos en Dipel, het 'n effens vinniger afname in besmetting as die virusbehandelings veroorsaak, maar besmetting was laer in al die virusbehandelings met die finale evaluasie drie weke na bespuiting. HearNPV-SA is die enigste behandeling wat 100% beheer gegee het alhoewel net in een van die drie proewe. Voor finale gevolgtrekkings gemaak kan word moet vrugskade- en oesevaluasies uitgevoer word. Indien daar besluit word dat een van die virusse vir gebruik op sitrus geregistreer moet word, sal verdere proewe in hierdie verband uitgevoer word.

Gedurende 1996-1998 is 'n Suid-Afrikaanse isolaat van *Helicoverpa armigera* nukleêre polihedrovirus (HearNPV) teen bolwurm (*Helicoverpa armigera*) met uitstekende resultate op sitrus getoets. Desondanks is navorsing daarop nie voortgesit nie omdat die virus nie kommersieel beskikbaar was nie. 'n Australiese maatskappy het onlangs 'n HearNPV-produk, Vivus, begin vervaardig. In bogenoemde eksperiment is Vivus in boordproewe met HearNPV-SA, Mevinphos en Dipel vergelyk. Resultate met Vivus op Valencias in Mpumalanga was teleurstellend. Dit is waarskynlik omdat die larwes te groot was ten tye van bespuiting en ook as gevolg van die relatiewe lae hoeveelheid virus wat per hektaar toegedien is. Al was bome by hierdie persele verder as van mekaar gespaseer as by die ander proefpersele, is bome baie groter as by die ander persele. Nietemin het die beste Vivus-behandeling twee weke na toediening bolwurmbesmetting met 77% verminder in vergelyking met die onbehandelde kontrole. In twee proewe wat op nawellemoene in die Oos-Kaap toegedien was, het HearNPV-SA 'n groter afname in besmetting as die Vivus-behandelings veroorsaak, al was dit nie statisties betekenisvol nie. Die twee standaardbehandelings, Mevinphos en Dipel, het 'n effens vinniger afname in besmetting as die virusbehandelings veroorsaak. Teen die tyd van die finale evaluasie (drie weke na behandeling) was die besmetting in al die virusbehandelings egter laer gewees. HearNPV-SA was die enigste behandeling wat 100% beheer gegee het (in een van die drie proewe). Voor finale gevolgtrekkings gemaak kan word moet vrugskade- en oesevaluasies uitgevoer word. As besluit word dat een van die virusse vir gebruik op sitrus geregistreer moet word, sal verdere proewe uitgevoer word.

Introduction

From 1996-1998 a South African isolate of *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) was tested against bollworm (*Helicoverpa armigera*) on citrus, with excellent results (Moore *et al.*, 2004). Despite this, work on this experiment was terminated, as the virus was not commercially available. Recently, an Australian company, Ag-Biotech Australia, began commercially producing HearNPV. The product is known as Vivus and is available in a suspension at 2×10^9 viral occlusion bodies (OBs) per ml. If similarly good results are achieved with this commercial product, its registration for the control of bollworm on citrus in South Africa can be considered. Alternately, local production of the South African isolate of HearNPV might prove to be a better option. If HearNPV could replace the organophosphate, carbamate or pyrethroid usually used for bollworm control in spring, growers will be greatly assisted towards a reduction in the use of chemicals and the implementation of a bio-intensive IPM programme.

Materials and methods

One Valencia orange orchard in Mpumalanga and two navel orange orchards in the Eastern Cape were selected for conducting field trials during the 2004/05 season. The Mpumalanga trial was conducted in an orchard of 18-year-old Valentine Valencia oranges on Crocodile Valley Citrus farm near Nelspruit. These trees were planted at a spacing of 8 x 7.5 m (rows x trees). However, every third tree had been removed. There were therefore 227 trees per hectare. The first Eastern Cape trial was conducted in an orchard of 19-year-old Robyn navel orange trees on Rough Lemon rootstock on Vergenoeg Farm in the Gamtoos River Valley. These trees were planted at a spacing of 6 x 3 m (rows x trees) giving 555 trees per hectare. The

final trial was applied in an orchard of 6-year-old Palmer navel orange trees on Lionel Jurgen's farm near Addo in Sundays River Valley. Ten trees, randomly selected in each orchard, were inspected for bollworm infestation, weekly from early in September 2004. Treatments were applied as soon as the first bollworm larvae were observed. The two Eastern Cape trials were laid out in single-tree random design, with each treatment replicated 10 times. The Mpumalanga trial was laid out in double-tree random design, as adjacent trees were so close that they were inseparable. As every third tree had been removed, trees were grouped in twos. Seven double replicates were used per treatment, although each tree was evaluated as a separate replicate, making 14 replicates per treatment.

At Crocodile Valley, three treatments were applied: Vivus (15 ml/100 ℓ), Vivus (30 ml/100 ℓ) and Mevinphos EC (100 ml/100 ℓ). An average of 12.0 ℓ of spray mix was applied per tree. The rates of virus per hectare (of Vivus) were therefore approximately 8.2×10^{11} OBs and 1.6×10^{12} OBs. An untreated control was retained. Applications were made on 8 September 2004. At this stage the average length of larvae was already about 12.5 mm. Larvae were therefore probably too large to expect optimum efficacy of Vivus.

Four treatments were applied in each of the other two trials (Vergenoeg and Jurgens): Mevinphos EC (100 ml/100 ℓ); Dipel 2X (12.5 g/100 ℓ) and Kynobuff (100 ml/100 ℓ); HearNPV-SA; Vivus (15 ml/100 ℓ); Vivus (30 ml/100 ℓ).

In the Vergenoeg trial, an average of 16 ℓ of spray mix was applied per tree. Therefore the rate of virus per hectare of HearNPV-SA was approximately 3×10^{12} OBs; the rates of virus per hectare of Vivus were approximately 3×10^{12} OBs and 6×10^{12} OBs. This trial was applied on 29 September 2004.

In the Jurgens trial, an average of 9 ℓ of spray mix was applied per tree. Therefore the rate of virus per hectare of HearNPV-SA was approximately 1.6×10^{12} OBs; the rates of virus per hectare of Vivus were approximately 1.6×10^{12} OBs and 3.2×10^{12} OBs. This trial was applied on 2 October 2004.

After application of treatments, bollworm infestation was evaluated weekly at each site: twice at Crocodile Valley and three times at the other sites. This was done by randomly inspecting 10 inspection points *viz.* blossom or fruitlet clusters, on each tree. Inspection points were recorded as either being infested with any live bollworm or not. A figure for proportion or percentage of inspection points infested was therefore obtained. Infestation was then averaged for each treatment and means were statistically compared. As the Parathion treatment at Daisydelle was applied shortly before the final evaluation, bollworm infestation in this treatment was not assessed.

In all trials, data were analysed using ANOVA and means compared using the Bonferroni LSD multiple range test at the 95% significance level. Proportions (percentages/100) were subjected to an arc sine transformation, where necessary, in order to normalise the data. A zero percentage was counted as $1/(4n)$ and 100% as $(n - 0.25)/n$ before transformation (Bartlett, 1947; Snedecor & Cochran, 1980).

Results and discussion

As expected, results with Vivus in the Crocodile Valley trial were disappointing (Table 3.3.8.1). This was probably because larvae were too large (approximately 12 mm long) at the time of spraying. As is the case with Dipel (and other *Bacillus thuringiensis* products) (Moore, 2003), larger larvae are less sensitive to virus than are smaller larvae (Hunter-Fujita *et al.*, 1998). Another factor contributing to the disappointing results could have been the relatively low rate of virus application per hectare or relative to tree size. Although on average, trees in this orchard might have been spaced further apart than at the other trial sites, the trees at this site were far larger.

By two weeks after application, infestation in the Vivus 2X treatment was significantly 77% lower than in the untreated control (Table 3.3.8.1). However, by this time infestation in the untreated control was also low. An evaluation of fruit damage at a later stage will indicate whether this reduction in infestation in the Vivus treatments occurred too late or not. The Vivus 2X reduced infestation by marginally more than did the Vivus 1X treatment.

Table 3.3.8.1. Efficacy of various treatments in controlling bollworm on navel orange trees.

Site	Treatment	Blossom/fruitlet clusters infested with bollworm (%) (mean ± SE)*		
		1 week later	2 weeks later	3 weeks later
Crocodile Valley		14 Sep	21 Sep	
	Control	47.9a ± 3.9	9.3a ± 2.4	-
	Mevinphos	2.9b ± 1.6	0.7b ± 0.7	-
	Vivus 1X	43.6a ± 5.2	3.6ab ± 1.3	-
	Vivus 2X	40.7a ± 5.2	2.1b ± 1.1	-
Vergenoeg		6 October	12 October	19 October
	Control	37a ± 4.9	33a ± 4.7	11a ± 2.8
	Mevinphos	13b ± 2.1	12b ± 3.3	6ab ± 2.2
	Dipel	18b ± 3.9	12b ± 3.3	5ab ± 2.2
	HearNPV-SA	22b ± 2.5	8b ± 2.5	0b
	Vivus 1X	27ab ± 3.3	14b ± 4.0	1b ± 1.0
	Vivus 2X	27ab ± 2.6	10b ± 3.9	2b ± 2.0
Jurgens		8 October	14 October	20 October
	Control	65a ± 3.7	57a ± 6.1	32a ± 3.6
	Mevinphos	10d ± 2.1	16b ± 4.0	17b ± 3.0
	Dipel	29cd ± 4.8	23b ± 3.3	15bc ± 3.1
	HearNPV-SA	44bc ± 3.4	9b ± 3.5	3cd ± 1.5
	Vivus 1X	54ab ± 4.8	28b ± 5.1	9bcd ± 3.1
	Vivus 2X	47bc ± 4.0	14b ± 2.7	2d ± 1.3

*Values in the same column followed by the same letter are not significantly different ($P < 0.05$; Bonferonni LSD multiple range test).

In the Vergenoeg trial (as in the Crocodile Valley trial), bollworm infestation one week after application was not significantly lower in the Vivus treatments than in the untreated control (Table 3.3.8.1). However, the difference in infestation was conspicuous. HearNPV-SA performed better than the imported virus in that bollworm infestation was significantly lower than in the untreated control from one week after application. Bollworm infestation was also lower for the HearNPV-SA treatment than for the Vivus treatments at each evaluation, albeit not significantly so (Table 3.3.8.1). Neither Mevinphos nor Dipel performed better than did the virus treatments. Although these two standard treatments caused a slightly more rapid reduction in infestation, at the final evaluation (three weeks after spraying) infestation in all three virus treatments was lower. The Vivus 2X treatment did not appear to be any more effective than the Vivus 1X.

Results in the Jurgens trial were similar to those recorded in the Vergenoeg trial. However, Mevinphos caused a significantly greater reduction in infestation at one week after application than did any of the virus treatments (Table 3.3.8.1). Infestation in the HearNPV-SA treatment was again lower than in the Vivus treatments, at one and two weeks after application. Vivus 2X, caused a greater reduction in bollworm infestation than did Vivus 1X, although not significantly so.

Conclusion

Before final conclusions can be drawn, evaluations of fruit damage (and possibly yield too) must be conducted. However, based on evaluations of infestation, Vivus appears to be almost as effective as the South African isolate of bollworm NPV. Both viruses also appear to be sufficiently effective to be used commercially for the control of bollworm on citrus, as long as they are sprayed before larvae are too large.

Future research

During 2005, bollworm damage to fruit will be evaluated at each site. If it is decided that one of the viruses should be registered for use on citrus, further trials will be conducted for this purpose.

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3.4 **PROJEK: VALSKODLINGMOT**

Projekkoördineerder: Hendrik Hofmeyr (CRI)

3.4.1 **Projekopsomming**

Navorsing om valskodlingmot (VKM) op 'n praktiese wyse te bestry, is die afgelope jaar voortgesit. 'n Groot verskeidenheid onderwerpe het aandag geniet en daar is vordering gemaak wat van praktiese belang vir produsente is.

Navorsing met 'n granulovirus het so 'n gevorderde stadium bereik dat dit vir die bestryding van VKM geregistreer is (3.4.2). Die raklewe van die produk is ondersoek en op minstens 10 maande onder ideale toestande vasgestel. Studies om die nabywende werking daarvan verder te ondersoek, word voortgesit. 'n Geneties-gemanipuleerde virus is ook getoets (3.4.3). Alhoewel dit aktief teen VKM-larwes is, is die doeltreffende dosis te onprakties hoog en word verdere werk daarmee nie beplan nie.

Etlieke honderde chemiese verbindings in 'n hele aantal chemiese groeperings, se potensiaal om VKM aan te lok of af te weer, is in olfaktometers ondersoek (3.4.4). 'n Aantal daarvan is geïdentifiseer en sal verder in lokvalproewe bestudeer word.

Navorsing op Steriele Insekloslatings (SIL) is voortgesit nadat ondersoeke na die bestralingsbiologie en F1-steriliteit van VKM afgehandel is (3.4.5). Merk-en-loslaatproewe is uitgevoer om meer kennis oor die motte se aktiwiteit in boorde in te win. Daar is vasgestel dat die motte se aktiwiteit temperatuur-afhanklik is en hulle slegs by hoër as 16°C aktief is. Die inligting wat ingewin is dui daarop dat die loslatingsprotokol wat vir 'n loodsprojek beplan word, in die kol is en min aanpassing nodig het. Groot probleme is met swam- en virusbesmettings in die insektarium, wat voorbereiding vir 'n beoogde loodsprojek gekniehalter het. Navorsing op dié aspekte is deur CRI in Port Elizabeth (3.4.6) en in Citrusdal (3.4.5.16) uitgevoer en dit lyk asof bruikbare oplossings gevind is.

Opnames om die biologie van larweparasitoïede van VKM in die Oos-Kaap te ondersoek, is uitgevoer (3.4.7). Pogings om die parasitoïede te teel het misluk, maar dié werk sal herhaal word sodat die organismes se vermoë om VKM te bestry, in sitrusboorde bepaal kan word.

Opnames van alternatiewe gasheerplante vir VKM is in die Oos-Kaap (3.4.8) en Wes-Kaap (3.4.10) uitgevoer. Daar is slegs enkele plante gevind wat nie as "makgemaak" bestempel kan word nie, waarin VKM aangetref is, te wete die wilde-amandel, *Brabejum stellatifolium* (Wes-Kaap) en die kerkeibos, *Crassula ovata* (Oos-Kaap). Veral laasgenoemde kom in groot getalle in die Oos-Kaap voor en kan derhalwe 'n probleem wees indien dit naby sitrusboorde aangetref word.

Die korrekte identifikasie van VKM in uitvoervrugte is uiters belangrik. Taksonomiese kenmerke van VKM se eiers, finale instarlarwes en papies is daarom vasgestel en beskryf om die verkeerde identifikasie van VKM te voorkom en sodoende die uitvoer van vrugte te vergemaklik (3.4.9). Daar word beplan om soortgelyke aandag aan verwante spesies soos die makadamieneutboorder en die lietsjiemot te gee.

Die radiosensitiwiteit van VKM-larwes vir gammabestraling is ondersoek en daar is weer eens gevind dat hul ontwikkeling erg gestrem word (3.4.11). Dié tegniek kan die sleutel inhou tot veiliger disinfestasielandelings vir vrugte wat na markte uitgevoer moet word wat VKM as 'n fitosanitêre plaag klassifiseer.

Project summary

Research to control false codling moth (FCM) commercially, was continued during the past year. A number of subjects received attention and progress was made that will be of practical importance to citrus producers.

Research on a granulovirus had reached such an advanced stage that it was registered as Cryptogran[®] for the control of FCM (3.4.2). The shelf life of the product Cryptogran[®], was investigated and established to be at least 10 months under ideal circumstances. Studies to determine the residual action of Cryptogran[®], are continuing. An imported genetically manipulated virus was also evaluated (3.4.3). Although it was active against FCM, the effective concentration would be too high and further work on the product was discontinued.

The potential of a few hundred chemical compounds in various chemical groupings, was evaluated in olfactometers as attractants and repellents for FCM. A number of these were identified and will be studied further in trap experiments.

Research on Sterile Insect Releases was continued once studies on the radiation biology and F1 sterility of FCM were concluded (3.4.5). Mark and release experiments were conducted to collect information about the moths' activity in citrus orchards. It was established that the moths' activity is temperature related and that they are only active at temperatures higher than 16°C. The accumulated information shows that the release protocol planned for a pilot SIT project is spot-on and will require little revision. Major problems were encountered with fungal and virus contamination in the insectary, which impeded preparations for the pilot project. Research on these aspects was conducted in the Eastern Cape (3.4.6) and Western Cape (3.4.5.16) and it seems that practical solutions for the problems have been discovered.

Surveys were conducted to investigate the biology of larval parasitoids of FCM in the Eastern Cape (3.4.7). Attempts to rear the parasitoids were unsuccessful, but the work will be repeated to enable research into their ability to suppress FCM in citrus orchards can be evaluated.

Surveys of host plants for FCM were conducted in the Eastern Cape (3.4.8) and Western Cape (3.4.10). Only a few plants were discovered which could not be classed as "domesticated", from which FCM was recovered, i.e. the wild almond, *Brabejum stellatifolium* (Western Cape) and *Crassula ovata* (Eastern Cape, indigenous). *C. ovata* occurs in large numbers in the Eastern Cape and may be therefore be a problem if it occurs in the vicinity of citrus orchards.

The correct identification of FCM in export fruit is of paramount importance. Taxonomic characteristics of the FCM egg, final instar larva and pupa were examined and described to prevent the incorrect identification of FCM and to facilitate the export of fruit (3.4.9). Similar attention is planned for related insects such as the litchi moth and macadamia nut borer.

The radio sensitivity of FCM larvae for gamma irradiation was investigated and it was shown yet again that their development was severely restricted (3.4.11). This technique can be the key to a safer disinfestation treatment for fruit that need to be exported to countries that classify FCM as a phytosanitary pest.

3.4.2 Evaluation of the efficacy of a granulovirus (GV) for the control of false codling moth Experiment 169 by Sean D. Moore, Wayne Kirkman and Peter Stephen (CRI)

Opsomming

'n Granulovirus (GV) van VKM-larwes is 'n paar jaar gelede geïdentifiseer. Met DNS-ontleding is die virus as 'n onbeskryfde isolaat van *Cryptophlebia leucotreta* GV (CrleGV) herken. Biotoetse teen pas-uitgebroeide larwes het die potensiaal van die virus as 'n biologiese beheermiddel bevestig. Sedert 2000 is 'n groot verskeidenheid boordproewe met die virus uitgevoer. Gedurende 2004 is die virus by die Departement Landbou (Wet 36 van 1947; registrasienommer L7598) onder die naam CRYPTOGRAN geregistreer om teen VKM op sitrus gebruik te word. Daar is vasgestel dat die raklewe van CRYPTOGRAN minstens 10 maande is indien die produk in die yskas gehou word teen 'n temperatuur van nie meer as 10°C nie. 'n Voorlopige proef om die invloed van nagemaakte reënval op CRYPTOGRAN-residu's op vrugte te bepaal, het getoon dat CRYPTOGRAN reënvas is. VKM-besmetting van nawellemoene is in boordproewe met tot 70% verminder. Waar CRYPTOGRAN in blokke bome gespuit is in teenstelling met enkelbome, was die onderdrukking van die VKM-besmetting nie net groter nie, maar het ook langer geduur. Die besmetting van Satsumas is met tot 60% verminder. Resultate met CRYPTOGRAN wat kort voor oes gespuit is, was swak, behalwe waar die CRYPTOGRAN-bespuiting baie deeglik as 'n voldek-filmbedekking met goeie indringing toegedien is. In so 'n geval is besmetting van Midnight-valencias met 70% verminder. Gedurende 2005 sal verdere boordproewe uitgevoer word om verskillende konsentrasies van CRYPTOGRAN en bymiddels te vergelyk. Biotoetse om die volle raklewe van CRYPTOGRAN te bepaal, sal voortgesit word. Die proefwerk sal daarna gestaak word. Navorsing op die nawerking van CRYPTOGRAN sal egter in 'n nuwe proef voortgesit word.

Introduction

Chemical control of FCM is fraught with problems. Simultaneously, the justification for adopting an IPM approach in the citrus industry is increasing. Consequently, an effective and IPM compatible means of controlling FCM has been sought. A few years ago a granulovirus (GV) was identified from FCM larvae from Goedehoop Citrus insectary at Citrusdal. Through restriction enzyme analyses the virus was identified as a novel isolate of *Cryptophlebia leucotreta* GV (CrleGV). Bioassays against neonate larvae, confirming its potential as a biocontrol agent, have led to fairly extensive field trials since 2000. These trials, in which FCM infestation was reduced by up to 80%, confirmed the concentration and coverage required to achieve the best results. During 2004 the virus was registered with the Department of Agriculture (Act 36 of 1947; registration number L7598) to be used against FCM on citrus. Throughout this report, reference is made both to CrleGV (the unformulated virus) and Cryptogran (the formulated virus product). Work conducted during 2004 on the shelf-life of the virus, the compatibility of CRYPTOGRAN with various products, the rainfastness of CRYPTOGRAN, and various field trials are described in this report.

Materials and methods

Virus shelf-life

A total of 222 aliquots of CrleGV were made. Each was 0.25 ml (250 µl), with a concentration of 1×10^{11} OBs/ml. There were six different treatments i.e. formulations of CrleGV. These were CrleGV with no additive; with 0.1% (w/v) sorbic acid; with 1% (w/v) boric acid; with 0.1% (w/v) sulphuric acid; with 1% (w/v) table salt; and freeze-dried. There were therefore 37 of each treatment prepared. One of each treatment was immediately frozen at -40°C as the test standard; 18 of each treatment were refrigerated at 10°C; the remaining 18 vials were individually wrapped in silver foil to protect them against any possible UV-inactivation and placed in a room at 27°C. One vial of each treatment, from each temperature, was removed and frozen at -40°C at monthly intervals from one month after initiation of the trial. This will continue for 18 months. However, at the time of writing this report, samples had only been incubated for 10 months. After incubation for the designated time, samples were bioassayed (dose-response) against neonate FCM larvae on artificial diet. This was done alongside a bioassay with the equivalent sample which had been frozen at day zero. Only the first CrleGV treatment i.e. the treatment with no additive, has thus far been bioassayed. Bioassays were conducted with CrleGV after seven, eight and 10 months at 10°C. Probit analysis was used to analyse the results of the bioassays. All other test samples (with the various additives) are available for testing, should this be considered necessary at any stage.

Compatibility with other products

Compatibility of the virus with certain other plant protection products is important. The products deemed most likely to be applied at the same timing as CRYPTOGRAN are abamectin with oil, and Dithane and Benlate with oil. Two detached fruit (out of season Valencia oranges) bioassays were conducted. In the first bioassay, oranges were treated with one of six different treatments (Table 3.4.2.1). Twenty fruit were dipped into beakers containing suspensions/solutions of each treatment. After dipping, fruit were placed onto a wire mesh rack until dried. Thereafter, four FCM neonate larvae were placed onto each fruit using a size 000 paintbrush. Fruit were then kept at 27°C for 14 days. Thereafter they were evaluated for decay and penetration marks (suspected to be caused by FCM larvae). They were then dissected and FCM infestation (number and instar of larvae) recorded.

Table 3.4.2.1. Detached fruit (Valencia orange) bioassay treatments, testing compatibility of CRYPTOGRAN (at a concentration of 9×10^6 OBs/ml) with certain plant protection products.

Treatment No.	Treatment*
1	Distilled water
2	Abamectin (20 ml) + oil (0.3%)
3	Mancozeb (200 g) + benomyl (50 g) + oil (0.3%)
4	CRYPTOGRAN + molasses (0.5%)
5	CRYPTOGRAN + molasses (0.5%) + abamectin (20 ml) + oil (0.3%)
6	CRYPTOGRAN + molasses (0.5%) + mancozeb (200 g) + benomyl (50 g) + oil (0.3%)

* Dosages are per 100 l water.

In the second bioassay, four treatments were used (Table 3.4.2.2). Bioassays were conducted in exactly the same way as described for the first bioassay, except that treated fruit were held for four days before initiating

bioassays, i.e. before placing larvae onto fruit. The reason is that it was found that abamectin, even without CRYPTOGRAN, was effective against FCM. The intention was to try to avoid any immediate knock down effect of abamectin in order to be able to test whether the addition of the abamectin to the CRYPTOGRAN had any detrimental effect on the CRYPTOGRAN.

Table 3.4.2.2. Detached fruit (Valencia orange) bioassay treatments, testing compatibility of CRYPTOGRAN (at a concentration of 9×10^6 OBS/ml) with certain plant protection products.

Treatment No.	Treatment*
1	Distilled water
2	Abamectin (20 ml) + oil (0.3%)
3	CRYPTOGRAN + molasses (0.5%)
4	CRYPTOGRAN + molasses (0.5%) + abamectin (20 ml) + oil (0.3%)

* Dosages are per 100 l water.

Rainfastness

Thirty-two Valencia oranges were dipped in distilled water and 64 were dipped in CRYPTOGRAN (10 ml/100 l water) + molasses (0.5%). Fruit were placed onto a wire-mesh rack to dry. Once dried, 32 of the fruit that were dipped in CRYPTOGRAN were then immersed in distilled water. Once these fruit had dried, three neonate FCM larvae were placed onto each of the 96 fruit. Fruit were kept at 27°C for 14 days and then inspected for decay, penetration marks and infestation. Counts of these were compared between treatments by using ANOVA and the Bonferonni multiple range test.

Field trials

After application, all trials were evaluated in the following manner. Fruit drop (from data trees) was evaluated from three weeks after application (unless stated otherwise), usually until harvest or until there was a substantial decline in efficacy. All trials were applied in semi-commercial block format, with two replicates per treatment. Five to 10 data trees were selected in the middle of each block (i.e. a total of 10 to 20 data trees per treatment). Dropped fruit from each data tree was collected and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was identified by the presence of the larva or its frass. Mean numbers of FCM infested fruit per tree per week for each treatment were compared using ANOVA and the Bonferroni multiple range test (or in one case Students' t-test), using Statgraphics Plus for Windows Version 2.0 (Statistical Graphics Corporation, 1996).

In total, six field trials were conducted by the authors during the 2003/04 season. The first four were designed to examine the efficacy of CRYPTOGRAN in reducing pre-harvest fruit loss and the last two investigated the value of CRYPTOGRAN for mitigating post-harvest risk.

The first trial was applied at Carden Farm in the Sundays River Valley, Eastern Cape Province. The orchard used, consisted of 11-year-old Palmer navel orange trees on rough lemon rootstock. The orchard was planted at a density of 555 trees per hectare. Eleven treatments were compared, including an untreated control (Table 3.4.2.3). Nine of the treatments (treatments 1-9 in Table 3.4.2.3) were laid out in single-tree format, each replicated ten times. The other two treatments (treatments 10 & 11 in Table 3.4.2.3) were laid out in a semi-commercial block format, each with two blocks consisting of an average of 68 trees per block. Although the CRYPTOGRAN concentrations used for each of the two layouts were the same, the number of OBs applied per unit area (i.e. per tree or per hectare) differed, due to the difficulty of applying exactly the same volumes with different machinery (Table 3.4.2.3). The blocks were sprayed with a mist-blower with an oscillating tower, and were applied after 17h30 on 3 December 2003, at an average of 15.3 l of spray mix per tree. The single-tree treatments were applied with hand-held spray guns after 17h30 on 3 December 2003, at an average of 20.1 l of spray mix per tree.

Table 3.4.2.3. Treatments applied on 3 December 2003 for the control of FCM on Palmer navel orange trees at Carden Farm.

Treat. No.	Product	Layout	Conc. per 100 ℓ water	Approximate rate per ha	Additive	Concentration per 100 ℓ water
1	Untreated	Single trees	-	-	-	-
2	CRYPTOGRAN	Single	10 ml	6.59×10^{13} OBs	Molasses	500 ml
3	CRYPTOGRAN	Single	10 ml	6.59×10^{13} OBs	Molasses	250 ml
4	CRYPTOGRAN	Single	10 ml	6.59×10^{13} OBs	Molasses + Agral 90	250 ml + 18 ml
5	CRYPTOGRAN	Single	5 ml	3.29×10^{13} OBs	Molasses	500 ml
6	CRYPTOGRAN	Single	5 ml	3.29×10^{13} OBs	Molasses	250 ml
7	CRYPTOGRAN	Single	5 ml	3.29×10^{13} OBs	Molasses + Agral 90	250 ml + 18 ml
8	CRYPTOGRAN	Single	10 ml	6.59×10^{13} OBs	-	-
9	CRYPTOGRAN	Single	10 ml	6.59×10^{13} OBs	Larval diet*	-
10	CRYPTOGRAN	Blocks	5 ml	2.60×10^{13} OBs	Molasses	250 ml
11	CRYPTOGRAN	Blocks	10 ml	5.20×10^{13} OBs	Molasses	250 ml

*Cryptogran was produced from virus homogenised with diet on which the infected larvae were fed after inoculation. (See Moore (2002) for the diet ingredients).

The second trial was conducted in an orchard of Robyn navel oranges at Schoeman Landgoed in Mpumalanga Province. The orchard was spaced at 340 trees per hectare. Four CRYPTOGRAN treatments were applied on 5 January 2004 (Table 3.4.2.4). An untreated control was retained. The trial was laid out in single-tree replicates in a randomised block design, ensuring that there were buffer trees between treatments in order to minimise any influence of spray drift. Ten replicates of each treatment were used. An average of 30 ℓ of spray mix was applied per tree, as a high-pressure spray, using hand-held spray guns. Spraying of the CRYPTOGRAN treatments was initiated at 16h55.

Table 3.4.2.4. Treatments applied on 5 January 2004 for the control of FCM on Palmer navel orange trees at Schoeman Landgoed.

Treatment number	Product	Concentration in 100 ℓ water	Approximate rate per ha	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-	-
2	CRYPTOGRAN	10 ml	6.37×10^{13} OBs	Molasses	500 ml
3	CRYPTOGRAN	10 ml	6.37×10^{13} OBs	Molasses + Agral 90	250 ml + 18 ml
4	CRYPTOGRAN	3.3 ml	2.14×10^{13} OBs	Molasses	500 ml
5	CRYPTOGRAN	3.3 ml	2.14×10^{13} OBs	Molasses + Agral 90	250 ml + 18 ml

The third trial was conducted on mature Palmer navel orange trees on Welgelegen Farm in Sundays River Valley, Eastern Cape Province. Five CRYPTOGRAN treatments, Alsystin, and Meothrin (both standard chemical treatments) were applied (Table 3.4.2.5) to blocks consisting of an average of 36 trees each. An average of 27.6 ℓ of spray mix was applied per tree, as a high-pressure spray, using an oscillating tower mist-blower. Sprays were applied between 15h00 and 17h30 from 13 to 21 January 2004. Treatments were applied over such a protracted period due to intermittent rainfall during that time.

Table 3.4.2.5. Treatments applied from 13-21 January 2004 for the control of FCM on Palmer navel orange trees at Welgelegen Farm.

Treatment number	Product	Concentration per 100 ℓ water	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-
2	Alsystin	10 ml/100 ℓ	-	-
3	Meothrin	30 ml /100 ℓ	-	-
4	CRYPTOGRAN	10 ml/100 ℓ	Molasses	250 ml
5	CRYPTOGRAN	10 ml/100 ℓ	Molasses	500 ml
6	CRYPTOGRAN	10 ml/100 ℓ	Molasses + Agral 90	250 ml 18 ml
7	CRYPTOGRAN	10 ml/100 ℓ	Molasses + Agral 90	500 ml 18 ml
8	CRYPTOGRAN	5 ml/100 ℓ	Molasses + Agral 90	500 ml 18 ml

The fourth trial was conducted on mature Satsuma mandarin trees on Pennyholme Farm in Sundays River Valley, Eastern Cape Province. Five CRYPTOGRAN treatments were applied (Table 3.4.2.6) to blocks consisting of an average of 40 trees each. An average of 9 ℓ of spray mix was applied per tree, as a high-pressure spray, using an oscillating tower mist-blower. All except one of the sprays were applied between 15h00 and 19h00 on 3 February 2004. The deviant spray was applied at 12h00 that day.

Table 3.4.2.6. Treatments applied on 3 February 2004 for the control of FCM on Satsuma mandarins at Pennyholme Farm.

Treatment number	Product	Concentration per 100 ℓ water	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-
2	CRYPTOGRAN	10 ml/100 ℓ	-	-
3	CRYPTOGRAN	10 ml/100 ℓ	Molasses	500 ml
4	CRYPTOGRAN	10 ml/100 ℓ	Milk powder	
5	CRYPTOGRAN	10 ml/100 ℓ	Brewers yeast	
6	CRYPTOGRAN	10 ml/100 ℓ	Molasses	500 ml
7	CRYPTOGRAN	10 ml/100 ℓ	Molasses + Abamectin + BP Medium oil	500 ml + 20 ml + 300 ml

The fifth trial was conducted on five-year-old Autumn Gold navel orange trees on Woodridge Farm in Sundays River Valley, Eastern Cape Province. An orchard of 1.3 ha was divided into four quarters. Two diagonally opposing quarters were each sprayed with CRYPTOGRAN (Table 3.4.2.7). Each block consisted of approximately 500 trees. An average of 9.25 ℓ of spray mix was applied per tree, as a high-pressure spray, using an oscillating tower mist-blower (Citromist Tower). Sprays were applied between 16h00 and 18h00 on 19 May 2004.

Table 3.4.2.7. Treatments applied on 19 May 2004 for the control of FCM on Autumn Gold navel orange trees at Woodridge Farm.

Treatment number	Product	Concentration per 100 ℓ water	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-
2	CRYPTOGRAN	10 ml	Molasses	500 ml

The sixth and final field trial was conducted on 10-year-old Midnight Valencia orange trees on Brandwacht Farm in Sundays River Valley, Eastern Cape Province. Three CRYPTOGRAN treatments were applied to blocks consisting of an average of 42 trees (Table 3.4.2.8). The purpose of this trial was to compare the efficacy of CRYPTOGRAN when applied with three different machines. Therefore different volumes of spray mix were applied per tree for each treatment. The three machines used were a Janisch, a Malray (both oscillating tower mist-blowers) and a handgun machine. Sprays were applied on 26 and 27 July after 16h00.

Table 3.4.2.8. Treatments applied on 26 July 2004 for the control of FCM on Midnight Valencia orange trees at Brandwacht Farm.

Treatment number	Spray machine	Product	Concentration per 100 ℓ water	Additive	Concentration per 100 ℓ water	Volume applied per tree
1	-	Untreated	-	-	-	-
2	Handguns	CRYPTOGRAN	10 ml	Molasses	500 ml	13.0
3	Janisch	CRYPTOGRAN	10 ml	Molasses	500 ml	18.0
4	Malray	CRYPTOGRAN	10 ml	Molasses	500 ml	13.0

Results and discussion

Shelf-life

LC₅₀ and LC₉₀ estimations from bioassays conducted with CrleGV kept at 10°C for both seven and eight months, indicated higher values than for CrleGV which had been frozen at zero days (Table 3.4.2.9). However, according to probit analyses, these differences were not significant. This was confirmed by the fact that there was once again no significant difference in LC values of CrleGV refrigerated at 10°C for 10 months and CrleGV frozen at zero days (Table 3.4.2.9). The values of the latter were actually higher than that of the former. This provides evidence that there is no loss of pathogenicity in CrleGV when kept at 10°C for at least 10 months.

Table 3.4.2.9. Results of dilution series bioassays with neonate FCM larvae and CrleGV refrigerated for various lengths of time.

Treatment (CrleGV concentration)	Larval mortality (%)					
	Control (0 days)	7 months	Control (0 days)	8 months	Control (0 days)	10 months
Distilled water control	16	16	20	20	12	12
1.219 x 10 ²	20	20	20	24	20	20
6.093 x 10 ²	36	44	28	24	48	40
3.046 x 10 ³	60	44	76	28	48	48
1.523 x 10 ⁴	80	72	72	64	64	88
7.616 x 10 ⁴	86	88	92	88	84	92
LC₅₀ (OBs/ml)	3.12x10³	4.88x10³	2.88x10³	1.22x10⁴	4.41x10³	2.43x10³
LC₉₀ (OBs/ml)	5.91x10⁴	1.66x10⁵	4.48x10⁴	1.15x10⁵	4.06x10⁵	3.51x10⁴

Compatibility with other products

It is envisaged that under commercial conditions, the first treatment of CRYPTOGRAN will be applied somewhere between late November and early January in a number of citrus producing areas of South Africa. This is because it has been confirmed that the first major peak in FCM levels occurs around this time, at least in the Eastern Cape (Moore & Fourie, 1999; Moore & Richards, 2000; 2001; 2002; SRCC, unpublished data; PSB, unpublished data) and Mpumalanga (Language, 2002). It is very possible that growers will need to control other pests around this time – one of the most important being citrus thrips. The treatment of choice is likely to be abamectin with oil. In some areas it might also be necessary to spray for the control of black spot. Mancozeb and benomyl with oil is likely to be the most popular treatment.

During December 2003 the compatibility of abamectin and the conventional black spot treatments was tested with CRYPTOGRAN in laboratory bioassays. Indications were that there was no incompatibility (Moore *et al.*, 2003). This bioassay was repeated, not only to confirm the results but also to test the effect of the chemical products on their own against FCM. There was again no indication that either the thrips treatment or the black spot treatment had any detrimental effect on the efficacy of the CRYPTOGRAN. Infestation was similarly low in fruit treated with the CRYPTOGRAN and molasses alone and in fruit treated with CRYPTOGRAN in conjunction with the chemical treatments (Table 3.4.2.10). As was suspected from the results of the bioassay conducted during 2003, abamectin on its own significantly reduced FCM infestation (Moore *et al.*, 2003).

Table 3.4.2.10. Damage to and infestation of fruit (Valencia oranges) treated with CRYPTOGRAN (9×10^6 OBs/ml) and certain other plant protection products to test compatibility. (Four neonate larvae were placed onto each fruit, and 20 fruit were used per treatment).

Treat. No.	Treatment	Mean number of penetration marks/fruit*	Mean number of larvae/fruit*
1	Distilled water	1.55a	0.95ab
2	Abamectin (20 ml) + oil (0.3%)	0.30b	0.05c
3	Mancozeb (200 g) + benomyl (50 g)* + oil (0.3%)	1.45a	1.05a
4	CRYPTOGRAN + molasses (0.5%)	0.60ab	0.25c
5	CRYPTOGRAN + molasses (0.5%) + abamectin (20 ml) + oil (0.3%)	0.45b	0.10c
6	CRYPTOGRAN + molasses (0.5%) + mancozeb (200 g) + benomyl (50 g)* + oil (0.3%)	0.55ab	0.40bc

*Values in the same column followed by the same number are not significantly different ($P < 0.05$; Bonferonni LSD multiple range test).

In the next compatibility bioassay, even though larvae were placed onto fruit four days after treatment, the abamectin was still highly effective (Table 3.4.2.11). This was surprising, as abamectin is known to have a very short residual period. This effect of abamectin on FCM larvae, therefore still potentially hid any possible negative effect which abamectin might have had on CRYPTOGRAN, although, this did not appear to be the case (Table 3.4.2.11). The next step will be to test these combinations, and possibly others too, in an orchard trial.

Table 3.4.2.11. Damage to and infestation of fruit (Valencia oranges) treated with CRYPTOGRAN (9×10^6 OBs/ml) and abamectin to test compatibility. (Four neonate larvae were placed onto each fruit four days after treatment of fruit; 20 fruit were used per treatment).

Treat. No.	Treatment	Mean number of penetration marks/fruit*	Mean number of larvae/fruit*
1	Distilled water	1.53a	1.27a
2	Abamectin (20 ml) + oil (0.3%)	0.40b	0.20b
4	CRYPTOGRAN + molasses (0.5%)	1.07a	0.40b
5	CRYPTOGRAN + molasses (0.5%) + abamectin (20 ml) + oil (0.3%)	0.40b	0.20b

*Values in the same column followed by the same number are not significantly different ($P < 0.05$; Bonferonni LSD multiple range test).

Rainfastness

The rather crude trial conducted to test for rainfastness of CRYPTOGRAN, indicated that CRYPTOGRAN is rainfast (Table 3.4.2.12). This confirms the observations made in a number of field trials with CRYPTOGRAN, where rainfall did not appear to reduce the efficacy of the product. Although the pressure of rainfall was not simulated in this trial, the drenching of the fruit could be construed as a type of "worst case scenario". It will nevertheless, be very beneficial if it can be determined exactly how rainfast CRYPTOGRAN is. This can be done by using the rainfall (and spray) simulation apparatus described by Ware *et al.* (1998) for non-target effect testing.

Table 3.4.2.12. Damage to and infestation of fruit (Valencia oranges) treated with distilled water, CRYPTOGRAN or CRYPTOGRAN with simulated rainfall. (Three neonate larvae were placed onto each fruit; 20 fruit were used per treatment).

Treatment	Fruit decayed (%)	Fruit with penetration marks (%)	Mean number of penetration marks/fruit	Fruit infested (%)	Mean number of larvae/fruit
Control	36.7	63.3a	0.90a	66.7a	0.90a
CRYPTOGRAN	10.0	33.3b	0.33b	30.0b	0.33b
CRYPTOGRAN	16.7	40.0b	0.47b	26.7b	0.30b

+ "rainfall"					
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*Values in the same column followed by the same number are not significantly different ($P < 0.05$; Bonferonni LSD multiple range test).

Field trials

Evaluation of most of the treatments in the Carden trial was continued until seven weeks after application (Table 3.4.2.13), at which time most of the treatments appeared to be relatively ineffective. Evaluations of only five of the treatments (i.e. treatments 1,2,8,9 and 11) were continued thereafter. A number of important observations and comparisons were made. Firstly, reduction in infestation where CRYPTOGRAN was applied at 10 ml per 100 l water was invariably better than the comparable CRYPTOGRAN treatment (i.e. applied with the same adjuvants at the same concentrations) where 5 ml per 100 l water was used. Secondly, reduction in infestation was greater where CRYPTOGRAN was applied with 0.5% molasses as opposed to 0.25% molasses (at both 5 ml and 10 ml CRYPTOGRAN per 100 l water). It was not clear from this trial whether Agral 90 made any difference to the performance of CRYPTOGRAN.

Table 3.4.2.13. FCM infestation of navel oranges for various CRYPTOGRAN treatments applied on 3 December 2003 in a trial at Carden Farm in Sundays River Valley.

Trt. no	CRYPTO in 100 l water	Additive	Reduction in infestation (%) relative to infestation in untreated control (infested fruit/tree/week)*					TOTAL
			3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	
1	-	-	3.3	2.2	2.3	3.3	2.1	12.1
2	10 ml	Molasses 500 ml	60.6	54.5	52.2	63.6	52.4	53.7
3	10 ml	Molasses 250 ml	48.5	59.1	56.5	36.4	33.3	41.3
4	10 ml	Molasses 250 ml + Agral 90 18 ml	54.5	54.5	52.2	24.2	23.8	36.4
5	5 ml	Molasses 500 ml	69.7	54.5	47.8	21.2	33.3	40.5
6	5 ml	Molasses 250 ml	21.2	36.4	39.1	15.2	9.5	16.5
7	5 ml	Molasses 250 ml + Agral 90 18 ml	48.	45.5	43.5	6.1	14.3	24.8
8	10 ml	-	54.5	40.9	47.8	42.2	19.0	37.2
9	10 ml	Larval diet*	36.4	36.4	56.5	48.5	38.1	38.0
10	5 ml	Molasses 250 ml	45.5	31.8	43.5	27.3	0.0	24.8
11	10 ml	Molasses 250 ml	81.8	81.8	65.2	72.7	76.2	73.6

*WAT = weeks after treatment.

Evaluation of treatments 8 and 9 (CRYPTOGRAN with and without diet) was continued for a further two weeks. It was hoped that results would be better with CRYPTOGRAN with diet, due to diet acting as a feeding attractant and a UV-protectant. However, reduction in infestation where diet was included was only 8.6% greater than where diet was not included. This difference was not significant.

Probably the most important observation in this trial was that of the superior performance of CRYPTOGRAN where applied in blocks (treatment 11) as opposed to being applied on single trees (treatment 2) (Fig. 3.4.2.1). By nine weeks after application, there was no longer any difference in FCM infestation between the single tree CRYPTOGRAN treatment (at the registered rate) and the untreated control. However, where CRYPTOGRAN was applied at the registered rate in blocks, by 19 weeks after application there was still a 70% reduction in infestation. By 21 weeks after application there was no longer any difference between this treatment and the untreated control. What makes this difference in duration of control between single tree and block treatments more impressive, is that a lower volume of spray mix was applied per tree in blocks and that only 0.25% molasses was used instead of 0.5%.

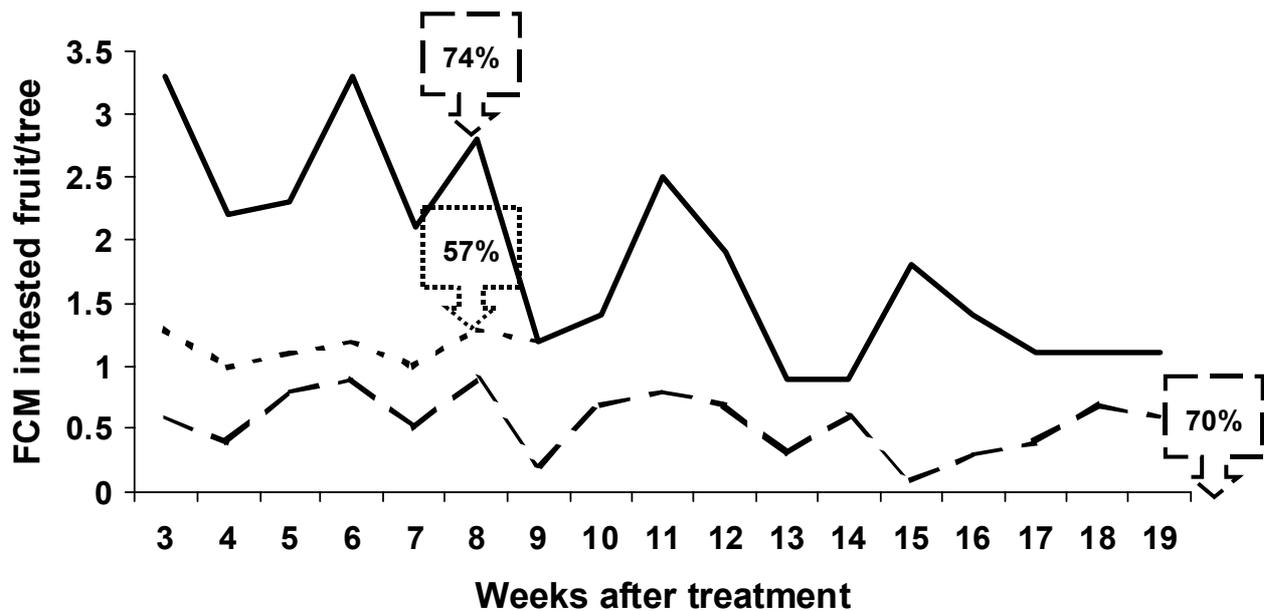


Fig. 3.4.2.1. Weekly FCM infestation of fruit (Palmer navel oranges) for CRYPTOGRAN treatments (10 ml/100 l water + 0.5% molasses) in single tree replicates (dotted line) and in blocks (dashed line) and for untreated control trees (solid line) at Carden Farm, Eastern Cape Province – sprays applied on 3 December 2003.

A principal disadvantage of the use of baculoviruses in the field is their short residual activity due to their inactivation by ultra-violet (UV) irradiation (Huber, 1990; Shapiro, 1995). This shortcoming is so striking with the use of the codling moth GV against codling moth on apples, that reapplication is required every 7-14 days (Dickler & Huber, 1988). Why then does FCM control persist for so long after one application of CRYPTOGRAN? It is speculated that there are four reasons for this. Firstly, a citrus tree provides substantial shading and therefore protection of virus against UV inactivation – more so than probably any other crop on which viruses have been tested for pest control. Secondly, it has been observed that during most of the growing season, the vast majority of FCM larvae penetrate a navel orange through its navel end. It is precisely here that CRYPTOGRAN could be protected against sunlight and possibly even rainfall. Thirdly, as demonstrated in the trial conducted at Carden Farm, FCM takes a long time to recolonise an area, even once the efficacy of a spray might have expired. Lastly, as CRYPTOGRAN would have little, if any, detrimental impact on the highly effective and naturally occurring egg parasitoid, *Trichogrammatoidea cryptophlebiae* (Newton & Odendaal, 1990), this biocontrol agent could aid in maintaining control of FCM once virus is no longer effective.

FCM infestation recorded in the trial at Schoeman Landgoed was abnormally high, peaking at 10.9 infested fruit per tree, four weeks after application and averaging 5.5 infested fruit per tree over a nine-week period (Fig. 3.4.2.2). Despite this, the registered concentration of CRYPTOGRAN and molasses still reduced FCM infestation by 63% over that period. At one third of the registered concentration CRYPTOGRAN was not much weaker, still reducing infestation by 60%. By reducing the molasses concentration, the efficacy of CRYPTOGRAN at both concentrations, also appeared to be reduced. By 10 weeks after application there was no longer much difference between infestation in any of the treatments and infestation in the untreated control. Duration of control achieved in this trial was not as long as in certain other trials. This might have had something to do with the tremendously high levels of rainfall experienced during the trial period, bringing into question the degree of rainfastness of CRYPTOGRAN.

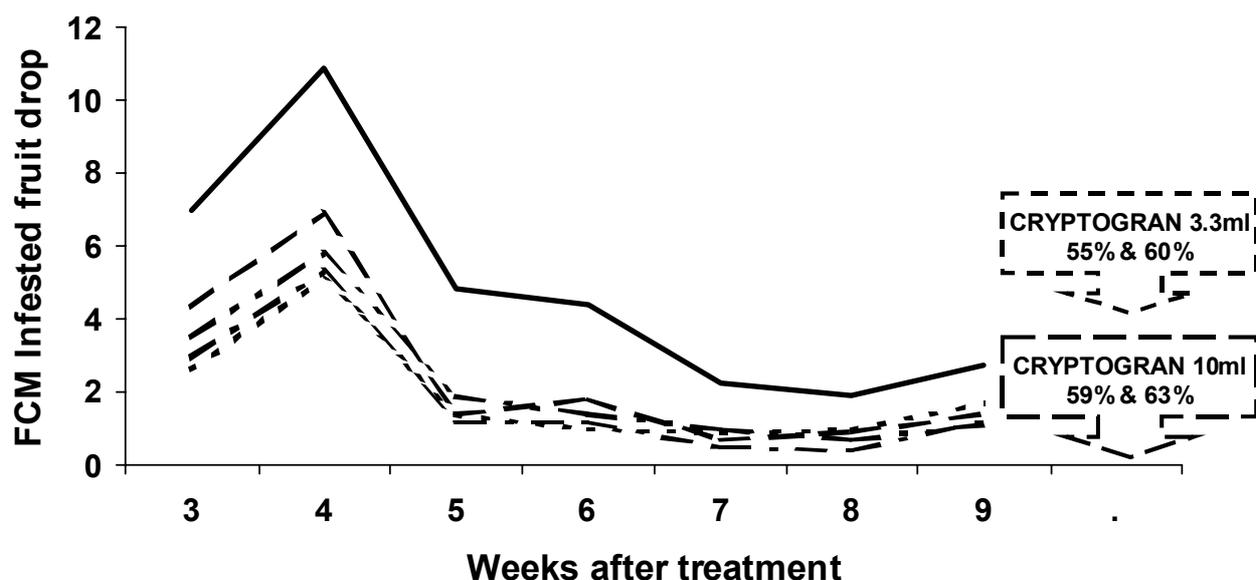


Fig. 3.4.2.2. Weekly FCM infestation of fruit (Palmer navel oranges) for treatments applied for control of FCM at Schoeman Landgoed, Mpumalanga Province, on 5 January 2004. (CRYPTOGRAN 10 ml + 0.5% molasses = dashed line; CRYPTGRAN 10 ml + 0.25% molasses + Agral 90 18 ml = dash-dot-dash line; CRYPTOGRAN 3.3 ml + 0.5%molasses = dash-dot-dot-dash line; CRYPTOGRAN 3.3 ml + 0.25 % molasses + Agral 90 18 ml = dotted line). Arrows indicate average (for each treatment) percentage reduction in FCM larval infestation of fruit, relative to untreated control trees. At each CRYPTOGRAN concentration the 0.5% molasses treatments caused the higher reduction in infestation.

In the trial conducted at Welgelegen Farm both of the standard chemical treatments worked poorly, as did the CRYPTOGRAN applied with only 0.25% molasses (Table 3.4.2.14). However, all of the other CRYPTOGRAN treatments reduced FCM infestation of fruit by more than 70% over the trial period.

Table 3.4.2.14. Comparison of the efficacy of various treatments against FCM on navel orange trees at Welgelegen Farm (evaluated from 10 February – 30 March 2004).

Treatment No.	Treatment	Mean infested fruit per tree per week*	Reduction in infestation relative to untreated control (%)
1	Untreated control	0.63ab	-
2	Alsystin	0.53b	15.9
3	Meothrin	0.86a	-36.4
4	CRYPTOGRAN + 0.25% molasses	0.73ab	-15.9
5	CRYPTOGRAN + 0.5% molasses	0.19c	70.5
6	CRYPTOGRAN + 0.25% molasses + Agral 90	0.19c	70.5
7	CRYPTOGRAN + 0.5% molasses + Agral 90	0.16c	75.0
8	0.5X CRYPTOGRAN + 0.25% molasses + Agral 90	0.17c	72.7

*Values in the same column followed by the same letter are not significantly different ($P>0.05$; Bonferroni LSD multiple range test).

In the trial conducted on Satsuma mandarins, all of the CRYPTOGRAN treatments reduced FCM infestation (Table 3.4.2.15). However, this reduction was not significant for two of the treatments: CRYPTOGRAN alone and CRYPTOGRAN with abamectin and oil. The most effective treatment was CRYPTOGRAN applied with molasses (0.5%) as registered. This treatment resulted in a 60% reduction in infestation.

Table 3.4.2.15. Comparison of the efficacy of various treatments against FCM on Satsuma mandarin trees at Pennyholme Farm (evaluated from 24 February - 9 March 2004).

Treatment No.	Treatment	Mean infested fruit per tree per week*	Reduction in infestation relative to untreated control (%)
1	Untreated control	3.13a	-
2	CRYPTOGRAN	2.17ab	30.9
3	CRYPTOGRAN + molasses	1.27c	59.6
4	CRYPTOGRAN + milk powder	2.03bc	35.1
5	CRYPTOGRAN + instant yeast	1.70bc	45.7
6	CRYPTOGRAN + molasses (applied midday)	1.83bc	41.5
7	CRYPTOGRAN + molasses + abamectin + oil	2.13ab	31.9

*Values in the same column followed by the same letter are not significantly different ($P>0.05$; Bonferroni LSD multiple range test).

The CRYPTOGRAN treatment applied in the trial at Woodridge Farm only reduced FCM infestation by 29%. However, overall fruit drop was reduced by 40%. In analysing the trial, some difficulty was experienced in determining the cause of fruit drop due to the rapid decaying of fruit once it had dropped. It is therefore possible that the actual difference in fruit drop between treated and untreated blocks is more reflective of the efficacy of the CRYPTOGRAN treatment against FCM than the infestation figures (Table 3.4.2.16) would reflect. The reduction in fruit drop in the CRYPTOGRAN treated blocks would extrapolate to 6456 fruit per hectare. The value of this saving would easily justify the cost of the CRYPTOGRAN application. It is nevertheless disappointing that fruit drop was reduced by only 40% and detectable infestation by only 29%. However, this raises two important points. Firstly, FCM control shortly before harvest is more difficult to achieve than earlier in the season. FCM should therefore not be at a high level when CRYPTOGRAN is applied shortly before harvest. Levels should have already been reduced by treatment earlier in the season. The treatment shortly before harvest should be applied against a relatively low level of FCM for the purpose of reducing post-harvest problems (i.e. waste and market interception risk). The second point is that coverage of trees, particularly at this stage of the season, is of paramount importance. It was patently clear in this trial that coverage of trees and consequently wetting of fruit was not adequate. Good spray coverage late in the season becomes more difficult to achieve than earlier in the season, due to the increase in tree density as a result of fruit and foliage growth. Growers therefore need to make an exceptional effort to achieve the required coverage at this time.

Table 3.4.2.16. Comparison of the efficacy of various treatments against FCM on Autumn Gold navel orange trees at Woodridge Farm (evaluated from 9-30 June 2004).

Treatment No.	Treatment	Mean infested fruit per tree per week*	Reduction in infestation relative to untreated control (%)
1	Untreated control	1.97a	-
2	CRYPTOGRAN	1.40b	29.0

*Values in the same column followed by the same letter are not significantly different ($P>0.05$; Student's T-test).

As a consequence of the unimpressive results achieved with the trial conducted at Woodridge Farm, and the reasons for those results, the trial at Brandwacht Farm was conducted. Here treatments were applied against a lower level of FCM presence for the purpose of reducing the risk of experiencing post-harvest problems. Three different modes of application were also compared in order to test the hypothesis that spray coverage of trees at this phenological period is of paramount importance. CRYPTOGRAN applied with the Janisch machine, which clearly achieved the best penetration and volume of coverage, reduced FCM infestation by more than 70% (Table 3.4.2.17). The other two CRYPTOGRAN treatments, in which 28% less spray mix was applied per tree, only reduced FCM infestation by 45% and 55%. The machinery that applied the highest spray volume and clearly the most thorough coverage, was the treatment which most markedly reduced FCM infestation. Consequently, the risk of experiencing post-harvest infestation problems with fruit subjected to this treatment would likely have been substantially reduced.

Table 3.4.2.17. Comparison of the efficacy of various treatments against FCM on Midnight Valencia orange trees at Brandwacht Farm (evaluated from 18 August – 1 September 2004).

Treatment No.	Treatment	Spray machine	Mean infested fruit per tree per week*	Reduction in infestation relative to untreated control (%)
1	Untreated control	-	0.37a	-
2	CRYPTOGRAN + molasses	Handguns	0.17bc	55.0
3	CRYPTOGRAN + molasses	Janisch	0.10c	73.0
4	CRYPTOGRAN + molasses	Malray	0.20b	45.0

*Values in the same column followed by the same letter are not significantly different ($P < 0.05$; Bonferroni LSD multiple range test).

Conclusion

It was confirmed that the shelf-life of CRYPTOGRAN is at least 10 months, if kept refrigerated at a temperature of no more than 10°C. A preliminary test to examine the effect of simulated rainfall on CRYPTOGRAN residues on fruit, indicated that CRYPTOGRAN is rainfast. FCM infestation of navel oranges was reduced by up to 70% in field trials. Reduction in infestation was substantially greater and more persistent where CRYPTOGRAN was applied to blocks of trees as opposed to single trees. Infestation of Satsumas was reduced by up to 60%. Results with CRYPTOGRAN applied shortly before harvest, were poor unless the spray was applied as a very thorough full cover spray, in which case infestation of Midnight Valencias was reduced by 70%.

Future research

Further field work will be conducted during 2005 to investigate the efficacy of different concentrations of CRYPTOGRAN and different adjuvants. Bioassays to determine the full shelf-life of CRYPTOGRAN will be continued. Thereafter, this experiment will be terminated. However, work on the field persistence of CRYPTOGRAN, under a new experiment, will continue.

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3.4.3 Evaluation of a genetically modified pathogen for control of false codling moth Experiment 569 by Sean D. Moore & Wayne Kirkman (CRI)

Opsomming

'n Virus wat geneties verander is deur Horticulture Research International (HRI) in die VK, is teen VKM getoets om te bepaal of dit vinniger as die wilde-tipe virus (CrleGV) doodmaak. Twee bio-toetse is met die geneties-gemanipuleerde (GM) virus teen pas-uitgeborede VKM-larwes op Cara-cara navellemoene uitgevoer. Die besmetting van lemoene is van 46.7% tot 10.0% verminder, maar dit is slegs met baie hoë konsentrasies van die GM-virus behaal. Die LC_{50} en LC_{90} is onderskeidelik as 8.31×10^9 OPs/ml en 5.66×10^{11} OPs/ml geskat. Die konsentrasies wat geskik is vir boordgebruik sal daarom baie hoog wees. Omdat die GM-virus nie veel kommersiële potensiaal het nie, word geen verdere werk met hierdie produk beplan nie.

Introduction

A granulovirus (CrleGV) is currently being investigated for control of FCM (Section 3.4.2). A typical drawback with wild type baculoviruses is their slow speed of kill. This can be remedied through genetic modification of the pathogen. A heterologous virus has been genetically modified by Horticulture Research International (HRI) in the UK. Coincidentally, this virus has been found to be highly effective against FCM in laboratory bioassays. This potential was further tested by CRI during 2002 and 2003 by conducting dose-response and time-response bioassays with neonate larvae on artificial diet. During 2004, detached fruit bioassays were conducted with the genetically modified (GM) virus against neonate FCM larvae. The results of these are described here.

Materials and methods

Two detached fruit bioassays were conducted with the GM virus against FCM larvae. For the first bioassay, 180 Cara-cara navel oranges were picked from trees on the Citrus Foundation Block (CFB), near Uitenhage, on 15 June 2004. Six treatments were prepared, one being a distilled water control. The other five were prepared as a five-fold dilution series of the GM virus, from 1.19×10^9 OBs/ml (the strongest treatment) to 1.90×10^0 OBs/ml (the weakest treatment). These were the same concentrations used to determine the LC_{50} and LC_{90} of the GM virus against neonate FCM larvae on artificial diet (Moore *et al.*, 2003). A volume of 400 ml of each treatment was prepared. Thirty fruit were then dipped into each treatment, starting with the weakest and ending with the strongest treatment, and placed onto a wire mesh rack to dry. Once dry, four neonate FCM larvae were placed onto each fruit (again starting with the weakest and ending with the strongest treatment), using a size 000 paintbrush. This was done on the 17 June. Eighteen days later, on 5 July, the trial was evaluated. Fruit were firstly inspected for any apparent penetration marks, which were recorded. Fruit were then dissected and inspected for FCM infestation.

From the results of the first bioassay, it appeared that virus concentrations were too low to obtain a good dose-response curve. Therefore, in the second bioassay, the three lower concentrations used in the first bioassay were eliminated and three higher concentrations were used. The lowest concentration was therefore 2.42×10^8 OBs/ml and the highest concentration was 1.51×10^{11} OBs/ml. Cara-cara navels from the CFB were again used. These were picked on 5 July 2004. Trials were initiated the following day and evaluated on 21 July. Results (penetration marks and larval infestation) were analysed using probit analysis (Finney, 1971). Total possible numbers of penetration marks and larvae considered per treatment were the values recorded for the distilled water treated control. This is called artificial truncation (Bliss, 1935) and is done in order to estimate a more meaningful dose-response relationship. Probit analysis calculated a dose-response relationship and estimated LC values.

Results and discussion

In the first bioassay, all treatments caused reductions in both penetration marks and infestation of fruit (Table 3.4.3.1). However, there was no satisfactory trend in levels of damage to the fruit, relative to the concentrations of virus. It was therefore not possible to conduct a probit analysis of the data. Also, the reduction in infestation, even with the highest concentration, was not satisfactory.

Table 3.4.3.1. Penetration and infestation of Cara-cara navels in a bioassay conducted with a dilution series of concentrations of a GM virus against neonate FCM larvae (trial conducted from 17 June to 5 July 2004).

	Treatment (in OBs/ml)	Fruit with penetration marks (%)	Penetration marks/fruit	Fruit infested (%)	Larvae/fruit
1	Distilled water control	70.0	1.00	66.7	0.97
2	1.90×10^6	60.0	0.93	53.3	0.83
3	9.50×10^6	46.7	0.57	46.7	0.50
4	4.75×10^7	50.0	0.60	33.3	0.37
5	2.38×10^8	50.0	0.60	50.0	0.60
6	1.19×10^9	50.0	0.60	46.7	0.50

Consequently, in the second bioassay, higher concentrations of GM virus were used. Good trends were obtained between both penetration marks and virus concentrations, and larval infestation and virus concentrations (Table 3.4.3.2). As a result, it was possible to determine the LC values of the virus against neonate FCM larvae on fruit, using a probit analysis. Probably the most relevant estimations were those calculated from the relationship between larval infestation and virus concentrations. This gave estimations of 8.31×10^9 OBs/ml and 5.66×10^{11} OBs/ml for the LC₅₀ and LC₉₀, respectively. These values can be considered to be very high. The LC₅₀ and LC₉₀ values estimated for CrleGV (the homologous FCM granulovirus) against neonate FCM larvae on fruit, were 5.342×10^6 and 7.052×10^8 OBs/ml, respectively (Moore, 2002).

Table 3.4.3.2. Penetration and infestation of Cara-cara navels in a bioassay conducted with a dilution series of concentrations of a GM virus against neonate FCM larvae (trial conducted from 6 to 21 July 2004).

	Treatment	Fruit with penetration marks (%)	Penetration marks/fruit	Fruit infested (%)	Larvae/fruit
1	Distilled water control	50.0	0.83	46.7	0.80
2	2.42×10^8	50.0	0.73	43.3	0.67
3	1.21×10^9	53.3	0.63	50.0	0.57
4	6.05×10^9	40.0	0.47	36.7	0.47
5	3.02×10^{10}	40.0	0.40	33.3	0.33
6	1.51×10^{11}	33.3	0.40	10.0	0.10
LC₅₀			4.22×10^{10}		8.31×10^9
LC₉₀			3.47×10^{13}		5.66×10^{11}

Therefore, the concentrations of the GM virus which would have to be used in the field would be inhibitory high. Not only would it be impossible to produce sufficiently large volumes of virus to cover a meaningful area, but the cost of the product would be unaffordable.

Conclusion

In bioassays conducted with the GM virus against neonate FCM larvae on Cara-cara navel oranges, infestation of oranges was reduced from 46.7% to 10.0% fruit infested. However, this was with very high concentrations. Consequently, LC₅₀ and LC₉₀ were estimated as 8.31×10^9 OBs/ml and 5.66×10^{11} OBs/ml, respectively. Therefore, the concentrations of the GM virus which would have to be used in the field would be inhibitory high.

Future research

No further work is planned on this experiment as it is concluded that the GM virus has little commercial potential.

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3.4.4 Development of semiochemical odorants for the attraction and repulsion of false codling moth in citrus

Experiment 648 by Christo Smit (Desense Pest Control)

Opsomming

In die proewe onder bespreking is uitsluitlik van olfaktometers gebruik gemaak om reukstowwe te identifiseer wat valskodlingmot aanlok of afstoot. Mannetjies en wyfies is van mekaar geskei onder verkoeling en afsonderlik in reukstofgelaaide olfaktometers ondersoek vir hulle reaksies ten opsigte van doelgerigte beweging.

Chemiese groeperings waarin verbindings gevind is wat aanloklik vir VKM was, is die volgende:

- Benseen-ringverbindings: Metoksie-bensaldehyede, propeniel-bensene en aminobensene
- Groep van suurstof-bevattende ringverbindings (O-heterosikliese verbindings): Twee hoofgroepe van VKM-lokmiddels, nl. laktone en suur-anhidriede wat heksagonale (=piraan) en pentagonale (furaan) ringverbindings is.
- Terpene-groep monoterpene en veral seskwiterpene.
- Onder die groep van alifatiese (reguit ketting) aldehyede, C-8 tot C-12-verbindings, wat versadig of onversadig mag wees; ook asetaatesters.
- Alkohole en moontlik ook aldehyede in die kortketting groep, C-4 tot C-6-, met en sonder metiel-vertakkings. Metiel-vertakte aldehyede in hierdie groep is belangrike reukbestanddele van VKM-aanlokkende paprika-plantmateriaal.
- Verskillende plantmateriale lok VKM aan, insluitende lemoen- en jasmynbloeisels, jong nawelruggies, paprika, borrie en heuning.
- Alle kleure van die ligspektrum wat ondersoek is, trek VKM aan met aanduidings van sterker aantrekking in die oranje en blou spektrum.

Introduction

The primary aim is to identify semiochemical odorants and light stimuli which attract or repel false codling moth (FCM) and to adapt this knowledge for FCM control on an orchard scale.

Materials and methods

In the past season, all experiments were conducted under controlled laboratory conditions with olfactometers.

- **Test insects:** FCM, 24 to 48 hours old, were obtained from Ceder Biocontrol's FCM insectary at Citrusdal. All moths were discarded after they'd been used once. Male and female FCM were separated while cold immobilized. Tests were initiated during early evening to coincide with the start of the normal nocturnal FCM activity pattern.
- **Test chemicals:** The test compounds were procured from R. C. Treatt (England), Bedoukian and Aldrich (USA) and Fluka (Switzerland).
- **Olfactometer installation:** Initially an olfactometer with six tubes was installed. The olfactometer tubes consisted of cylindrical transparent Perspex material, 2 000 mm long x 110 mm external diameter. A 40 mm

diameter hole was located in the centre of the tube through which the test insects were introduced into the olfactometer tube.

Air was supplied by means of an air conditioner which introduced fresh air from outside the laboratory and delivered it to both the olfactometer tubes and the laboratory. FCM tests were conducted at 22°C to 24°C. A 110 mm diameter in-line extractor fan was used to draw a low speed air stream through the olfactometer tubes. At the point of air introduction, a control valve was installed to regulate airspeed by using a simple airspeed indicator. Connection of the upwind (intake) and downwind (exhaust) sides of the olfactometer tubes was by means of 40 mm swimming pool cleaner tubes. They were attached on both sides to a six tube air-distribution manifold - on the intake side to the air conditioner and on the exhaust side to the air extractor fan.

To keep the moths from escaping both ends of the olfactometer tubes were closed off by wrapping pieces of nylon insect mesh around the openings. The mesh was kept in place by a PVC fitting pressed over both ends of the olfactometer tubes. Alternatively, mesh fitted over suitable diameter PVC pipe rings, were introduced internally into the ends of the tubes.

The olfactometer tubes were marked and numbered at 100 mm intervals, starting from the centre of each tube where the moths were introduced, to the ends. Positive numbers were used for the intake side and negative numbers for the exhaust side.

- **Olfactometer preparation:** Loading of the olfactometer tubes with FCM was done at 16°C so as to sedate them temporarily and to keep them immobile at the starting position. After loading the moths, the odorant dispensers were loaded at the intake end of the olfactometer tubes with a previously prepared odorant dispenser. The latter consisted of a six-ply square of tissue or toilet paper, 25 mm x 25 mm, stapled together, onto which the odorants were applied, liquids as droplets and solids as powders pressed with a blade onto a paper square which was previously wetted with a neutral mineral oil (Sunspray 7 E citrus oil). A strip of 30 mm wide aluminium foil was folded double over each loaded paper square, pressed flat and its contents marked on an adhesive strip. According to the volatility of the particular odorant, the slit between the aluminium layers at both sides of the dispenser square could be adjusted to control vapour emission to some extent. This method also reduced the risk of contamination between odorant dispensers.

After loading the olfactometer at 16°C air temperature was increased to 23°C to reactivate the moths. From that point onwards the laboratory was kept dark for the duration of the experiment, except when readings were taken with the aid of a mains-powered torch.

The same method was followed to expose moths to the light emitting diodes, except that they were introduced at the intake end of the olfactometer tubes. When working with light, each olfactometer tube was wrapped with dark material to screen it from its neighbour.

- **Minitunnels:** Minitunnels were designed to speed up the rate of odorant screening. These tunnels consisted of rolled-up A3 transparency sheets, 420 mm x 300 mm, which resulted in transparent tubes, 420 mm long x 50 mm diameter. The tubes were held together with adhesive tape. Two of these minitunnels fitted into one of the larger 110 mm olfactometers. The olfactometers were therefore effectively increased to 12, instead of six. Only four zones were marked on each tube. The tubes were cleaned in the same way as the larger olfactometers, but were discarded after approximately six uses, as the plastic material became too opaque to allow observation.

Because of the shorter tube length of the minitunnels, only attraction could be evaluated as the moths had to be applied to the exhaust end of the tubes. Readings were shortened to 45 minutes to compensate for the shorter tubes.

- **Cleaning olfactometer tubes between treatments:** Upon completion, the moths were removed and discarded. The olfactometers and their fittings were separated and cleaned twice with boiling water to remove any adsorbed odours. The tubes were then dried and put in the sun to assist degradation of any remaining odours.

- **Observation intervals:** Observations were repeated four times at one-hour intervals during the course of each experiment. This interval was shortened to 30 min. in the case of very attractive odorants. The total observation period was restricted to the time it took untreated control moths to migrate through the length of a tube.

- **Recording results:** Data recording consisted of counting the numbers of moths in each marked zone over the length of the olfactometer tube. Moths in the intake side of the tubes were deemed to have been attracted, and moths in the extractor side, repulsed.

If the moths were too active to allow counting, they were temporarily immobilized by reducing the air temperature supplied to the olfactometer. The temperature was increased again immediately afterwards. Counting was also sometimes facilitated by immobilizing the moths in a particular segment with torch light.

- **Requirements for light attraction experiments:** In order to determine the optimal colour/wavelength peaks of light for FCM attraction, pure, monochromatic sources are needed. Such sources are light emitting diodes (LEDs) of which almost all colours of the spectrum, except purple and ultraviolet, can be obtained commercially. For purple and ultraviolet, fluorescent tubes were used instead. An added advantage was that the LEDs were small enough to be used in the olfactometer tubes.

- **Screening strategy:** Because of the large numbers of candidate test chemicals, it was necessary to narrow the research focus to chemical groupings within which the best chance of success could be expected. Initial decisions were based on literature studies. Increasing personal experience dictated later test directions. The results for FCM attraction below are presented under the indicated chemical groupings. In the discussion of the results, the more promising research directions will be indicated.

Initial screening involved one replication of each odorant. However, when even slight indications of activity were observed, the particular substances were repeatedly tested, and perhaps used as reference standards against which new odorants within that specific chemical group were compared.

- **Calculation of relative attraction or repellence:** Data was transferred to Microsoft Excel for the following calculations:

(i) The percentage of moths present in each of the 20 zones marked on the olfactometer tubes, i.e. 10 upwind (+1 to +10) and 10 downwind (-1 to -10) counted from the release point in the centre of the olfactometer.

(ii) The percentage moths in each zone was multiplied with its zone value number (1 to 10) for each observation. These figures were added together to obtain a dispersion index for the specific odorant. This figure was subtracted from the dispersion index of the untreated control to obtain a **relative dispersion index**. The means for each treatment were calculated at the end of the four observations.

- **Interpretation of results:** For unknown reasons, the experimental moths were not always equally lively and reactive, causing dispersal to vary between experiments. Time intervals at which results were taken sometimes differed, and different types of olfactometer tubes were used. There were therefore variations in dispersion indexes between experiments, involving even untreated controls. Thus, in interpreting the results, comparisons could at best be made within the same experiment as is indicated by similar test dates, the same sex and the same type of olfactometer used. The attraction indexes from mini-tunnels, indicated by an asterisk, were also mostly smaller for the minitunnels than for the larger 110 mm olfactometer tubes. For the same date series, experiments with males and females were conducted separately on consecutive days.

Results and discussion

The results expressed as relative attraction as explained above, are those of odorants which in the screening process, indicated moth attraction. The two sexes of FCM were tested separately. The results marked with an asterisk (*) were obtained from the minitunnels and the remainder with the larger olfactometer tubes.

Chemical groupings in which FCM attractants were discovered are the following:

Table 3.4.4.1. Attraction of false codling moth males and females to Benzene/phenolic compounds.

Test date	Odorant	Relative dispersion index of FCM	
		males	females
	Methoxy benzenes		
	• Mono-methoxy benzaldehydes		
12-13/01/05	Benzaldehyde	-	16*
	2-Methoxy benzaldehyde	-	9*
	4-Methoxy benzaldehyde	-	4*
	4-Methoxy 2-hydroxy benzaldehyde	-	13*
	4-Methoxy 3-hydroxy benzaldehyde	-	-39*
	3-Methoxy 2-hydroxy benzaldehyde	-	31*
	3-Methoxy 4-hydroxy benzaldehyde	-	-28*
05-06/01/05	4-Methoxy benzaldehyde	-	24*
12-13/10/04	4-Methoxy benzaldehyde	129	55
	• Dimethoxy benzaldehydes		
12-13/01/05	3,4 Dimethoxy benzaldehyde (=Veratraldehyde)	-	22*
12-13/01/05	3,5-Dimethoxy 4-hydroxy benzaldehyde (=Syringaldehyde)	-	14*
	• Other methoxy benzenes - See under propenyl methoxy benzenes below		
	Propenyl benzenes (2C= and -3-C=)		
	• 2-Propenyl benzenes (Double bond at 2-C)		
20-21/01/05	Cinnamic alcohol (Benzyl propen 1-ol)	8*	21*
	Cinnamic aldehyde	23*	-
	Cinnamic acid	10*	3*
	Ethyl cinnamate	24*	10*
	• 2-Propenyl methoxy/ethoxy benzenes		
25-26/01/05	Iso-eugenol (4-Propenyl 2-methoxy 1-hydroxy benzene)	-	38*
	Methyl iso-eugenol (4-Propenyl 1,2-dimethoxy benzene)	-	33*
	Propenyl guaethol (5-Propenyl 2-ethoxy 1-hydroxy benzene)	-	15*
20-21/01/05	Propenyl guaethol	20*	26*
	Anethole (4-Propenyl 1-methoxy benzene)	9*	-4
	• 3-Propenyl (=allyl) benzenes (Double bond at 3-C)		
20-21/01/05	Methyl chavicol (4-(allyl 1-methoxybenzene)	13*	13*
	Eugenol en methyl eugenol (4-Allyl 2-methoxy 1-hydroxybenzene)	<0	<0
05-06/01/05	Methyl chavicol	-	8*
12-13/10/05	Methyl chavicol	117	38
	Amino benzenes		
09/10/04	Butyl 4-aminobenzoate	-	91
	2-Isopropenyl 1-amino benzene (=2-Isopropenyl aniline)	-	81
	Methyl 4-aminobensoaat	-	-62
02-04/08/04	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	24	196
	• Amine-aldehyde Schiff base combinations		
02-04/08/04	Triplal methyl anthranilate	139	423
	Lilial methyl anthranilate	5	207
07/09/04	Triplal methyl anthranilate	159	-
05-06/01/05	Triplal methyl anthranilate	-	13*
	Lilial methyl anthranilate	-	9*
	Anisaldehyde methyl anthranilate	-	27*

• **Methoxy benzenes:** Best FCM attraction seemed to be obtained with the methoxy groups in the 3-C, 4-C and/or 5-C positions.

• **Propenyl benzenes:** FCM attraction is much more common in the case of the 2-propenyl benzenes with the double bond in the middle than the allyl (=3-C) benzenes with the double bond at the end of the

molecule. The former includes the cinnamyl and iso-eugenol compounds and the latter the eugenol compounds.

- **Amino benzenes:** Keeping in mind the field experiments from 2003, proper amino benzenes seem to be good candidates for synergists for other odorants. The synergism between ethyl anthranilate and the FCM sex pheromone is an example.

Table 3.4.4.2. Attraction of false codling moth males and females to O-heterocyclic compounds (oxygen containing ring structures).

Test date	Odorants	Relative dispersion index of FCM	
		males	females
	<u>Lactones</u>		
	• Benzopyranones		
27-28/10/04	Coumarin	20	131
	7-Methoxycoumarin	-44	266
	Dihydro coumarin	71	65
	• Pyranones		
23-24/09/04	Delta dodecalactone	156	63
02-03/02/05	Indalone (=Butopyronoxyl)	12*	19*
	• Furanones		
30/31/08/04	Gamma decalactone	-	166
	Gamma undecalactone	114	83
	Gamma dodecalactone	131	108
23-24/09/04	Gamma hexalactone	-	36
05-06/01/05	Jasmolactone extra C (=gamma 3-hexenyl butyrolactone)	-	26*
	Lactone of cis-jasmone	-	28*
26/07/04	Jasmolactone extra C	-	88
	Lactone of cis-jasmone	-	79
22-23/12/04	Lactone -"Aldehyde C-19"	-	11
23-24/09/04	Lactone -"Aldehyde -29"	135	-26
	<u>Acid anhydrides</u>		
02-03/02/04	2-Dodecenyl succinic anhydride	34*	43*
02-03/02/04	2-Octenyl succinic anhydride	28*	44*

- The coumarins and several C-10 to C-12 lactones are naturally occurring compounds in citrus and as such may be part of the FCM host identifying odorants.
- O-heterocyclic compounds are also active in odour perception as anti-oxidants. The presence of the in-ring oxygen restricts the oxydative breakdown of odorant molecules at the site of odour perception in preparation for the perception of the next odorant molecule. This causes the system to be exposed for longer to the effects of other perceived odorants which also may give these compounds a synergistic role to play.

Table 3.4.4.3. Attraction of false codling moth males and females to terpenes.

Test date	Odorant	Relative dispersion index of FCM	
		males	females
	Monoterpenes		
11-12/08/04	Neryl acetate	156	28
25-26/01/05	Linalyl acetate	-	15*
	Sesquiterpenes		
11-12/08/04	Farnesol	423	85
02-04/08/04	Farnesene	15	-
	Farnesyl acetone	77	15
	Nerolidol	192	10
15/12/05	Copaene in ginger oil	151	-
22-23/12/04	Copaene in ginger oil	92	40
	Methoprene (Farnesene derivative)	-43	56
05-06/01/05	Farnesol	-	12*
	Farnesyl acetone	-	17*
	Farnesene	-	6*
	Sinensal 40A	-	11*

02-03/02/05	b-Damascone	22*	27*
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- Monoterpenes are very well represented in the natural range of citrus odorants, but very few of them attract FCM. In contrast, the success rate with the sesquiterpenes, which are also well represented in citrus, is much higher. However, the pure laboratory substances are very often expensive. A cheaper alternative will be to obtain the specific natural plant oils in which they occur.
- Sesquiterpenes are less volatile than the monoterpenes because of their bigger molecules and thus do not evaporate so easily. Sinensal is an example which attracts FCM and is also perceptible to humans at very low concentrations. It is thought that the prime host plant identifying odorants for FCM fall in the sesquiterpene group.

Table 3.4.4.4. Attraction of false codling moth males and females to long chain aliphatic aldehydes and esters (C-8 to C-12).

Test date	Odorant	Relative dispersion index of FCM	
		males	females
	Unsaturated aldehydes (C-8 to C-12): This subgroup includes some important natural citrus and soft fruit flavours of which one related decadienyl ester is used to attract codling moth in pome fruit orchards in the USA.		
25-26/01/05	Tr-2-Octenal	40*	61*
	Tr-2,4-Nonadienal	12*	49*
	10-Undecenal	35*	-
	Tr-2-Dodecenal	-2*	18*
	Saturated aldehydes and esters (C-8 to C-12)		
25-26/01/05	Octanal	48*	7*
	Decanal	5*	44*
02-03/02/05	Dodecanal	17*	16*
	Decyl acetate	50*	4*
	Dodecyl acetate	18*	7*
	Tetradecyl acetate	0*	-10*

Some of the strongest moth reactions in terms of almost instantaneous activation and directional upwind movement to the odorant source, were obtained from trans 2-octenal and 2,4-nonadienal. This chemical group should therefore be fully investigated. Humans perceive these odours as typically fruity. A representative of this chemical group, i.e. ethyl 2,4-decadienate, has the typical smell of Bon Chretien pears. It also seems to be a host plant identifying substance for Codling Moth, as it is used in more recent commercial attractants for males and females of this pest in the USA. For this reason, odorants of this group may very well be discovered in the range of host plant identifying odorants for FCM that would attract both sexes.

Table 3.4.4.5. Attraction of false codling moth males and females to short chain aliphatic alcohols and aldehydes (C-4 to C-6).

Test date	Odorant	Relative dispersion index of FCM	
		males	females
14-15/12/04	Butanol	52	72
	3-Methyl 1-butanol	112	-
	3-Methyl 3-buten 1-ol	-	29
25-26/01/05	Hexanol	16*	-
	Tr-2- Hexenol	18*	-
	Hexanal	18*	-
	Tr-2-Hexenal	23*	-

- Butanol is a well represented ingredient of natural citrus volatile substances and as such may play a strong role in the attraction of FCM to citrus trees.
- The C-6 alcohols and aldehydes are typical leaf odorants in most plant species which are thought to enable insect herbivores to identify host plants.
- Methyl branched aldehydes in this group constitute an important part of the flavour bouquet of paprika, which in itself strongly attracts FCM.

Table 3.4.4.6. Various odorants attractive to false codling moth males and females.

Test date	Odorant	Relative dispersion index of FCM	
		males	females
09-11/11/05	Water	132	83
05-06/01/05	Water	-	18*
06/10/04	Water	107	-
26/07/04	Water	-	227
02-04/08/04	Poly-unsaturates: Retinyl palmitate	80	1
12-13/10/04	Aromatic/benzyl esters: Phenyl ethyl propionate	48	-13

- FCM withheld from water, can detect water vapour upwind. They can survive without water but the females' egg laying capabilities are improved when supplied with water. This phenomenon creates the possibility that poisoned flavoured and sugared baits can be developed in conjunction with a "master" attractant.
- The C-18 to C22+ group of polyunsaturated olefins with double bonds at the C-3, C-6 and C-9 positions, is an important chemical group of Lepidoptera attractants cited in literature. Until now it has not been evaluated for FCM attraction. It is a priority group for future evaluation.

Table 3.4.4.7. Plant materials attractive to false codling moth males and females.

Test date	Odorant	Relative dispersion index of FCM	
		males	females
23-24/09/04	Orange blossoms	207	78
	Jasmin blossoms	143	38
27-28/10/04	Small navel orange fruits	52	171
	Honey (ex Eucalyptus)	-27	131
27-28/10/04	Lina navel (fruit one month old)	203	49
	Palmer navel (fruit one month old)	293	21
09-11/11/04	Navel fruitlets	28	65
	Navel orange fruitlets + water	154	99
	Navel orange fruitlets + water + blue LED	96	-
	Navel fruitlets + blue LED	-	173
	Blue LED only	578	-
	Water only	132	83
	Navel fruitlets + Jasmolactone	-	-5
06/10/04	Navel orange blossoms	118	-
	Water alone	107	-
	Navel blossoms + Lorelei sex pheromone (SF)	-6	-
	Navel blossoms + Lorelei SF + water	-111	-

FCM of both genders are also attracted to small immature navel orange fruits in the olfactometers, as would be expected in the orchards. This would constitute strong competition for the perceived master attractant odorants as it would compete for the moths' attention. It would be interesting to know all the constituents of the emitted volatiles from citrus flowers and young fruit. The critical attractive odorants could be supplied in "killing stations" in high concentrations to out compete the natural odorants. The critical attractive components of orange blossom flavour could also be used any time after blossoming when there is no natural competition. Jasmine substances are common odour ingredients of both orange and jasmin flowers. As such they may be part of an attractant odorant composition.

There seems to be additional attraction effects operative in the case of odours from small oranges plus water vapour, but not in combination with blue LED light, which was best on its own. Water only also did well.

For some unknown reason the odours from a combination of orange blossom plus FCM sex pheromone seem to cause a strongly antagonistic reaction.

Table 3.4.4.8. Attraction of false codling moth males and females to light emitting diodes.

Test date	Colour of light emitting diode (LED)	Relative dispersion index of FCM	
		males	females
28-29/09/04	Infra red	-48	12
	Red	89	125
	Orange	517	230
	Yellow	43	171
02-03/11/04	Orange	49	233
	Yellow	38	221
	Green	11	252
	Blue	301	309
	Ultraviolet	87	337

Although all colours of the light spectrum attracted FCM of both sexes, there seems to be attraction peaks in the case of orange and blue LEDs. In comparison with the olfactory signals from most of the attractive odorants, the attraction from these visual light signals seem to be stronger. Light could perhaps be developed as part of the attraction potential of so-called “killing stations”.

Table 3.4.4.9. Synergists to enhance the attraction of male FCM to natural and synthetic female sex pheromone.

Test date	Sex pheromone ¹ plus synergist	Relative dispersion index of FCM males
07/09/04	15 Female FCM in mesh wire cages	4221
	Lorelei sex pheromone (SF) only	203
	Lorelei SF + farnesyl acetone	130
	Lorelei SF + nerolidol	-511
	Lorelei SF + gamma dodecalactone	-428
08/10/04	20 Female FCM in mesh wire cages	246
	FCM SF only	13
	FCM SF + ethyl anthranilate	32
	FCM SF + dodecyl acetate	40
	FCM SF + neryl acetate	-1

¹The “Lorelei” sex pheromone was evaluated in the Lorelei pheromone dispenser as supplied commercially. The “FCM” sex pheromone was liquid pheromone impregnated into the paper dispensers as described in the section “Olfactometer preparation” under “Materials and methods”.

The degree of attraction obtained from the natural sex pheromone and other odours that may have been emitted by the female FCM, is of a much higher order than that of the synthetic sex pheromone attractant mix. The best synergists for enhancing the attractive power of the synthetic pheromone mixture in the olfactometers were dodecyl acetate and ethyl anthranilate. The latter synergism with ethyl anthranilate was confirmed in field experiments of the previous season. Dodecyl acetate constitutes a major part of the natural female FCM sex pheromone and may play its role in this regard, but it is not part of the artificial sex pheromone attractant which contains only the major constituents of the three unsaturated dodecenyl acetates and has prove ineffective in trap experiments (Hendrik Hofmeyr, pers. com.). None of the other odorants enhanced attraction; in fact, compounds such as nerolidol and gamma dodecalactone, which are FCM attractants on their own, were actually repellent.

Combining clues from nature, literature and own experience to construct a master FCM attractant

One of the better clues from nature which had been followed up until now with good success with regard to the choice and identification of odour active chemical groupings for FCM, was that of the aphrodisiac odorant composition of the Oriental fruit moth (OFM), *Grapholita molesta*, a close relative of FCM. This aphrodisiac includes ethyl cinnamate (a propenyl benzene), mellein (hydroxy chromone; a benzo-pyranone heterocyclic substance) and two jasmone substances, i.e. methyl jasmonate and methyl epi-jasmonate which are related to the jasmone lactones, which are furanones, and are attractive to FCM. The aphrodisiac odorant composition of FCM itself is not yet known.

Another example of synergism from nature that has also been confirmed personally, is that of the main components of the natural female sex pheromone, dodecenyl acetate and dodecyl acetate.

Other clues from nature could be to select from the mass of odorants which have been identified with gas chromatography and mass spectrography in citrus, with reference to those chemicals included in the already identified chemical groupings active to FCM. The attraction of many of these have been confirmed, including strong attractants such as gamma dodecalactone, sinensal, 2-octenal and the other unsaturated and saturated C-8 to C-12 aliphatic aldehydes mentioned.

Taking all of the above into consideration, there is a very strong possibility that maximal FCM attraction will be achieved with an odorant composition rather than from single substances. The above-mentioned OFM aphrodisiac is a good example.

The overall aim thus will be to identify odour ingredients for an ideal master attractant once the relative attraction of as many as possible various potential candidates has been established. With accumulated present knowledge, it is possible that such a master attractant would contain a host plant identifying substance(s), which may be especially sesquiterpenes and probably also unsaturated C-8 to C-12 aldehydes. If similar to the OFM aphrodisiac, there would also be included a propenyl benzene and one of the O-heterocyclic substances, probably a benzopyranone or furanone. It is suspected that an amino-benzene component as well as one of the group of C-18 to C-21 triene long chain unsaturates may be involved.

Conclusion

Results of the past season indicated the chemical groupings where the best attractants should be found. The applicability of the above results still has to be investigated under orchard conditions. One of these results which has in fact been confirmed previously under orchard conditions (2003 CRI Annual Report), is the synergistic effect of ethyl anthranilate on the attractiveness of the female FCM sex pheromone for males.

Methods will have to be developed to maximise the attractiveness of odorants when applied under natural conditions in an orchard as there will be strong competition with natural odorants to which FCM normally react. Such methods may include a search for synergistic combinations. This will probably also include implementation of light attraction e.g. with orange and/or blue diodes.

Future research

- **Olfactometers:** The use of olfactometers is a quicker way than orchard tests of screening hundreds of candidate odorants. The comparability of results can be improved by using a larger number of minitunnels per experiment as conditions are likely to be similar for at least that particular experiment. Therefore, the number of minitunnels will be increased. The screening process will progress quicker and the more promising treatments can be confirmed by using a number of replicates per experiment.
- **Selection and procurement of candidate test odorants:** The range of FCM attractive odorants within the main and subgroups of chemicals, will be extended to all available compounds from the companies mentioned.
- **Application of laboratory results to practical implementation in orchards:** Field experiments of the most promising attractants will be conducted during the 2005-2006 season. Test chemicals for this will be procured shortly.

Acknowledgements

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3.4.5 Control of false codling moth with sterile insect releases Experiment 662 by Hendrik and Marsheille Hofmeyr (CRI)

General introduction

Studies into the potential of the Sterile Insect Technique (SIT) to control FCM were initiated in July 2002. Since then the fundamental requirements of SIT for Lepidoptera, viz. inherited F1 sterility, as well as the radiation biology have been confirmed for FCM. These investigations were followed by a field cage experiment that was conducted to confirm the findings of the basic research under more natural conditions. The experiment confirmed the irradiation rate to be 150 to 200 Gy and an optimum release rate of 10

irradiated males to 1 feral male. These results indicated that a practical pilot project on semi-commercial scale would be a logical next step. However, to enable this, there were many additional requirements involving logistics and infrastructure that had to be tested and, in many cases, created. This involved, *inter alia*, testing the mass production capabilities of the insectary responsible for the supply of the moths required for mass release. Also, the longevity and mobility of irradiated moths had to be established to enable effective Sterile Insect Releases (SIR). Equipment needed for the whole process of moth handling, manipulation and releasing also had to be acquired, or alternatively, designed and manufactured.

Some of these experiments date back to 2003, and were not reported on earlier as the results (i) did not have any value for the practical control of FCM and (ii) the data were better evaluated and compared with experiments that followed at a later date.

3.4.5.1 Distinguishing between fluorescent powders of different colours

Opsomming

Probleme is met die onderskeiding van fluoriserende poeier met verskillende kleure ondervind nadat dit saam in dieselfde merk&loslaat boordproef gebruik is. Sewe verskillende kleure poeier is daarom in die laboratorium ondersoek en 'n tabel is opgestel wat gebruik kan word om die beste kleurkombinasie te kies.

Results and recommendaions

Fluorescent powders are available in various colours and if used correctly, are ideal to mark insects when conducting mark and release experiments involving different treatments. However, in spite of the variety of colours available, some colours are sometimes impossible to distinguish under ultraviolet (UV) light, even when using different wavelengths. This can lead to a situation where differently treated insects are indistinguishable from each other upon recapture. The following colour combinations were tested for their compatibility under long (365 Nm) and short (254 Nm) wavelength UV light (Fig. 3.4.5.1):

	Rocket Red	Fire Orange	Saturn Yellow	Signal Green	Invisible Blue	Horizon Blue	Arc Yellow
Rocket Red		X	✓	✓	✓	✓	✓
Fire Orange			✓	✓	✓	✓	✓
Saturn Yellow				X	✓	✓	✓
Signal Green					✓	✓	✓
Invisible Blue						X	✓
Horizon Blue							✓
Arc Yellow							

Fig. 3.4.5.1. Compatibility of various colours of fluorescent powders (X = incompatible; ✓ = compatible).

Useful combinations of up to four colours (the maximum with colours available to the author) are as follows:

- Rocket Red + Arc Yellow + Saturn Yellow **or** Signal Green + Invisible Blue **or** Horizon Blue
- Fire Orange + Arc Yellow + Saturn Yellow **or** Signal Green + Invisible Blue **or** Horizon Blue
- Saturn Yellow + Arc Yellow + Rocket Red **or** Fire Orange + Invisible Blue **or** Horizon Blue
- Signal Green + Arc Yellow + Rocket Red **or** Fire Orange + Invisible Blue **or** Horizon Blue
- Invisible Blue + Arc Yellow + Rocket Red **or** Fire Orange + Saturn Yellow **or** Signal Green
- Horizon Blue + Arc Yellow + Rocket Red **or** Fire Orange + Saturn Yellow **or** Signal Green
- Arc Yellow + Rocket Red **or** Fire Orange + Saturn Yellow **or** Signal Green + Invisible Blue **or** Horizon Blue

Avoid using together:

- Rocket Red and Fire Orange
- Saturn Yellow and Signal Green

- Invisible Blue and Horizon Blue

3.4.5.2 Effect of irradiation rate on FCM males attracted to female moths under orchard conditions

Opsomming

Die vermoë van gammabestraalde VKM-mannetjies om lokvalle in die boord te vind, is bestudeer. Lokvalle is met óf wyfies óf sintetiese lokmiddel toegerus. Die paringsvermoë van bestraalde VKM-mannetjies met maagdelike wyfies op paringsplatforms, is terselfdertyd ondersoek.

Die mededingingsvermoë van mannetjies wat met 150 Gy en 200 Gy bestraal was, was dieselfde as dié van onbestraalde mannetjies in terme van hulle aanlokking na sintetiese feromoon én maagdelike wyfies. Bestraalde mannetjies kon ook suksesvol met wyfiemotte paar. Lokvalle wat met sintetiese feromoon toegerus was, het beter presteer as lokvalle waarin maagdelike wyfies as lokmiddel gebruik was.

Introduction

This field experiment was conducted to study the ability of irradiated male moths to detect and reach traps.

Materials and methods

The experiment was conducted on the farm Rivierplaas, Citrusdal during February 2003. Forty-eight traps (16 traps per each of three treatments) were distributed in a 6 x 8 pattern in a navel orange orchard one ha in size. One trap was placed in every third tree in every third row. The treatments were repeated in a fixed pattern and were not changed for the duration of the experiment.

FCM larvae are reared in diet in glass honey jars, which are fitted with cotton wool stoppers that serve two purposes:

- The stoppers allow a certain amount of air exchange and also release moisture fairly slowly over the three week period which the larvae use to develop fully. This ensures that the diet does not deteriorate because of excessive moisture and maintains the diet in a fairly soft texture, which otherwise would have dried to an undesired rock hard consistency.
- The stoppers act as filters to prevent the ingress of fungal spores and other air-borne contaminants.

In the standardized FCM breeding process, mature larvae leave the diet in the breeding jars (hereafter "diet" jars) and crawl up to the cotton wool stoppers, where cocoons are formed. The stoppers are usually removed in the insectary and placed into emergence boxes for the pupae to eclose. With this system it is extremely tedious to collect pupae, as individual cocoons have to be manually opened to remove the pupae. In all FCM experiments where the two genders had to be accumulated separately, the cotton wool stoppers on the diet jars were therefore replaced with composite stoppers. Each stopper consisted of a cap and an SFK (single face corrugated; "C" flute) cardboard roll (Fig. 3.4.5.2). Each cap was made from a 25 mm wide segment of PVC pipe, one end of which was closed off by stainless steel mesh (30# x 0,27 mm wire diameter). A roll of SFK cardboard (35 mm wide) was pressed into the cap, the remaining 10 mm of SFK roll protruding from the PVC cap tightly fitted into the jar mouth. This system stopped larvae escaping from the diet jars by crawling through the SFK corrugations and offered ample space for pupation. After pupation, the SFK roll was removed, and the two layers of paper pulled apart, which split the cocoons and allowed the vast majority of pupae to drop out without further manipulation. After removal from the stoppers, the pupae were individually sorted into genders with the aid of a stereomicroscope. They were then incubated at 26°C until moth emergence.



Fig. 3.4.5.2. Cotton wool stoppers replaced with composite PVC/SFK cardboard stoppers to facilitate removal of false codling moth pupae for experimental purposes.

Male moths, not more than 24 hours old, were treated with 150 Gy or 200 Gy in a gamma irradiation facility at ARC-Infruitec, Stellenbosch. Two lots of males, a total of 1208 males, were irradiated on successive days, transported to Citrusdal and released the same day at 17:00-18:30. The males were lightly dusted with fluorescent powder of different colours to distinguish between treatments. The males were divided equally and released on all trees in the centre row between trap rows.

The following treatments were compared:

- Delta trap from Chempak (Drakenstein, Paarl) supplied with a single Lorelei pheromone dispenser.
- Similar Delta trap each with two caged virgin females, 18 to 24 hours old. The females were replaced daily.
- Mating table with two virgin females as above, also replaced daily. Pherocon wing traps were modified to create a flat bottom centrally fitted with a round open-topped container 85 mm diameter x 50 mm high, to confine the females. The top wing of each trap, forming a roof, was separated from the bottom by spacers to create a 80 mm high opening with 360 degree access to the moth container. The inside of the container wall was lightly dusted with talcum powder to prevent the females escaping. Each female was additionally wing-clipped by clipping off half of one of the forewings. All pairs noticed *in copulo* during inspections, were carefully removed so as not to disturb copulation, and placed in sample jars. The females were dissected the next day to confirm successful transfer of sperm.

All traps and mating tables were removed every morning to, respectively, remove the sticky bottoms for counting purposes and to replace females. All trapped males were inspected with a Raytech UV lamp at 254 Nm and 365 Nm.

Observations started at 19:00 and were conducted at 60-minute intervals until no fresh moth activity occurred in the mating tables during two successive inspections. Red torch light was used to avoid disturbing moths around the traps and mating tables. Ambient temperatures were recorded every hour with a maximum/minimum thermometer. The observations lasted for six nights until the activity of released males had dwindled to almost none and only the odd male was still trapped.

Results

There was very little difference in the total number of males trapped in the various treatments (Fig. 3.4.5.3). Slightly more males treated with 200 Gy were trapped in pheromone-baited traps, but less of these moths were trapped when females were used as bait. Many more males were trapped in the pheromone-baited

traps than in the female baited traps. This result is inexplicable and also at odds with data accumulated when the Lorelei pheromone trapping system for monitoring FCM was developed by the author during the early eighties.

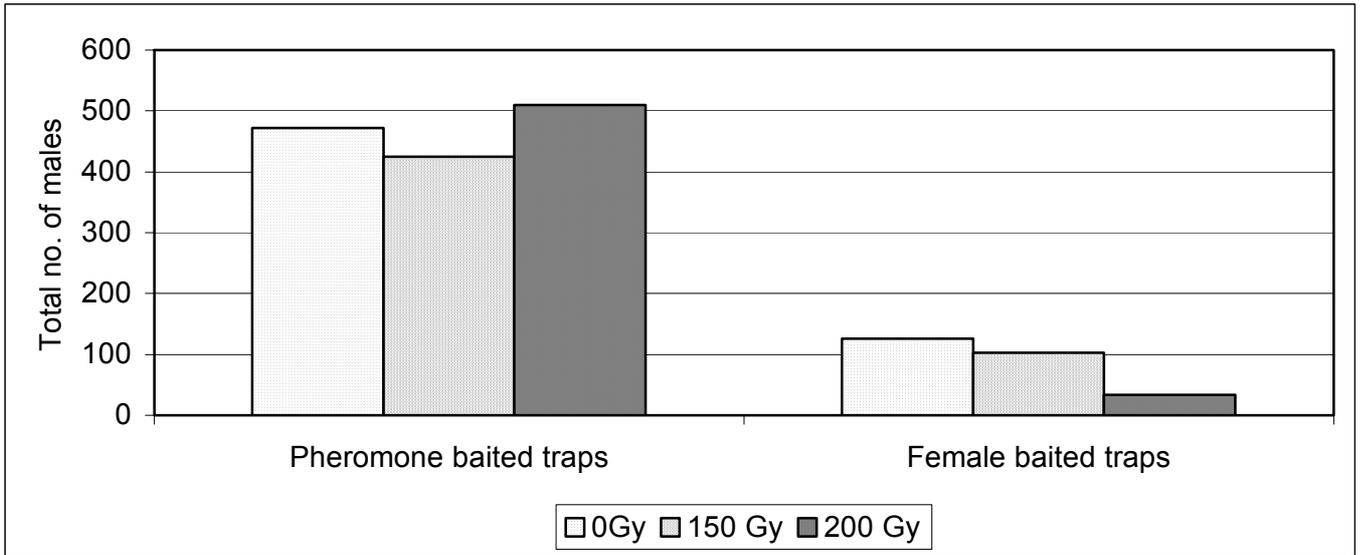


Fig. 3.4.5.3. Number of irradiated and unirradiated false codling moth males trapped in pheromone and female baited traps.

Most males were trapped during the first two nights after release, with catches peaking on the second night. The trap catches dropped sharply from the third night on. The mean temperatures during the period of moth activity, viz. ca. 1800 to 0100, was in the range 21° to 29°C (Fig. 3.4.5.4). This is well outside the minimum temperature considered to be acceptable for moth activity, viz. 14-15°C. Recapture of irradiated males in the 0 Gy, 150 Gy and 200 Gy treatments, were respectively 49,5%, 45,0% and 43,7%. These recovery percentages are regarded to be fair for this type of experiment.

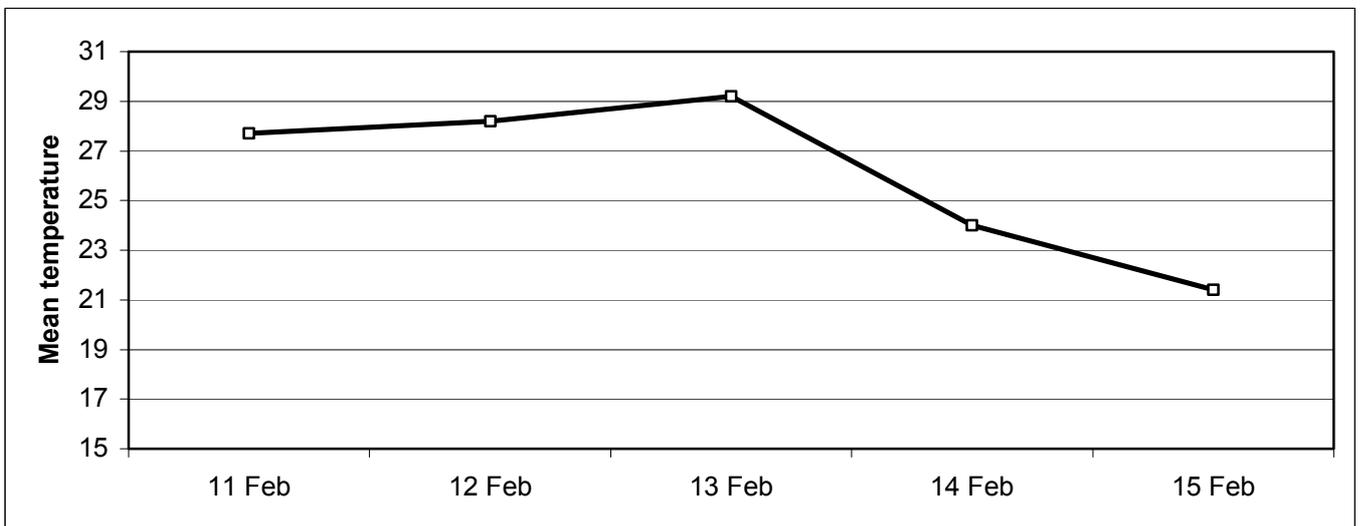


Fig. 3.4.5.4. Mean temperatures during the period 1700 to 0100 in a mark and release experiment on the farm Rivierplaas, Citrusdal, during February 2003.

A total of respectively four, four and three mating pairs were collected in the 0 Gy, 150 Gy and 200 Gy treatments. Spermatophores were found in all the females, an indication of successful mating. All matings occurred from 23:00 to 01:00. The mating rate was unexpectedly low, and could possibly be ascribed to the design of the mating tables – the containers being too deep for pheromone vapour to distribute freely, and also perhaps preventing males from freely entering the containers.

In this orchard experiment, there were little differences between the two radiation doses tested with respect to the ability of treated males to find and copulate with females. In a field cage experiment (2003 CRI annual report) data were collected which showed that treatments involving males treated with 150 Gy were significantly better than treatments where the males had been treated with 200 Gy. Therefore, it may be that the debilitating effect on males irradiated with 150 Gy or 200 Gy is relatively unimportant in practice. If so, it would be desirable to use the 200 Gy dose when preparing for mass releases, as the sterilizing effect on P1 males is greater than with 150 Gy, and will further reduce the already relatively small risk of fruit being damaged by F1 larvae.

3.4.5.3 Testing release rate and release pattern of FCM for use under commercial SIR conditions

Opsomming

Die hervangs van gammabestraalde VKM-mannetjies waarvan 'n vasgestelde aantal in 'n sekere patroon twee keer per week vier weke lank saam met dieselfde aantal bestraalde wyfietotte in 'n nawelboord losgelaat was, is ondersoek om die toepaslikheid daarvan in 'n Steriele Insekloslatingsprogram (SIL) vas te stel. Die hervangspersentasie was oor die algemeen swak weens probleme wat met die vervoer van die motte onder verkoelde toestande opgeduik het. Die inligting wat ingewin is, dui egter daarop dat die aantal loslatings per week en die loslaatpatroon geskik vir 'n SIL-program sal wees. Delta-lokvalle het 1,3 tot 1,4 keer meer mannetjies as die standaard Lorelei-lokvalle gevang, wat die gebruik daarvan in SIL-programme sal regverdig.

Introduction

Trap interaction is known to occur when FCM traps are located within 100 m of each other. It is therefore assumed that a pheromone trap may be attractive to males within an area of at least 60 m wide around the trap, or approximately one ha. A trap catch of 50 feral males per week can be considered to be the maximum mean number of FCM that will be caught under normal circumstances. If these males were accumulated from the surrounding one ha of citrus, it follows that 500 irradiated males must be released per ha per week to attain a 10:1 release ratio. However, until further research shows otherwise, it was decided to standardize the release rate at 1000 males per ha per week, thus doubling the suspected effective number of males, and also mirroring standard practice in Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), SIR.

This experiment was conducted to study the recapture pattern of males when they are released as envisaged under commercial SIR circumstances and, also, to compare two types of traps.

Materials and methods

The experiment was conducted in November 2003 on the farm, Rivierplaas, Citrusdal, in the same navel orange orchard as in Section 3.4.5.2. The experimental site consisted of 19 rows with 23 trees each. Eight traps, four Lorelei pipe traps and four Chempak delta traps, were alternatively distributed in a 4 x 2 pattern in the orchard. One trap of each type was placed approximately 50 m apart in row numbers 1, 7, 13 and 19. Equally divided numbers of moths were released on the trees in row numbers 4, 10 and 16.

Eight batches of moths, each consisting of 500 females and 500 males, were transported to ARC Infruitec, Stellenbosch and treated with 200 Gy irradiation. They were placed into Petri dishes which were then placed into a "six-pack" Coleman type cool box together with a "standard" sized freezer block (145 mm x 858 mm x 30 mm). Several layers of newspaper were put in between the moths and the freezer blocks to prevent direct exposure to the cold. The Petri dishes from the first four batches were removed for irradiation purposes and replaced in the cool box afterwards. For the remainder of the batches (5-8), the moths were only placed inside the cool box during the journey to Stellenbosch. After irradiation, the moths were put into larger 2 l plastic jars, and transported back to Citrusdal under ambient temperature and light conditions. Following treatment, 500 females and 500 males were released during late afternoon on the same day in the designated rows. All moths were marked with fluorescent powder, using a different colour for each release. Moths were released twice per week at three to four day intervals for a total of 2 000 mixed gender moths per week. Eight releases were made over a four-week period. Traps were inspected on every release date. Trap liners were replaced and the removed liners were transported to the laboratory to inspect the trapped moths under UV light.

Results and discussion

Moths from the second and third batches that were to be released, were affected detrimentally by the cold transportation process. Respectively, approximately 70% and 10% of the moths were already dead when the Petri dishes were opened in the orchard just prior to release. The remainder of the moths, although still showing signs of life, were partially immobilized and dropped to the ground when released by hand. It is unclear why moths from the first and fourth batches were not also as obviously affected, although the recapture rate was very low (Table 3.4.5.1). This may be an indication that, although the moths were not noticeably affected, their viability was nonetheless detrimentally influenced. It is clear from the data that the number of males recaptured increased greatly from batch 5 onward (moths partially transported under ambient conditions). The moths were completely inactive due to the daylight, and this technique became standard procedure in following experiments whenever it was possible to keep the genders separate (if put together mixed genders more than 12 to 18 hours old tended to mate even under daylight conditions).

Table 3.4.5.1. Total numbers of irradiated false codling moth males recaptured after being released together with irradiated females in a navel orange orchard.

Date*	Release batch							
	1	2	3	4	5	6	7	8
4 Nov								
7 Nov	61							
11 Nov	19	0						
14 Nov		0	33					
17 Nov			5	32				
20 Nov				5	135			
24 Nov					3	332		
27 Nov						0	147	
2 Dec							26	143
5 Dec								5
Total	80	0	38	37	138	332	173	148
% males recaptured	16.0	0.0	7.6	7.4	27.6	66.4	34.6	29.6

*Releases lasted from 4 to 27 November.

Only two counts were conducted per week, and it is therefore unknown exactly how many nights the males were active for after release. Throughout the experiment males were still noticed in the second count after every release, which indicates that they had to be active for at least three nights after release. However, irradiated males from successive releases were registered in the same traps, which shows that they were continuously present in the orchard when they were released twice a week. This is obviously favourable from an SIR point of view as it improves the probability that irradiated males will be able to locate feral virgin females.

It must be noted that the above male recaptures occurred in spite of females being released simultaneously. This suggests that the males should be able to find feral females despite the large number of released females present in the orchard. It is possible that the released females' attractiveness to males had been reduced by irradiation, which caused the males to prefer the traps, or perhaps even feral females. From a SIR point of view, this would of course be a great advantage. It is, however, quite speculative, as the performance of irradiated females have yet to be investigated under orchard conditions.

The Chempak delta trap trapped more males than the Lorelei pipe trap. As a commercial monitoring tool, the Lorelei trap is a better option to use, as a set trap threshold value exists that can be used for pest management purposes. The delta trap lacks a threshold value. However, in the mark&release experiments, a threshold value is unnecessary and the ease with which the much lighter delta traps can be suspended in trees, is useful. Although trap catches varied from count to count, it seemed that approximately 1,3 to 1,4 times more males were trapped in the delta traps than in the pipe traps (Fig. 3.4.5.5).

The data from this experiment suggest that no more than two releases of irradiated males per week are sufficient to maintain a continuous FCM male presence in the release orchard.

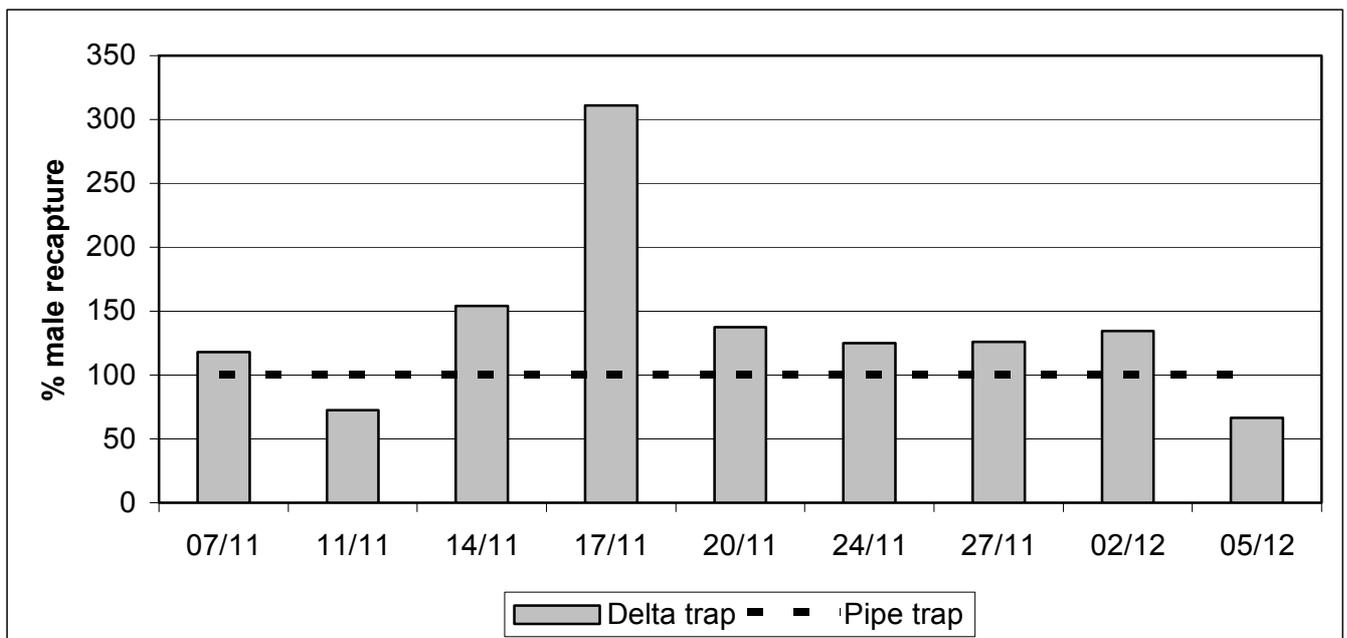


Fig. 3.4.5.5. Percentage recapture of irradiated false codling moth males in delta traps relative to Lorelei pipe traps (represented as 100%).

3.4.5.4 The dispersal distance and lifespan of irradiated FCM males and their recapture in the presence or absence of released FCM females.

Opsomming

Die lewensduur en verspreiding van gammabestraalde VKM-mannetjies wat by vier geleenthede saam met en sonder bestraalde wyfietotte in 'n nawelboord losgelaat was, is bestudeer. Die meeste mannetjies is binne 50 m vanaf die loslaatpunt in lokvalle met maagdelike wyfies as lokaas, gevang, alhoewel 'n hele aantal tot 100 m ver gevang is. Mannetjies is tot 13 dae na loslating in beduidende getalle gevang. 'n Groter persentasie mannetjies is weer gevang nadat hulle saam met wyfies losgelaat was as daarsonder, wat 'n aanduiding kan wees dat gesamentlike loslatings die soekaktiwiteit van die mannetjies verhoog. Al die inligting wat ingewin was, dui daarop dat die algemene gedrag van bestraalde, losgelate VKM die toepassing van SIL moontlik sal maak.

Introduction

This study was conducted to gain more insight into the competitiveness of irradiated FCM males under orchard conditions. It is impractical to separate mass-produced FCM into genders before field releasing. It is therefore important to study the possible effect of such mixed gender releases on the ability of mass-released male moths to find feral females.

Materials and methods

The experiment was conducted in October 2003 on the farm, Kweekkraal, Citrusdal. A nine-hectare block of navel orange trees was used. Thirty-six Delta traps were arranged in four concentric squares containing (inside to outside) respectively four, eight, 16 and eight delta traps, around a moth release point in the centre of the block (Fig. 3.4.5.6). Trap distances from the release point can be seen in Fig. 3.4.5.8. Two virgin females were used as attractants in each trap and were replaced every three days. The females were inspected daily and dead individuals were replaced.

29						36							35
		13	28			27			26	25			
		14	5			12			11	24			
						1		4					
30		15	6			2	♂		10	23			34
								3					
		16	7			8			9	22			
		17	18			19			20	21			
31													33

♂ = Moth release point.

Fig. 3.4.5.6. Diagrammatic representation of trap lay-out. North is to the left of the diagram.

The males were irradiated with 200 Gy. Transportation to and from Stellenbosch was similar to the procedure described in Section 3.4.5.3. Moths were released on four occasions – the first two were male only (920 males per release) and in the last two releases both genders (1320 individuals of each) were released. The two male and two mixed gender releases were respectively made with a two day interval, while an interval of three days was allowed between the male only and mixed gender releases. To enable differentiation, the males from each release were dusted with a different fluorescent powder before release. All releases were made by hand in the late afternoon on two citrus trees at the centrally located release point.

Trap counts were conducted daily and lasted until seven days after the last release. Trap liners with trapped males were removed and replaced with new liners. The used liners were placed inside Glad Wrap® envelopes, stacked, and transported to the laboratory where they were inspected under UV light. A Hobo datalogger was placed inside one of the traps nearest the release point for the duration of the experiment.

Results and discussion

- **Dispersal of released males:** All trap catches for each trap position for the duration of the experiment were added together (Fig. 3.4.5.7). Three distinctive clumps of catches are recognizable. If the position of the traps responsible for the higher catches are compared to their respective positions in the orchard, it is clear that the traps were mostly located north-easterly and south-easterly from the point of release. These traps were downhill from the release point, and in an opposite direction to what would normally be the prevailing wind direction (south-westerly) in the area. An anemometer was not available, and this anomaly cannot be explained, unless (i) the winds blowing during the experiment were, in fact, predominantly from the opposite direction to what would be expected, or (ii) the ground slope had an effect.

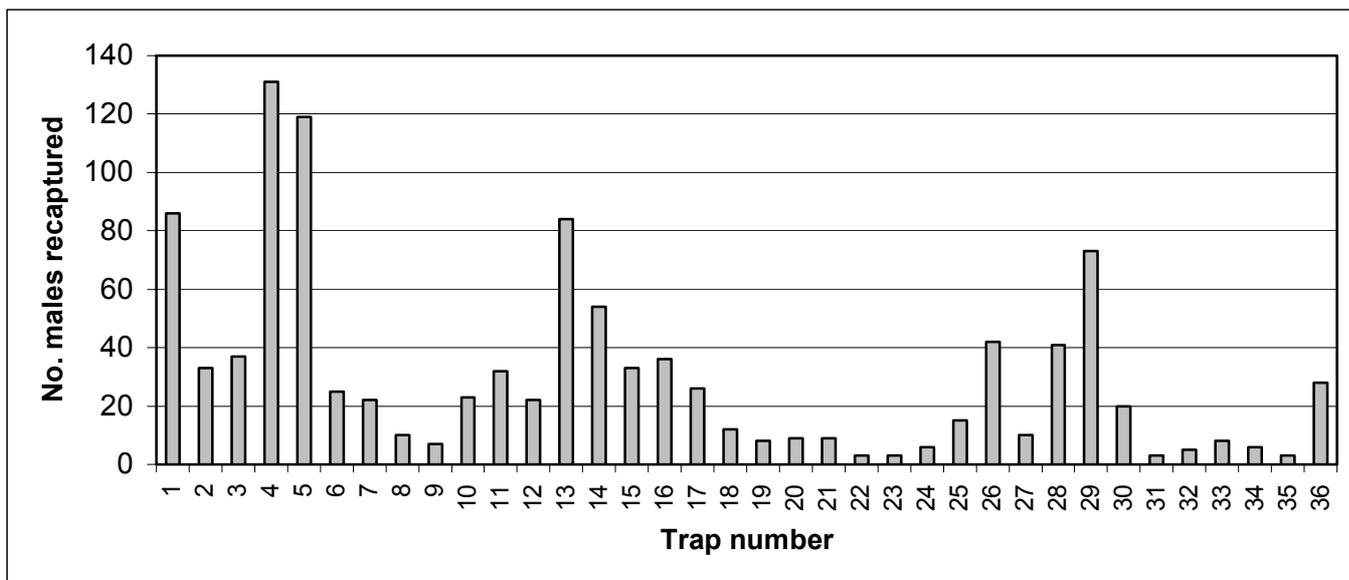


Fig. 3.4.5.7. Total catches of false codling moth males per trap.

Relatively large numbers of males were recaptured up to 100 m from the release point (Fig. 3.4.5.8). However, most of this apparent wide distribution was recorded following a night of strong south-westerly winds which possibly resulted in the moths being physically carried away further than they would normally have dispersed. Apart from this probable abnormality, the males dispersed quite well under the circumstances prevailing during the experiment, and shows that in practice release lanes can possibly be at least 50 m apart to ensure good distribution and overlap.

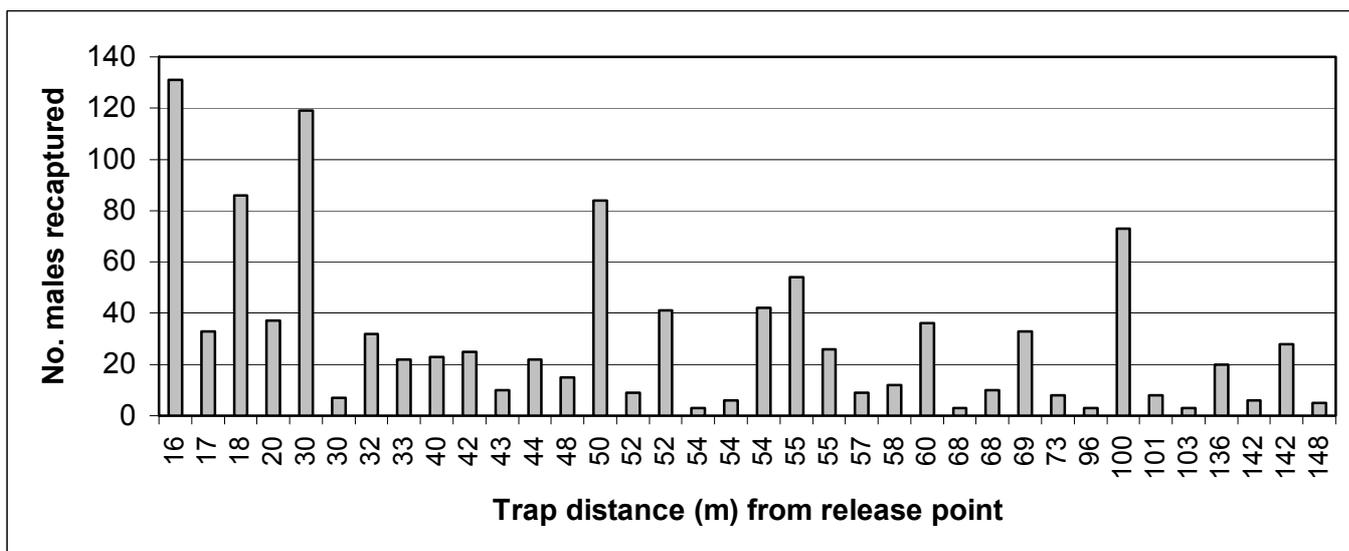


Fig. 3.4.5.8. Total catches of false codling moth males in 36 traps located at various distances from the point of release.

- **Lifespan of released males:** Males were recaptured for up to 13 days after release (Table 3.4.5.2, release 2). Trap catches were totally suppressed by bad weather during the night of 25 October, but recovered slightly during the next two nights. Male activity was extensive, and lasted much longer than recorded in mark and release experiments conducted during other times of the year. This could be an indication that the extended activity is linked to the time of year – the males seem to be active for longer periods when the ambient daily temperatures have not yet increased to summer levels.

A higher percentage of males were recaptured when released together with females. The reason for this is unsure, but could possibly be attributed to increased male sexual activity caused by the surplus of females.

Table 3.4.5.2. Number of false codling moth males recaptured when released with and without females.

Date	Males only released		Males plus females released		Total released males recaptured	Total feral males trapped
	Release 1 (12 Oct)	Release 2 (14 Oct)	Release 3 (17 Oct)	Release 4 (19 Oct)		
12 October						
13 October	1				1	109
14 October	0				0	42
15 October	24	0			24	56
16 October	0	0			0	27
17 October	0	0			0	2
18 October	7	3	0		10	35
19 October	32	35	15		82	95
20 October	29	74	223	28	354	145
21 October	1	10	43	91	145	34
22 October	3	23	113	114	253	58
23 October	1	14	50	80	145	71
24 October	2	3	12	29	46	51
25 October	0	0	0	0	0	0
26 October	0	1	0	0	1	32
27 October	0	4	8	11	23	130
Total	100	167	464	353	1084	887
% recaptured	10.9	18.2	35.2	26.7	-	-

The trapping pattern of the released males is virtually identical to that of the feral males also trapped. It probably reflects the influence of ambient conditions on male and/or female activity. Temperatures during the activity period of the moths were generally lower than 15°C (Fig. 3.4.5.9). The recapture rate was commensurately low (less than 35%), which suggest that there is a correlation between low temperature and a poor recapture rate.

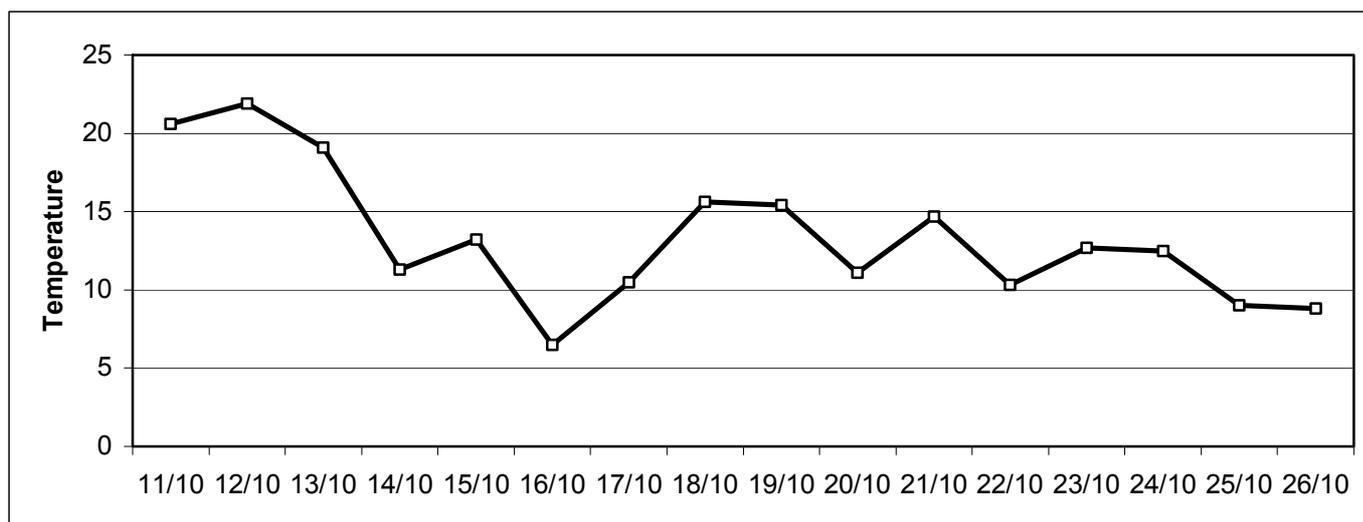


Fig. 3.4.5.9. Mean temperatures during the period 1800 to 0100 in a mark and release experiment on the farm Kweekkraal, Citrusdal, during October 2003.

In general the results from this experiment suggest that:

- Irradiated FCM can be released once a week. These findings are in contrast to those of the experiments described in Sections 3.4.5.2 and 3.4.5.3, which were conducted in February and November respectively and where males were only recaptured for two to three days after release. It is therefore possible that, with further refinement, a release routine can be developed to release males once or twice a week depending on

the time of year. Releasing moths only once a week will obviously be cheaper and more productive than having to, under current circumstances, travel 350 km twice a week to irradiate moths in Stellenbosch, and spending 12 hours in the process, instead of six.

- Release lanes of possibly 45 m apart may be appropriate to ensure good distribution of males. A lack of competition from the absence of irradiated males if “gaps” are created when release lanes are too far apart, will thus be avoided.

3.4.5.5 Additional assessment of irradiated FCM quality under orchard conditions

Opsomming

Verdere navorsing is uitgevoer om die gedrag van bestraalde VKM onder boordtoestande te bestudeer. Lokvalle en paringsplatforms wat met wyfies as lokaas toegerus was, is in 'n nawelboord versprei. Eenduisend mannetjies per behandeling is met 150 Gy en 200 Gy bestraal en eenmalig losgelaat. Minder mannetjies wat met 200 Gy bestraal was, is weer gevang, terwyl onbehandelde mannetjies en dié wat met 150 Gy bestraal was, naastenby dieselfde gevaar het. Bietjie minder bestraalde (beide 150 Gy en 200 Gy) as onbestraalde kontrolemannetjies is op die paringsplatforms aangetref. Mannetjies is tot agt dae na loslating weer gevang.

Introduction

This experiment was conducted to further assess irradiated male moth performance in the orchard in terms of their searching and mating capabilities.

Materials and methods

The experiment was conducted in October 2003 on the farm, Dankert, Citrusdal. Twelve Chempak delta traps and four mating arenas were distributed in a 4 x 4 pattern in a navel orange orchard one ha in size. One trap was placed in every sixth to eighth tree in every fourth row. The mating arenas' positions were allocated at random and were not changed for the duration of the experiment. Each arena consisted of a pie-dish shaped container, 500 mm in diameter, manufactured from double sided corrugated plastic sheets (“Correx”). The sides were 50 mm high and consisted of stiff, condensed polystyrene material, one mm thick, coated on the inside with Teflon tape to inhibit moth escape (Fig. 3.4.5.10). The inside of the arena walls were additionally lightly dusted with talcum powder to prevent the females from escaping.



Fig. 3.4.5.10. Three stacked mating arenas with wing-clipped FCM females.

Each arena was fixed horizontally to the top rung of a picking ladder, approximately 2,5 m high (Fig. 3.4.5.11). This set-up enabled easy access to the arena, and without unduly disturbing the moths inside.



Fig. 3.4.5.11. Fruit picking ladder with mating arena attached.

Each arena was supplied with 20 females, approximately 18 hours old. Each female was wing-clipped by clipping off half of one of the forewings. The mating arenas were inspected at 30-minute intervals from 1900 until no more matings were noticed. This usually occurred when the temperature had dropped to below ca. 14-15°C and visibly cold immobilized females and both irradiated and feral males were noticed sitting in the mating arenas without attempting to mate. All pairs noticed *in copulo* during inspections, were carefully removed so as not to disturb copulation, and placed in sample jars. The females were dissected the next day to confirm successful transfer of sperm.

Each trap was supplied with two virgin females approximately 18 hours old, and were replaced with moths of the same age every second night. Traps were inspected daily and trap liners with males were removed and replaced with clean liners. The liners with trapped males were transported to the laboratory and inspected under UV light.

Male moths were released by hand on all trees in each of three release lanes, 24 m apart (Fig. 3.4.5.12). A single release consisting of 1 000 irradiated males, approximately 18 hours old, was made per treatment. Two irradiation doses, viz. 150 Gy and 200 Gy, were compared. All males were lightly dusted with fluorescent powder before release, using a different colour for each treatment.

Temperature readings were taken every 30 minutes with the aid of a maximum/minimum thermometer placed in a tree.

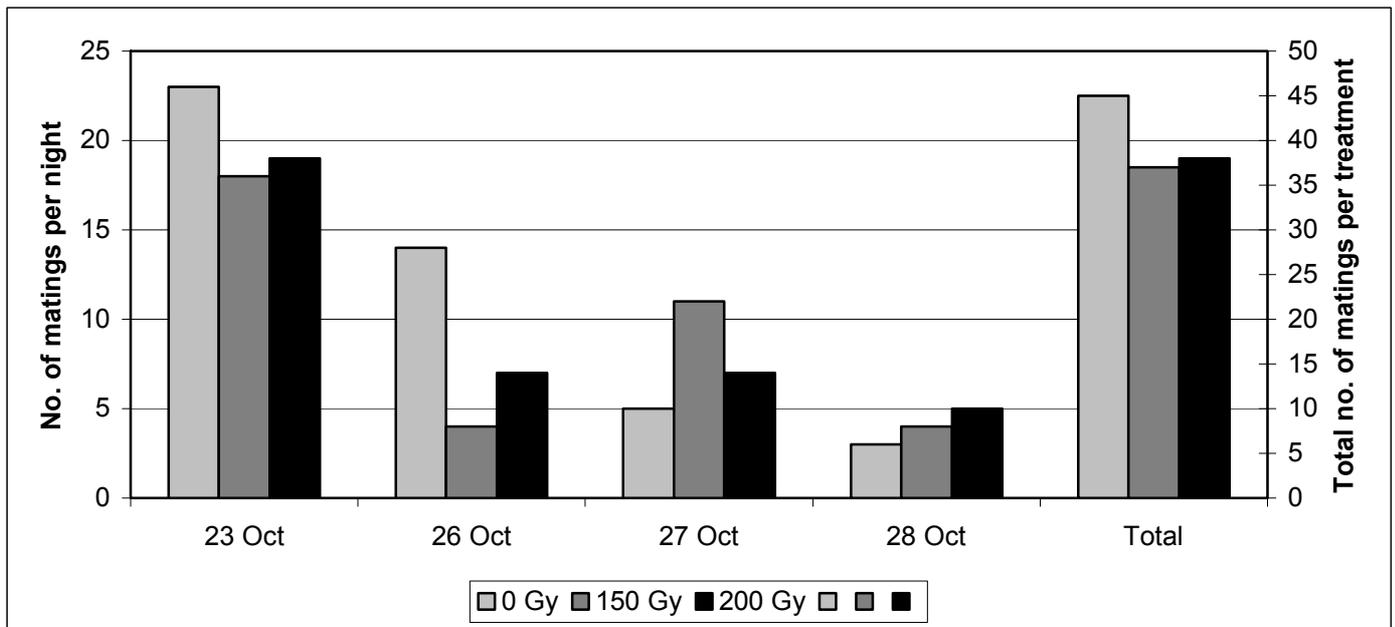


Fig. 3.4.5.13. Effect of gamma irradiation on the mating competitiveness of false codling moth males in an orchard.

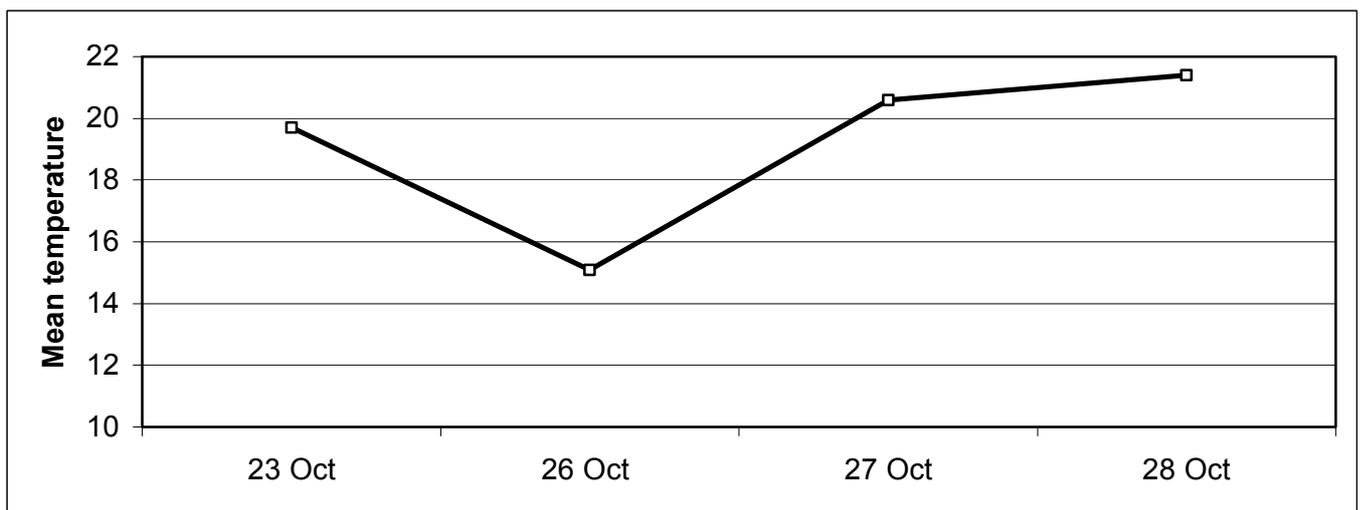


Fig. 3.4.5.14. Mean temperatures during the period 1700 to 2200 in a mark and release experiment on the farm Dankert, Citrusdal, during October, 2003.

Temperatures during the activity period of the moths varied from approximately 15°C to 21°C (Fig. 3.4.5.14). This correlated well with the relatively high male recapture rate.

The overall results from this experiment are similar to that from previous experiments and show that irradiated males can compete successfully with unirradiated males, despite a fairly consistent, but relatively minor, penalty paid by irradiation. This penalty will depend on the radiation dose and will be smaller with 150 Gy than with 200 Gy.

3.4.5.6 The dispersal distance and lifespan of irradiated FCM males

Opsomming

Die lewensduur en verspreiding van gammabestraalde VKM-mannetjies is vir 'n tweede keer ondersoek. Die meeste mannetjies is tot ongeveer 55 m vanaf die loslaatpunt gevang. Baie min mannetjies is verder van die loslaatpunt gevang, in teenstelling met die vorige proef. Mannetjies is slegs vir drie tot vyf dae na loslating gevang, wat heelwat korter as vantevore is. Dit is moontlik dat die warm somersdae (Februarie) veroorsaak dat die motte korter lewe – die vorige proef is in Oktober uitgevoer toe dit heelwat koeler was. Die data dui daarop dat bestraalde motte waarskynlik in die somer twee keer per week losgelaat sal moet

word om te verseker dat daar altyd lewenskragtige mannetjies in die boorde aanwesig is. Loslaatrye sal nagenoeg 50 m tot 60 m van mekaar geplaas word om te verseker dat daar 'n redelike mate van oorvleueling sal wees wanneer die motte na loslating versprei.

Introduction

This experiment was repeated (see Section 3.4.5.4) to gain more insight into the competitiveness of irradiated FCM males under orchard conditions.

Materials and methods

The experiment was conducted on the farm Kweekkraal, Citrusdal during March 2004 and differed in a few details from the previous experiment, as follows: (i) The experiment was conducted in March, (ii) Females used as bait in the traps were replaced every two days, (iii) Moths were released three times at intervals of four days and each release consisted of 935 males each, and (iv) males only were released – no females. The experiment lasted until no more moths were trapped, which was four days after the third and final release.

Results and discussion

- **Dispersal of release males:** The males in the experiment described in Section 3.4.5.4 were mainly recaptured in a north-easterly to south-easterly direction from the release point (Fig. 3.4.5.7). The catches in the current experiment were not as clearly directional in relation to the centre point, and most males were more evenly recaptured around the release point (Fig. 3.4.5.15). Again, data on wind strength and direction during the experiment was not available and it must be assumed that the wind was either less strong and/or not clearly predominant. This tendency is an advantage as the distribution of released males will be more even under such conditions and competitiveness will be enhanced.

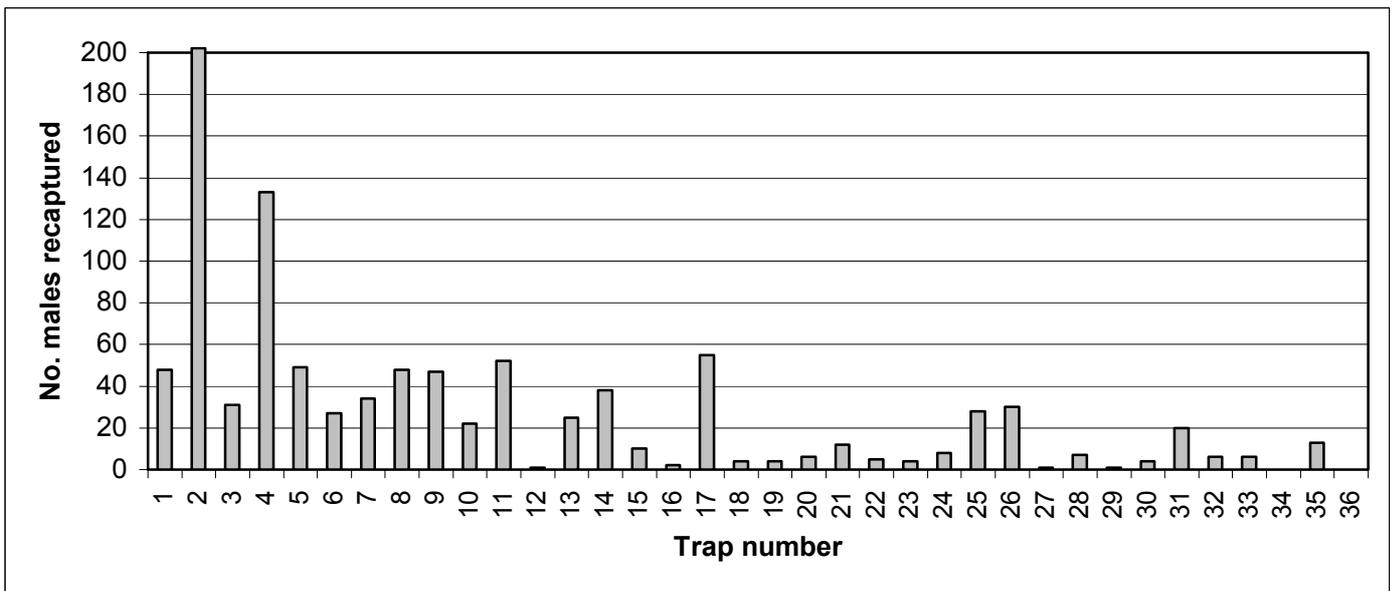


Fig. 3.4.5.15. Total catches of false codling moth males per trap.

Most males were recaptured up to ca. 55 m from the release point (Fig. 3.4.5.16). This trend is very similar to that in the previous experiment (Section 3.4.5.4) and confirms the currently held belief that release lanes need not be less than ca. 50 m apart.

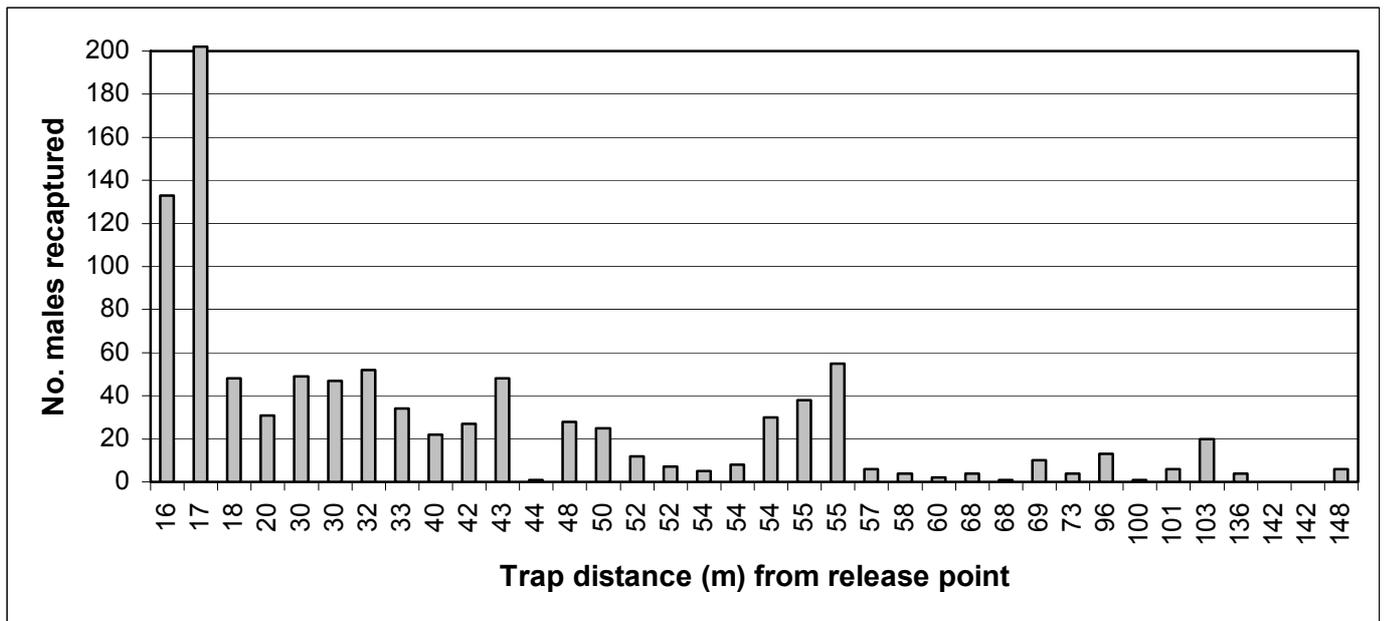


Fig. 3.4.5.16. Total catches of false codling moth males in 36 traps located at various distances from the point of release.

- Lifespan of released males:** Only a few males (1,9%) were recaptured from the first release which was again due to problems encountered with the transport of the cold immobilized moths to Stellenbosch for irradiation (Table 3.4.5.3). Up to 57% of males released subsequently were recaptured, which is more acceptable. The reason for these males apparently better tolerating cold transportation is unknown, but demonstrates the inherent variability resulting from the cooling technique as used. The temperature inside cool boxes has to be low enough to prevent excessive movement of the closely packed moths in the Petri dishes used for transport, irradiation and release, i.e. 4-5°C. Conversely, it should not be so low that the moths are detrimentally influenced. It was impossible to regulate temperature inside the cool boxes with the commercially available freezer blocks commonly used. It also proved difficult to create a repeatable set-up with regard to number of freezer blocks used, the amount of insulating material used to protect the moths from overexposure to cold, and also the influence of ambient conditions outside the cool boxes.

Table 3.4.5.3. Number of false codling moth males recaptured in a mark and release experiment on the farm Kweekkraal, Citrusdal, during March 2004.

Date	Male release (935 males /release)			Total released males recaptured	Total feral males trapped
	Release 1 (4 March)	Release 2 (8 March)	Release 3 (12 March)		
4 March					
5 March	0			0	112
6 March	2			2	286
7 March	7			8	90
8 March	6			5	76
9 March	1	82		83	116
10 March	0	272		272	83
11 March	1	15		16	119
12 March	1	41		42	137
13 March	0	22	125	147	125
14 March	0	0	388	388	88
15 March	0	1	20	21	43
16 March	0	0	0	0	28
Total	18	433	533	984	1303
% recaptured	1,9	46,3	57,0	-	-

Moths were recaptured for only three to five days after release – a much shorter period than in the previous similar experiment. From the experiments described up to now, the recapture rate seems to be quite variable even at approximately the same time of the year, and it does not seem likely that this factor has a strong influence on the survival of the males. The recapture success (% released males recaptured) also seems to be independent of the time of year, as any obvious correlation has not yet been noticed in the various experiments. However, when ambient temperatures during the period of maximum moth activity (1800-0100) are studied, it becomes clear that less males are captured when it is cold – and more specifically less than 14-15°C (Fig. 3.4.5.17).

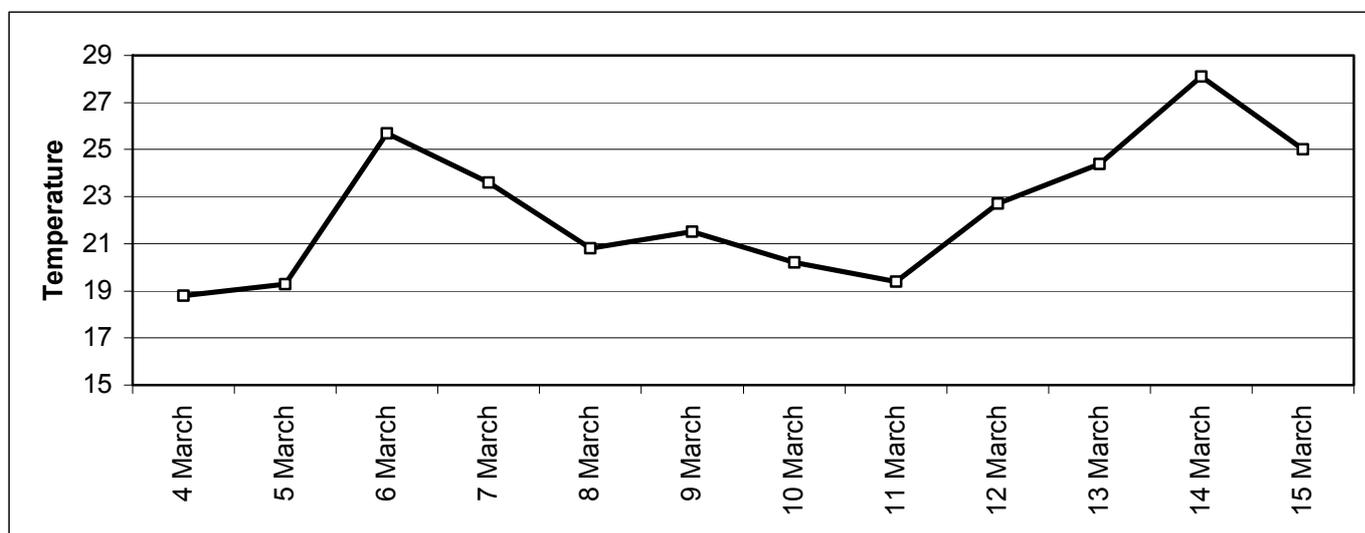


Fig. 3.4.5.17. Mean temperatures from 1800 to 0100 in a mark and release experiment at the farm Kweekkraal, Citrusdal during March, 2004.

3.4.5.7 Competitiveness of FCM males of different ages

Opsomming

Dit is belangrik dat die kwaliteit van motte behou word wat vanaf Citrusdal na Stellenbosch (en terug) vir bestraling vervoer moet word. Dit is dus belangrik vir besluitnemingsdoeleindes om te weet of motouderdom in ag geneem moet word wanneer motte oor 'n tydperk wat etlike dae lank kan wees, bymekaar gemaak word voor dat hulle behandel en losgelaat word.

VM-mannetjies van een tot vier dae oud, is met 200 Gy bestraal en in 'n nawelboord losgelaat. Meer as 40% van die losgelate motte is weer gevang, alhoewel vangste vir slegs twee dae lank geduur het. Daar was geen verskil in die vangspersentasie tussen die verskillende ouderdomme mannetjies nie, wat daarop dui dat motouderdom, binne die bestek van dié proef, nie 'n invloed gehad het nie. Indien nodig, sal mannetjies daarom vir minstens vier dae lank in 'n koelkamer gehou kan word totdat hulle losgelaat moet word.

Introduction

For lack of a gamma irradiation facility in Citrusdal, all moths used in SIT research, and eventually for semi-commercial or commercial SIR purposes, have to be irradiated at ARC-Infruitec, Stellenbosch. If moths have to be released twice a week, it would involve two round trips to Stellenbosch, with a resultant loss of time and increased cost. It is therefore important to know whether FCM males can be irradiated, cold stored and released, or alternatively, cold stored, irradiated and released. A preliminary experiment was conducted to investigate this aspect.

Materials and methods

An orchard experiment was conducted on the farm Kweekkraal, Citrusdal, during May 2004. Three treatments, each consisting of a number of FCM males of different ages, were compared. Emergence of male moths was planned to enable simultaneous release of all males, as follows:

- 148 Newly emerged males were placed in a coldroom on 30 April at 4-5°C for 24 hours, irradiated with 200 Gy, stored for three days and released in the orchard on 4 May.

- 211 Newly emerged males were placed in a coldroom on 1 May at 4-5°C for 24 hours, irradiated with 200 Gy, stored for two days and released in the orchard on 4 May.
- 352 Newly emerged males were placed in a coldroom on 3 May at 4-5°C for 24 hours, irradiated with 200 Gy and released in the orchard on 4 May.

The males were therefore respectively four, three and one day old. No unirradiated males were released. Males from the three treatments were dusted with differently coloured fluorescent powders to enable comparison.

The same site and trap lay-out as in Section 3.4.5.4 were used, except that only the two inner circles of traps, 12 in all, and up to 44 m from the centre release point, were used. Lorelei FCM pheromone was used as attractant in delta traps.

All moths were transported to Stellenbosch inside a cool box containing three freezer blocks. Two blocks were placed at the bottom, and one at the top, with the Petri dishes containing the moths in between. The Petri dishes and freezer blocks were separated from each other by 10 layers of newspaper and one layer of “bubble wrap” plastic sheet. A Hobo datalogger was placed inside an otherwise empty Petri dish to record temperatures in transit. After irradiation the males were transported back to Citrusdal in larger containers under ambient temperature and light conditions.

Results and discussion

The age of the males had no effect on their ability to locate the traps and 42% to 44% of the released males were recaptured in the various treatments (Fig. 3.4.5.18).

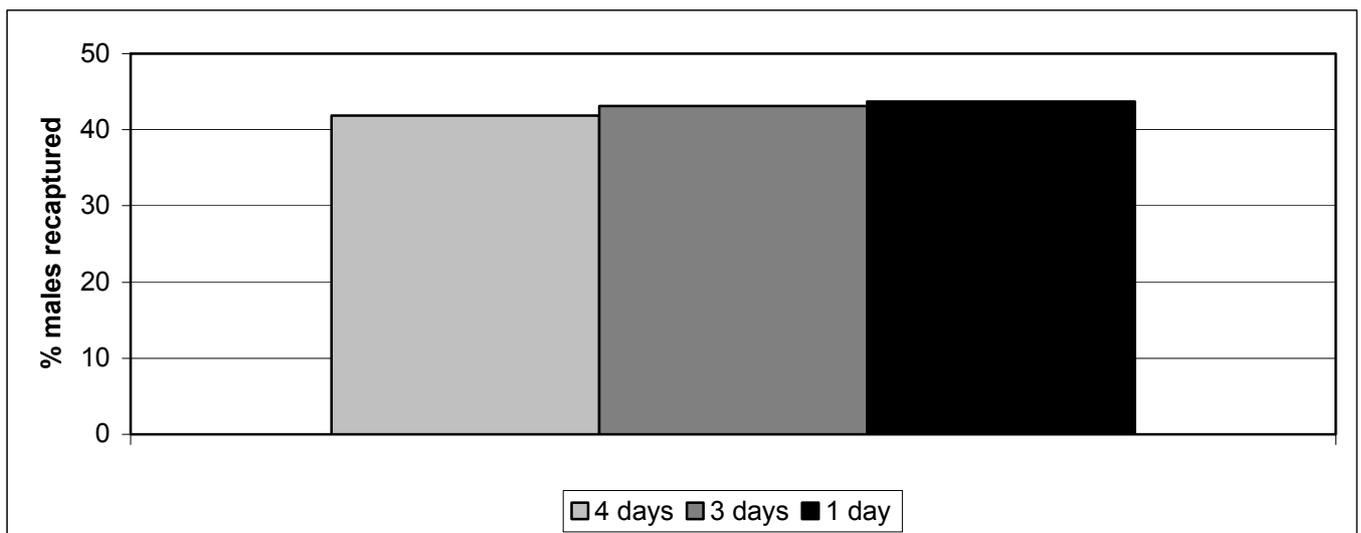


Fig. 3.4.5.18. The influence of age on the ability of male false codling moths to locate traps in an orchard on the farm Kweekkraal, Citrusdal, during May 2004.

Males in all treatments were recaptured for two days only, after which the trap rate decreased sharply (Fig. 3.4.5.19). A temperature study showed that temperatures during the period of activity (1800–0100) were consistently higher than 14-15°C (Fig. 3.4.5.20). This is an indication that ambient nocturnal temperature was not an inhibiting factor.

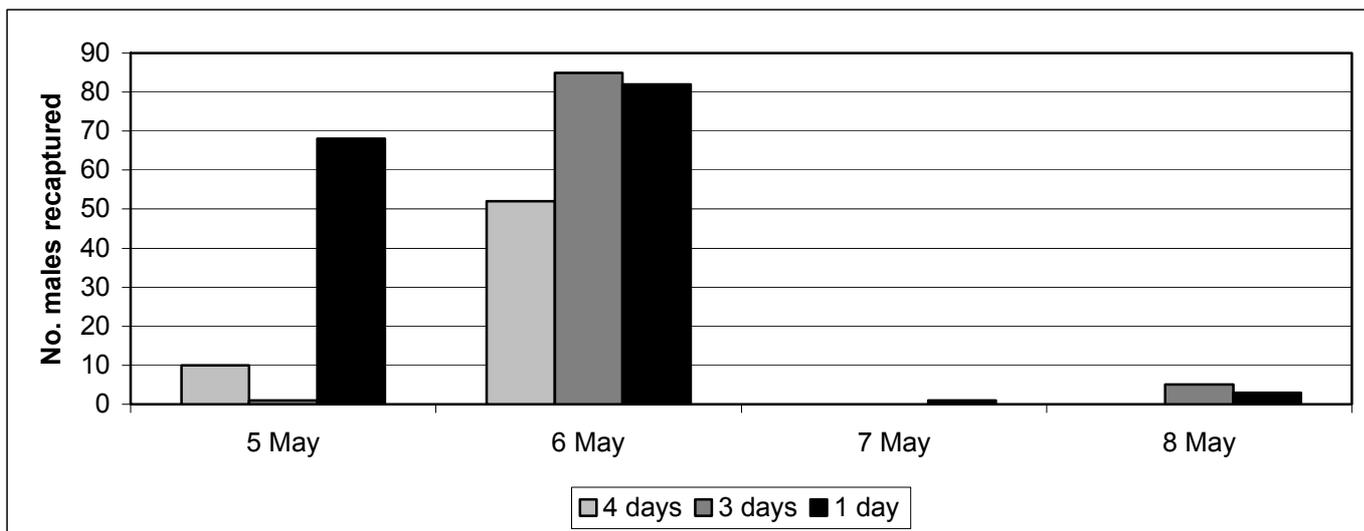


Fig. 3.4.5.19. Recapture rate of male false codling moth of different ages in an orchard on the farm Kweekkraal, Citrusdal, during May 2004.

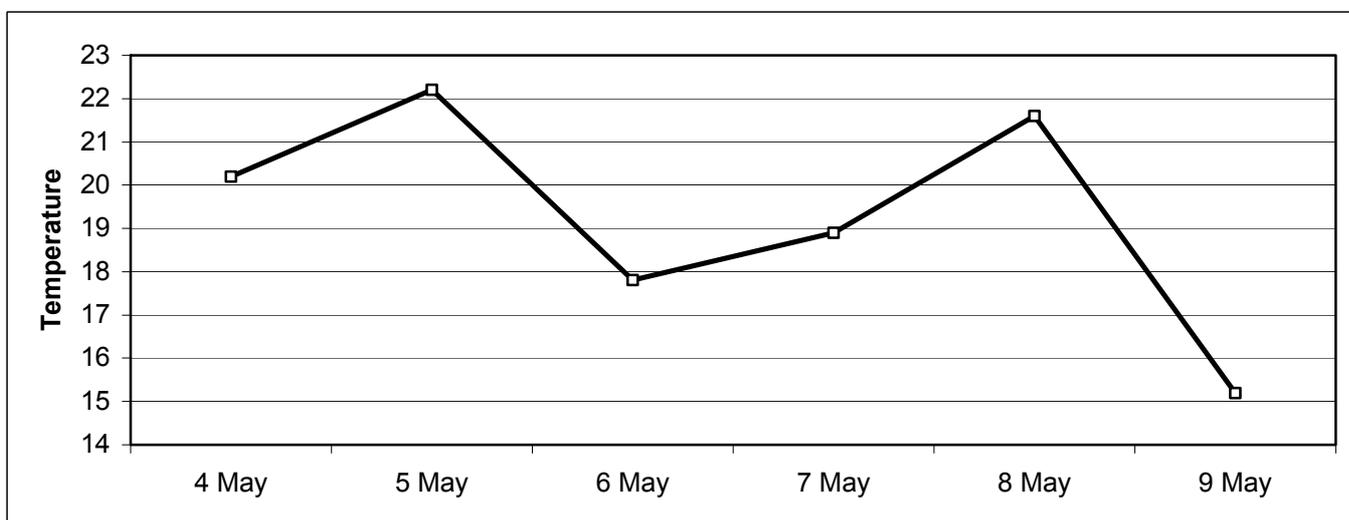


Fig. 3.4.5.20. Mean temperatures from 1800 to 0100 in a mark and release experiment on the farm Kweekkraal, Citrusdal, during May 2004.

Temperatures recorded inside the cool box used to transport the males, demonstrated that the freezer blocks could potentially reduce the ambient temperature inside the cool box to, in this particular instance, close to 0°C (Table 3.4.5.4).

Table 3.4.5.4. Temperatures recorded inside a cool box cooled with three freezer blocks used to transport false codling moths.

Time	Temperature
0900	23.2
0915	19.8
0930	14.9
0945	11.4
1000	9.0
1015	7.4
1030	6.2
1045	5.4
1100	5.8

1115	2.9
1130	1.2
1145	1.2
1200	1.2
1215	0.7
1230	0.7
1245	0.3
1300	0.3
1315	0.3
1330	0.3
1345	0.7
1400	1.2
1415	1.2
1430	1.6
1445	1.6
1500	1.6
1515	1.6
1530	1.6

The cooling capabilities of the cool box as used, was inadequate in almost all aspects:

(i) The contents of the cool box only reached a temperature that would adequately immobilize the moths after 60 minutes (<10°C). During this time tightly packed moths would have been very active and they would have lost most of their scales, and possibly other appendages as well.

(ii) Temperatures that would keep the moths immobile to a large degree, were only maintained for another 60 minutes. Disregarding problem (i), two hours are sufficient under normal circumstances to reach Stellenbosch from Citrusdal. If males only are transported, they can then be irradiated, transferred to a bigger container and then transported back under ambient conditions. If large numbers of moths are required, separating them into different genders is impractical and they have to be kept cold immobilized to prevent mating.

(iii) If moths have to be cold immobilized for longer than two hours, they will be exposed to cold damage. Temperatures in the cool box dropped to below 3°C after two hours and almost reached 0°C. These temperatures are potentially detrimental to the moths if maintained for any length of time (this statement is based on experience only; specific data on what this temperature is and the time involved has not been investigated fully).

This method of moth transportation when cold immobilized is therefore inadequate, and a different system that will deliver consistent results will have to be developed.

3.4.5.8 Colouring false codling moth for mark and release experiments

Opsomming

VKM wat vir proef- of SIL-doeleindes losgelaat word, moet gemerk wees sodat hulle van wilde motte in lokvalle onderskei kan word om die doeltreffendheid van SIL vas te stel. 'n Inwendige kleurstof, Calco Oil Red, is daarom met uitwendige kleurstowwe wat onder ultravioletlig fluoriseer, vergelyk. Mannetjies wat van 1-2 dae tot 4-5 dae ouderdom gewissel het, is na bestraling met 200 Gy losgelaat. Die hervangspersentasie was baie swak – waarskynlik as gevolg van lae nagtemperatuur (Julie). Daar was egter onverklaarbare wisseling tussen die verskillende ouderdomme motte wat niks met ouderdom of behandeling te doen gehad het nie. Die hoogste persentasie mannetjies wat weer gevang was, was 1-2 dae oud. Mannetjies is vir tot sewe dae na loslating gevang, terwyl meer uitwendig as inwendig gekleurde motte gevang is.

Introduction

A prerequisite for mark and release experiments is the ability to differentiate between moths from different treatments. Preliminary experiments to test internal dyes such as Calco Oil Red have already been reported

on (CRI Annual Report for 2003). This experiment was conducted to further investigate any possible detrimental effect that internal dyeing may have on moths. The effect of moth age on recapture success was simultaneously studied.

Materials and methods

The experiment was conducted on the farm Kweekkraal during July 2004. The same experimental site, 12 traps and lay-out as before were used (Section 3.4.5.7).

Male FCM were prepared using the same technique as before (Section 3.4.5.7). They were accumulated as the pupae eclosed and kept in a coldroom at 4-5°C. The moths were respectively 4-5 days, 3 days and 1-2 days old and were irradiated with 200 Gy on the day of release. Two batches of males were used for each age group, viz. moths that were (i) internally dyed with Calco Oil Red (reared on a diet containing 0,035 g Calco Oil Red per culture jar) and (ii) externally dusted with fluorescent powder. The internally dyed moths from the first two age groups were additionally dusted with differently coloured fluorescent powders to enable differentiation between the three internally dyed age groups.

Two hundred to 400 males per treatment were released by hand at the centre release point on 5 July. Traps were inspected daily and the trap liners removed and replaced. The removed liners were taken to the laboratory to inspect trapped moths under UV light. All moths were then crushed to inspect for any discoloration due to Calco dye.

A Hobo datalogger was placed in a trap to record ambient temperatures.

Results and discussion

- Lifespan of release males:** All ages of males were recaptured for up to 7 days after release at which point the catches decreased markedly and the experiment was terminated (Fig. 3.4.5.21). This is a relatively long period and may be attributable to the cooler conditions prevailing at that time of the year (maximum daily temperatures did not exceed 26°C during the experiment).

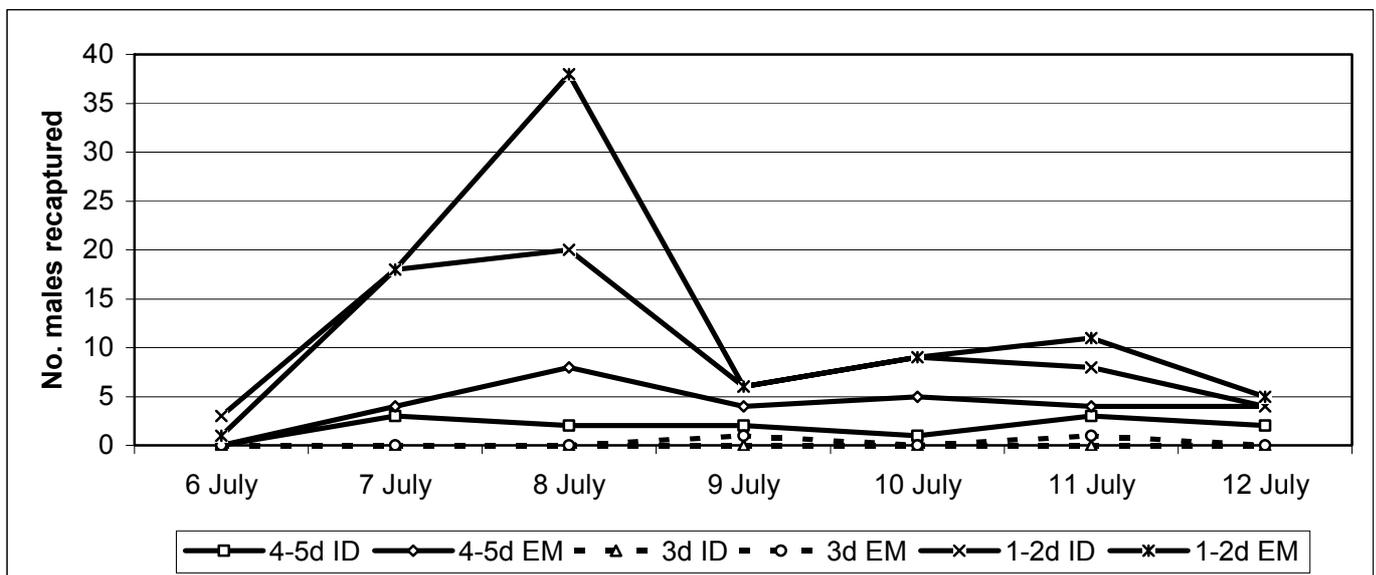


Fig. 3.4.5.21. Number of male false codling moths in different age groups (4-5d, etc.) and internally dyed (ID) or externally marked (EM), recaptured in an orchard on the farm Kweekkraal, Citrusdal during July 2004.

- Recapture success:** In general the percentage of males recaptured after release was very low (Table 3.4.5.5).

Table 3.4.5.5. Percentage males recaptured in an orchard on the farm Kweekkraal, Citrusdal, during July 2004.

Age	% Males recaptured	
	Internally dyed	Externally marked
4-5 days	3,9	13,9
3 days	0,0	1,0
1-2 days	21,5	22,7

There are differences between the various treatments, viz.:

- More younger than older males were recaptured.
- More externally marked than internally dyed males were recaptured.

There are, however, discrepancies – the exceptionally poor performance of the 3 day old males cannot be explained as they were treated the same as the other moths. Apart from that, the low catches prevent firm conclusions. All the nights were also quite cold during the main activity period of the moths (1800-0100). The mean minimum temperatures were much lower than what is accepted to be their bottom activity threshold (Fig. 3.4.5.22), which may explain the generally poor recapture.

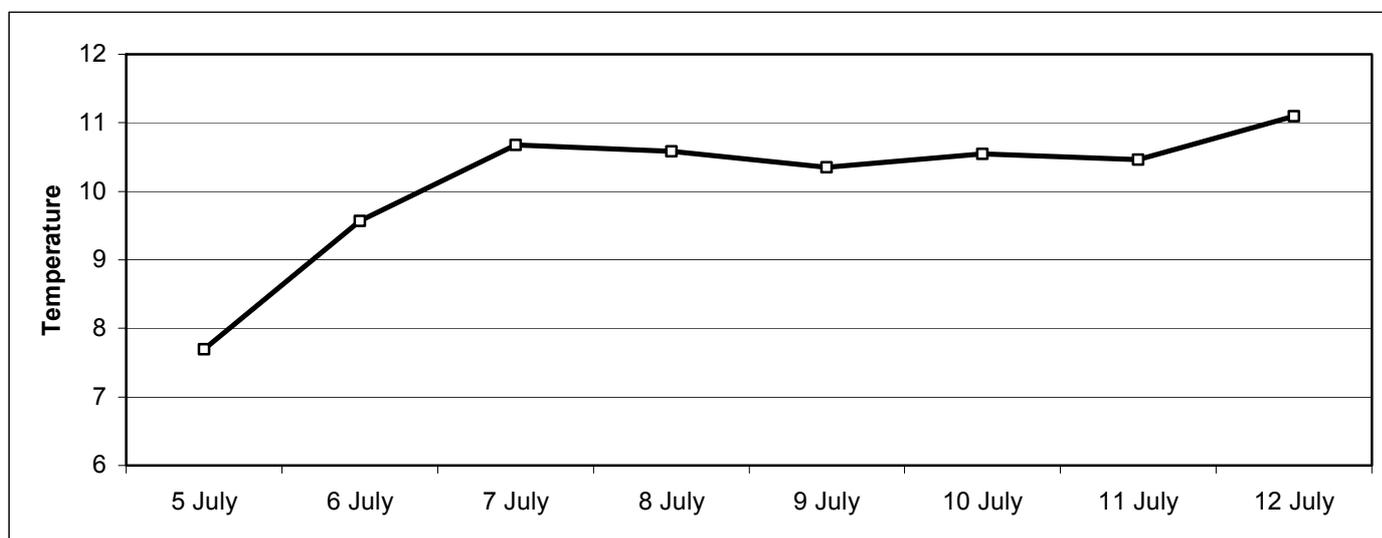


Fig. 3.4.5.22. Mean temperatures from 1800 to 0100 in a mark and release experiment on the farm Kweekkraal, Citrusdal, during July 2004.

3.4.5.9 The effect of cold storage on the competitiveness of false codling moth males

Opsomming

Die naaste gammabestralingseenheid aan Citrusdal, wat vir die sterilisasie van VKM gebruik kan word, is in Stellenbosch. Motte moet daarom deur afkoeling vir die rit geïmmobiliseer word sodat hulle in goeie toestand kan bly. Wisselvallige resultate is vantevore met verskeie koelkiste behaal wat die motte benadeel het. 'n Studie is derhalwe uitgevoer om die invloed van verskeie kombinasies van ouderdom, verkoeling en bestraling op VKM te bepaal. Daar is vasgestel dat motte wat vir ses dae in 'n koelkamer by 2-3°C gehou word en dan bestraal word, swak in die boord presteer. Dit was ook van toepassing op motte wat drie dae lank verkoel is, gammabestraal is, weer drie dae verkoel is en toe losgelaat is. Kontrolemotte wat dieselfde behandeling ondergaan het, die bestraling uitgesluit, het ook swak gevaar. Die enigste motte wat hul lewenskrag behou het, was motte wat slegs 1-3 dae oud was, bestraal is en toe losgelaat is.

'n Gewysigde koelkiststelsel, wat uit 'n koelkis-in-'n-koelkis bestaan, is getoets en toon potensiaal vir verdere ontwikkeling.

Introduction

The biggest problems facing a pilot project for the release of sterile false codling moth on a commercial scale, are (i) the rearing of sufficient numbers of moths for mass release and (ii) the transportation of moths

to and from the irradiation facility in Stellenbosch. The ability of moths to withstand cold immobilization for several days, which is either preceded or succeeded by irradiation, is a major concern. This is particularly as FCM's ability to tolerate cold can mean the difference between travelling to Stellenbosch either 40 or 80 times during the 10 month duration of the project. An experiment was therefore conducted to study the ability of FCM to react normally upon release in an orchard after cold immobilization lasting up to six days long.

Materials and methods

The experiment was conducted in a navel orange orchard on the farm Kweekkraal, Citrusdal, during September 2004. The same experimental site, traps and lay-out as used, *inter alia*, in Section 3.4.5.8, were used.

Four thousand FCM pupae were sorted into genders. The females were discarded and the male pupae were incubated at 26°C for development and eclosion. Male moths were collected over a six-day period and divided into four groups of 280 moths each. Moths were placed into a cold room at 2-3°C on the day of their emergence. The collection and treatment schedules were as follows (Table 3.4.5.6):

Table 3.4.5.6. Different schedules used to study the effect of cold immobilization and irradiation on the subsequent orchard performance of false codling moth males.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1 (Control)	Emergence; Kept in cold room	Emergence; Kept in cold room	Emergence; Kept in cold room	Kept in cold room	Kept in cold room	Kept in cold room	Released
2	Emergence; Kept in cold room	Emergence; Kept in cold room	Emergence; Kept in cold room	Kept in cold room	Kept in cold room	Kept in cold room	Irradiated and released
3	Emergence; Kept in cold room	Emergence; Kept in cold room	Emergence; Irradiate males from day 1-3	Kept in cold room	Kept in cold room	Kept in cold room	Released
4	-	-	-	Emergence; Kept in cold room	Emergence; Kept in cold room	Emergence; Kept in cold room	Irradiated and released

Moths in treatment 1 (unirradiated control) and treatments 2 and 3 were therefore 4 to 6 days old at the time of release. Moths from treatment 4 were 1 to 3 days old. Before release, the moths were dusted with differently coloured fluorescent powders to enable differentiation. The moths were released at the centre release point and traps were inspected daily. Counts of trapped males were conducted in the laboratory under UV light.

In an attempt to circumvent the variably detrimental effect of the freezer blocks in the coolbox containing the moths in transit, a different cooling system was tested. A small (280 mm x 210 mm x 240 mm) and large (600 mm x 340 mm x 370 mm) Coleman coolbox were pre-cooled at 2-3°C in the coldroom. Petri dishes containing the moths (also immobilized in the coldroom) were placed into the small coolbox. The small coolbox was placed into the large coolbox, together with two (first irradiation) and three (second irradiation) freezer blocks respectively. With this layout the moths in the small coolbox were protected from direct cold. The freezer blocks were intended to maintain the temperature of the smaller coolbox and its contents from the outside. A Hobo datalogger in a Petri dish was placed in each of the small and large coolboxes.

Results and discussion

Observations at the time of release showed that the moths were variably active in the different treatments (Table 3.4.5.7). Moths were mainly recaptured during the second to fourth nights following release, with catches in all treatments peaking on the third night. The recapture percentage varied substantially between treatments (Table 3.4.5.7).

Table 3.4.5.7. Activity and recapture rate of older and younger false codling moth males irradiated and unirradiated in an orchard on the farm Kweekkraal, Citrusdal, during September 2004.

Treatment	Activity upon release	% male recapture
1) Control: Moths emerged over three days, kept in cold for another three days and released; unirradiated	Relatively inactive; some moths dropping to the ground upon release	21.8*
2) Moths emerged over three days, kept in cold for another three days, irradiated and released.	Relatively inactive; some moths dropping to the ground upon release	2.9
3) Moths emerged over three days, irradiated, kept for another three days and released.	Very inactive; almost all moths dropping to the ground upon release	0.7
4) Moths emerged over last three days, irradiated and released.	Very active and flying off upon release	21.8*

*Unable to distinguish between these two treatments due to similarity in fluorescent powders used (Signal Green and Saturn Yellow: See Section 3.4.5.1). The two treatments were therefore added together.

- Effect of moth age:** The comparison of fluorescent powders described in Section 3.4.5.1, was conducted in response to the inability to distinguish between the two fluorescent powders, Signal Green and Saturn Yellow, used in this experiment for the control (treatment 1, 4-6 day old moths) and treatment 4 (1-3 day old moths). It is therefore largely a matter of conjecture whether most moths trapped were from treatments 1 or 4. The relative inactivity of the older control moths (as well as the moths from treatments 2 and 3) when released, was very conspicuous and in direct contrast to the intense activity of the younger moths, and this suggests that almost all trapped males were from treatment 1. This explanation is also supported by the fact that the recapture rate in treatments 2 and 3 was very poor, suggesting that age was an important limiting factor. Although the difference is not great, the moths that were kept in the coldroom for three days after irradiation, were in worse shape, both at the time of release and from a recapture point of view. This suggests that moth viability deteriorates with age after being irradiated. Whether age has an independent effect, and whether low temperature has an additional influence, is not known at this stage. Another factor still needing investigation is the holding temperature of the moths. This issue has not been adequately researched, but observations suggest that 2-3°C may be more detrimental than 4-5°C.

- Performance of cool boxes:** Readings of ambient temperature during the two days that FCM were transported to Stellenbosch for irradiation, and back to Citrusdal, were not collected. However, it was recorded that the second day was significantly hotter than the first day. This is reflected in the inability of the large cool box to keep the inside temperature down to similar levels as during the first day (Fig. 3.4.5.23 and Fig. 3.4.5.24). This failure is mirrored in the smaller cool box where the temperatures varied according to the environment in the large cool box.

Apart from this problem, the adapted two-box system seemed to function well. Temperatures inside the small cool box were kept at or above 5°C on both days, despite the fact that temperatures inside the large cool box were reduced to below 0°C for a short time on the first day. The insulation properties of the cool boxes are probably inadequate for what is required of them, and this problem will have to be investigated.

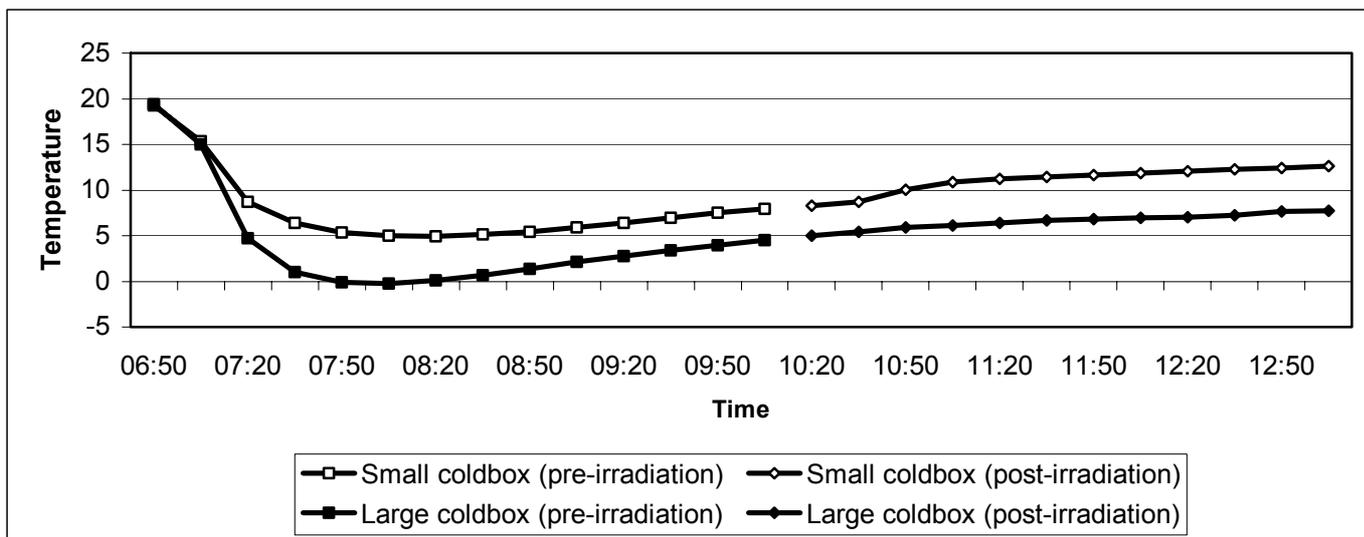


Fig. 3.4.5.23. Temperature fluctuations in cool boxes used to transport false codling moth to Stellenbosch and back to Citrusdal (cool day).

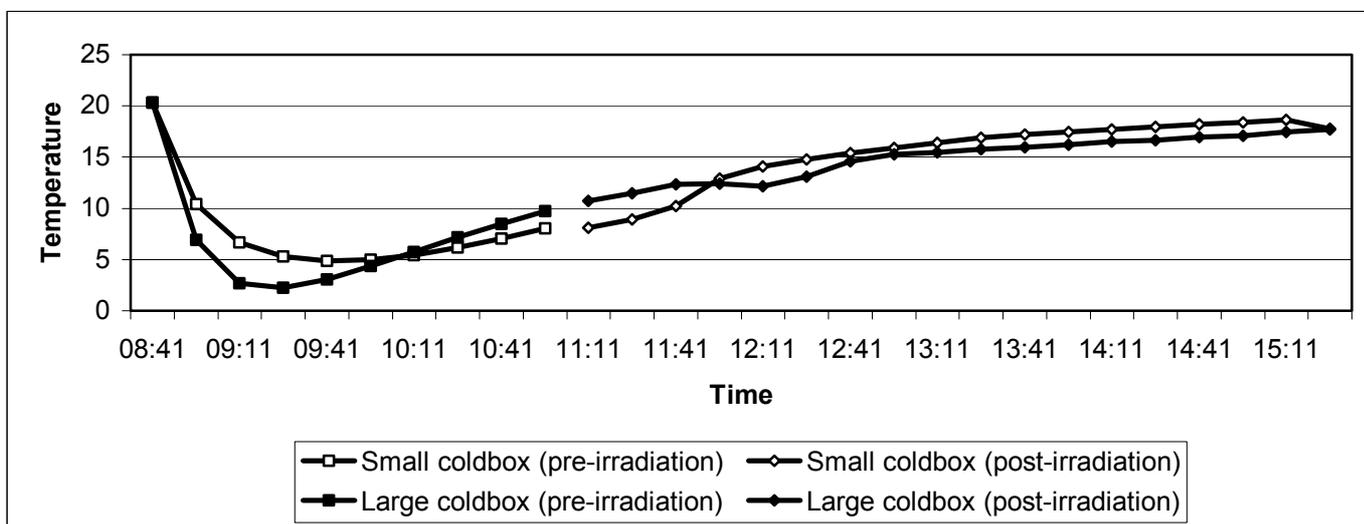


Fig. 3.4.5.24. Temperature fluctuations in cool boxes used to transport false codling moth to Stellenbosch and back to Citrusdal (hot day).

- Effect of ambient temperature on recapture success:** The catches in this experiment do not follow a similar pattern to previous experiments described in this report. The experiment was conducted in September, and the ambient minimum temperatures were relatively low, although slightly above the lower threshold for activity (Fig. 3.4.5.25). Despite this, the recapture percentages were low. This is, however, ascribed to the general influence of age and possibly, temperature, already discussed.

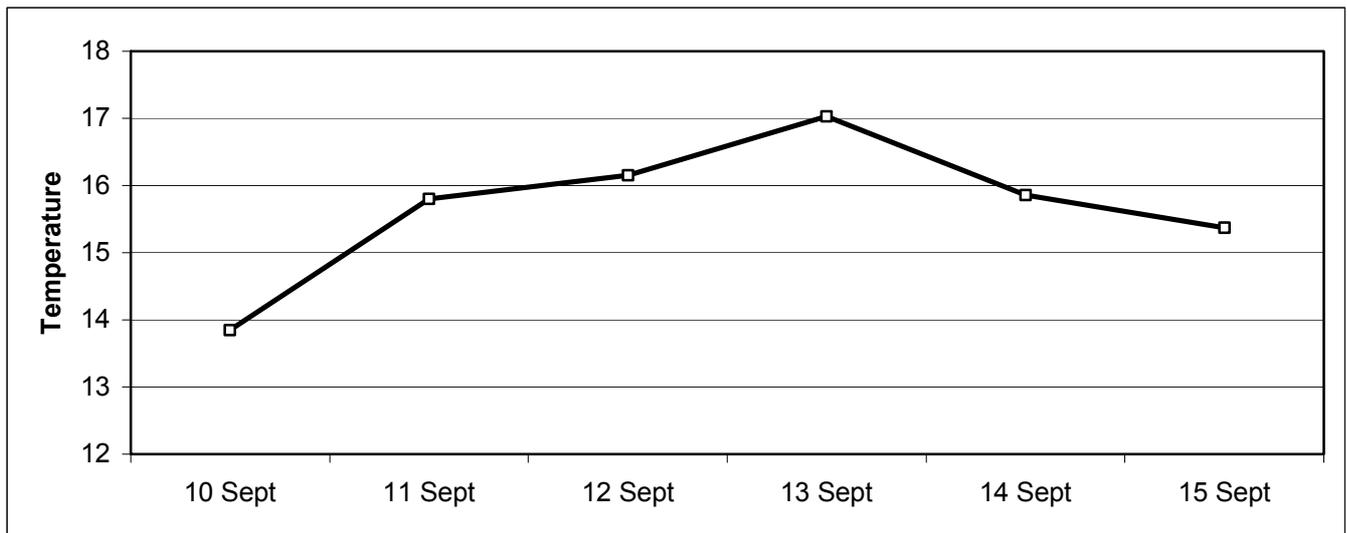


Fig. 3.4.5.25. Mean temperatures from 1800 to 0100 in a mark and release experiment on the farm Kweekkraal, Citrusdal, during September 2004.

3.4.5.10 The augmentative effect of egg parasitoids in a sterile insect release program

Opsomming

Dit is moontlik dat die doeltreffendheid van Steriele Insekloslatings van VKM aanvanklik deur massaloslatinge van die eierparasitoïed, *Trichogrammatoidea cryptophlebiae*, verbeter sal kan word. Dit was die doel van dié studie om onder beheerde toestande vas te stel of loslatings van die parasitoïed enige kommersiële voordeel inhou. 'n Hokproef is derhalwe uitgevoer waarin bestraalde motte en eierparasitoïede op ingehokte nawelbome met vrugte, losgelaat is.

Eierlegging deur onbestraalde en bestraalde motte was relatief swak. Parasitisme van dié eiers deur die parasitoïede was eweneens swak en het geen patroon gevolg in terme van die verskillende behandelingsalternatiewe wat vergelyk was nie. Die redes vir die onbevredigende resultate is onbekend en die proef sal gedurende 2005 herhaal word.

Introduction

It is possible that the efficacy of an SIT program may be improved upon in the initial stages while the natural populations of FCM in the orchards are still being reduced with SI releases. For codling moth, the combined release of sterile insects and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) egg parasitoids was first suggested by Nagy (1973), and experiments by Bloem *et al.* (1998) demonstrated that an additive suppressive effect can be realized when sterile moths are released together with *Trichogramma platneri* Nagarkatti inside field-cages when compared with cages receiving moths or parasitoids only.

Research was previously conducted to study sterile FCM/egg parasitoid interactions (2002 CRI Annual Report). The results suggested that *Trichogrammatoidea cryptophlebiae* would accept, successfully develop in, and emerge from FCM eggs laid by the different crosses that would theoretically be present in the orchard under a sterile insect release program for FCM (Normal ♀ x Irradiated ♂, Irradiated ♀ x Normal ♂, Irradiated ♀ x Irradiated ♂). These data demonstrated that further evaluations combining releases of irradiated moths and parasitoids were warranted and a field cage experiment was subsequently conducted.

Materials and methods

The experiment was conducted on the farm Boontjiesrivier, Citrusdal, during April 2004. An orchard was used with 7-yr old bearing Lina navel orange trees, on average 1,8 m in height. A framework consisting of wooden poles and steel beams was erected over 15 adjoining trees. A cage, 2,6 m x 2,6 m x 2,6 m, constructed from nylon material, with a mesh size of 125 µm, was suspended over each tree and the skirt buried 300 mm deep in the ground. A 1,2 m long zipper in one side gave access to the cage. One tree in a cage was regarded as an experimental unit. Four treatments were compared, consisting of four replicates each, except for the control, which had three replicates only. One replicate per treatment was randomly allocated in each of four blocks (three blocks for the control).

The trees were caged on 19 April 2004, approximately 10 weeks before harvest. Fifty fruit per tree were selected at random, inspected *in situ* and marked with a coloured sticker. All other fruit were removed and discarded.

Virgin female and male moths, 12-18 hours old, were collected in the insectary. A number of these were irradiated with 200 Gy on 22 April 2004. The two genders were then released on opposite sides of each tree on the same day. A 5 ml pill vial, containing 50-70 FCM eggs deposited on wax paper and parasitized with *T. cryptophlebiae* was tied to a convenient branch at head height inside the outer leaf canopy on each tree, except for the control, which received no parasitoids. The various treatments are described in Table 3.4.5.8. The parasitoids started emerging on the day of placement. One batch each of parasitoids was released on 24 and 25 April. The cages were sealed and only opened when all fruit were picked on 28 April. They were transported to the laboratory where they were individually placed into 500 ml clear plastic tubs without lids. The fruit were kept at 26°C and inspected on 10 May 2004. All eggs deposited per fruit were inspected for hatch and parasitism, and the number of larval scars per fruit was recorded.

Table 3.4.5.8. Numbers of false codling moths and parasitoids released in a field cage experiment on the farm Boontjiesrivier, Citrusdal, during April 2004.

Treatment	Number of moth pairs released per tree		Mean number of egg parasitoids released per tree
	unirradiated	irradiated	
1	4	0	0
2	4	0	77
3	4	40	0
4	4	40	68

A datalogger was placed in the outer leaf canopy of one data tree and temperatures were recorded for the duration of the experiment.

Results and discussion

No results of any importance were collected. Egg laying in the experiment was poor and the total numbers of eggs deposited on fruit varied between certain treatments that should at least theoretically have received approximately the same number of eggs, e.g. treatments 1 and 2 (4 pairs of moths per tree) and treatments 3 and 4 (44 pairs of moths) (Table 3.4.5.9). There was also no discernable pattern in egg laying between moths that had been irradiated or not.

Table 3.4.5.9. Numbers of eggs parasitized by *Trichogrammatoidea cryptophlebiae* and numbers of fruit infested with false codling moth larvae.

	Treatment			
	(1) 4 prs of moths; unirradiated	(2) 4 prs of moths; unirradiated + parasitoids	(3) 44 prs of moths; 40 prs irradiated	(4) 44 prs of moths; 40 prs irradiated + parasitoids
No. of fruit/treatment	150	200	200	200
No. of fruit with >0 eggs/fruit	50	24	33	51
Total eggs deposited	154	68	110	158
% Eggs parasitized	0.0	4.4	0.0	28.5
% Fruit infested	30.7	8.5	13.0	13.0

Similarly, the success of parasitization varied in treatments 2 and 4 for no apparent reason. The success with which fruit should have been additionally protected by the parasitoids, apart from the effect of irradiation, is also not reflected in the percentage of fruit infested with FCM larvae (13% in treatments 3 and 4, both irradiated but respectively without and with parasitoids).

The reason for the lack of moth and parasitoid performance is unclear. The experiment was conducted in April, almost a month earlier than a previous field cage experiment that provided excellent results during May

2003. It was relatively cold in the cages during the nights and ambient temperatures of ca. 11°C to 18°C were recorded, which varied around the lower threshold for moth activity (Fig. 3.4.5.26). It is not impossible that cold suppressed moth activity. However, it is unlikely as feral moths are also exposed to similar temperatures and commercial damage is often caused during this time of the season, and even later.

The procedure that was used for parasitoid application during the experiment deviated from what the data in Section 3.4.5.13 suggested. In that particular experiment the parasitoids' lifespan was probably at most 72 hours, while moths in this experiment were allowed to deposit eggs for up to 96 hours after the second parasitoid release. A number of eggs could therefore potentially have been deposited after all parasitoids have died. The number of eggs the 44 pairs of moths would have been able to deposit, would also have potentially exceeded the average parasitization potential by at least 2:1. As it was, not many eggs were laid by the females, and parasitization was so low in general that both factors were unimportant. However, when the experiment is repeated, the numbers of parasitoids released into the cages will have to be calculated more carefully to match the egg laying pattern and potential of the FCM females.

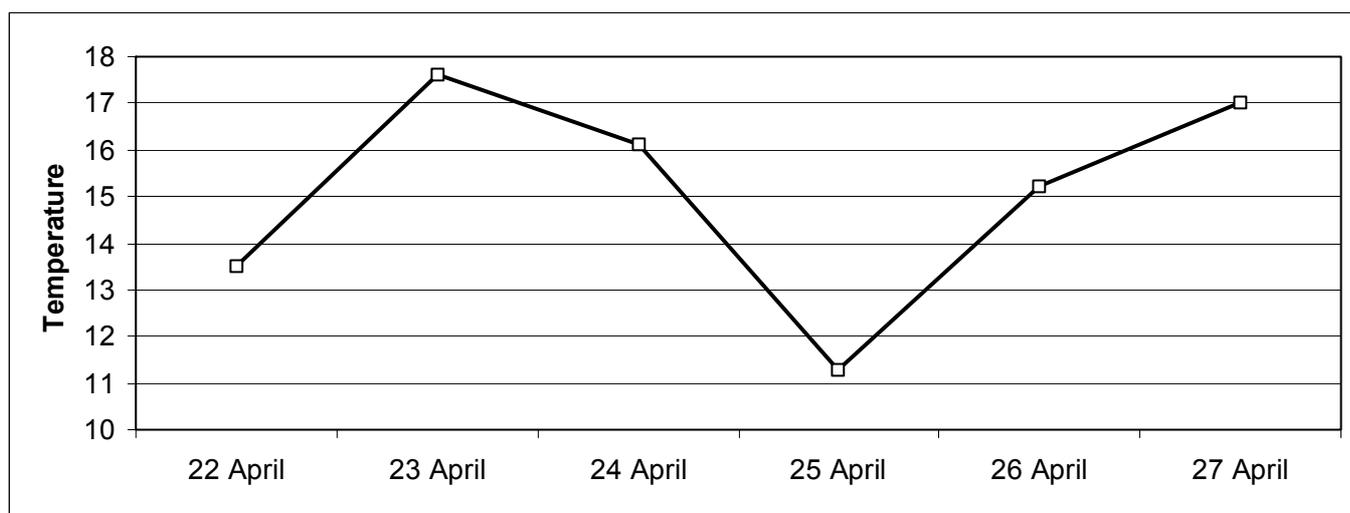


Fig. 3.4.5.26. Mean temperatures from 1800 to 0100 in a field cage experiment on the farm Boontjiesrivier, Citrusdal, during April 2004.

This experiment will be repeated during 2005, but at an earlier time in the season to avoid the potential detrimental influence of low temperatures.

3.4.5.11 The ovicidal activity of the fungicides Sporekill and Virkon S

Opsomming

Verskillende produkte word in die teelproses van VKM gebruik in pogings om die kunsmatige dieët te steriliseer en eiers te vrywaar van ongewenste swamme en virusse. Formalien is vantevore in die standaard teelproses gebruik vir die oppervlakkige sterilisasie van eiers. Die produk is egter onaangenaam en giftig om mee te werk en gebruikersvriendeliker produkte is nodig. 'n Sporekill/Virkon S-mengsel is ontwikkel wat die Formalien gedeeltelik vervang het. Daar is egter vermoed dat die produk wisselend giftig vir die eiers is en 'n proef is uitgevoer om dit te ondersoek. Ouer en jonger eiers is met die mengsel behandel. Tellings het getoon dat Sporekill/Virkon inderdaad giftig is en tot 40% meer eiers doodmaak as waarvoor natuurlike mortaliteit verantwoordelik is. Die produk is dus nie 'n goeie plaasvervanger vir Formalien nie.

Introduction

Formalin is used in the FCM rearing process to surface sterilize egg sheets before inoculation onto artificial diet in culture jars. It has been a standard treatment for many years but has never been favoured as it was (i) variably detrimental to FCM eggs and (ii) hazardous for workers exposed to its fumes. An alternative treatment consisting of, initially, Sporekill only, and later on with the addition of Virkon S, was developed by Moore & Richards (2001) to replace, or alternate with, Formalin. However, this treatment seemed to be readily toxic to FCM eggs during preliminary usage and an experiment was conducted to investigate the effect of the mixture on the eggs.

Materials and methods

A mixture of 0,15 % Sporekill plus 1% Virkon S in water, was used. An egg sheet, 170 mm in diameter, was divided in half. One half was placed in a cold room at 9°C to retard development, while the second half was allowed to develop at 26°C. Both halves were removed 60 hours later, when the eggs kept at ambient temperature were in the red stage of development. Each half was then further divided in half. One quarter sheet of each egg age was immersed in the Sporekill/Virkon mixture for 15 minutes, removed and left to dry. The second quarter was left untreated. Each of these quarters was divided into four. The pieces were incubated at 26°C until all viable eggs had hatched. The individual egg sheets were placed on a paper grid consisting of 10 mm² squares. All eggs in each of two squares were inspected and mortality counts conducted.

Results and discussion

Approximately 1 000 eggs of each age were inspected. Undeveloped and developed eggs of both ages responded unfavourably to the Sporekill/Virkon treatment and mortality was respectively 10% to 16% higher than with the untreated eggs (Fig. 3.4.5.27). This aspect will therefore have to be taken into consideration to compensate accordingly with regard to the numbers of eggs inoculated when the mixture is used.

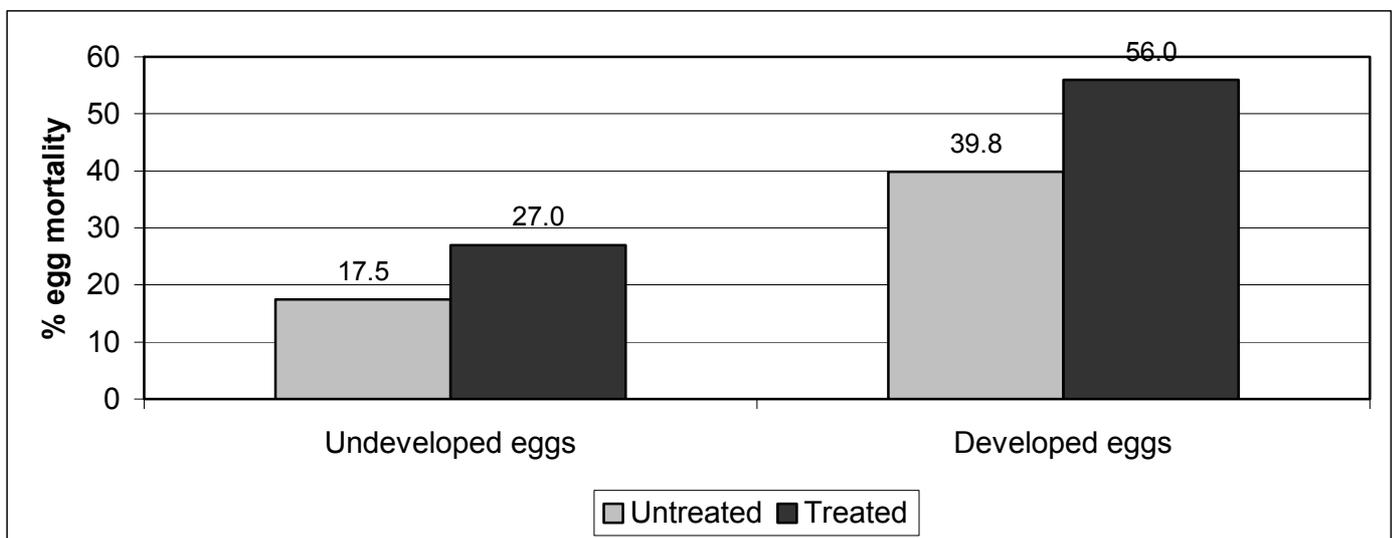


Fig. 3.4.5.27. Effect of a Sporekill/Virkon mixture on undeveloped and developed false codling moth eggs.

It is unclear why the natural mortality of eggs allowed to develop naturally after being cold stored for 60 hours, should be lower than eggs that were allowed to develop naturally at ambient temperature. This aspect will be investigated again at a later stage.

3.4.5.12 The effect of egg age on the production of false codling moth in artificial diet

Opsomming

VKM-eiers tot 24 uur oud, word meesal in die teelproses gebruik. Dit is egter somtyds nodig om eiers 'n paar dae lank te hou voordat dit gebruik word. 'n Proef is uitgevoer om vas te stel of dit voordeliger is om ouer eiers te gebruik. Jonger en ouer eiers is in kunsmatige dieët ingeënt en toegelaat normaal te ontwikkel. Twee keer meer motte is verkry uit flesses wat met jong eiers ingeënt was. Dit blyk dus dat ouer eiers nie vir dié doel gebruik behoort te word nie.

Introduction

The study described in the previous Section 3.4.5.11 was continued to determine whether the aging of eggs will have any significant detrimental effect when inoculated in diet jars.

Materials and methods

A standard egg sheet (170 mm diameter) with freshly laid eggs was divided in half. One half was refrigerated at 9°C. The second half was incubated at 26°C until the eggs had developed to the black stage. Both halves were cut into pieces, each containing approximately 300-400 eggs. The pieces of egg sheet

were surface sterilized with Sporekill for 15 minutes, and inoculated into 20 diet jars each. An alternative FCM diet (hereafter the “enriched” diet) developed by Moore & Richards (2001) was used. The jars were incubated at 26°C until the larvae had developed to the 5th instar stage and were ready to pupate. The standard cotton wool jar stoppers were then removed and replaced with SFK rolls. The larvae pupated in the SFK, which was subsequently pulled apart, the pupae removed and counted. Pieces of egg sheets were removed from 5 diet jars of each treatment. All eggs on each piece were counted and the egg mortality calculated.

Results and discussion

Natural mortality of the undeveloped and developed eggs collected from the diet jars was 39,8% and 35,3% respectively. This result differs from the previous experiment where natural mortality of the undeveloped eggs was much lower than that of the developed eggs, and can't be explained. The natural mortality is quite high but is probably the result of two factors, viz. (i) the Sporekill treatment used for surface sterilization of the egg sheet pieces and (ii) egg mortality due to the clumped deposition of eggs by densely packed moths while laying eggs.

Despite the similarity in egg survival in the two treatments, there was a big difference in the ultimate development to moths of the larvae hatching from the two types of eggs. A mean of respectively 61 and 37 moths developed from the jars containing the undeveloped and developed eggs. The only explanation for this trend is that the surface sterilant had a delayed effect on the well developed eggs that was transferred to the larvae, and increased the larval mortality, rather than killing the treated eggs. These data suggest that eggs should preferably not be aged before inoculation, but used when freshly laid.

3.4.5.13 The lifespan and parasitization potential of the egg parasitoid, *Trichogrammatoidea cryptophlebiae*

Opsomming

Min is oor die lewensduur en parasiteringspotensiaal van die eierparasitoïed, *Trichogrammatoidea cryptophlebiae*, bekend. Dié faktore is in 'n laboratoriumondersoek bestudeer. *T. cryptophlebiae* is saam met vars VKM-eiers in klein hokkies geplaas en by 26°C gehou. Die eiers is 72 uur later met vars eiers vervang. Die proef is 120 uur na aanvangs beëindig en tellings is van eierparasitisme en parasitoïedoorlewing uitgevoer.

Daar is vasgestel dat amper alle parasitoïede minder as 72 uur lank gelewe het. In dié tydperk het elke wyfieparasitoïed 'n gemiddeld van 9,6 eiers geparasiteer. 'n Maksimum van 17 eiers is deur 'n enkele wyfie geparasiteer.

Introduction

This study was conducted to investigate the lifespan and ability of the egg parasitoid, *T. cryptophlebiae* to parasitize FCM eggs. The information was necessary to calculate the number of parasitoids to be released into field cages (see Section 3.4.5.10).

Materials and methods

100-200 fresh FCM eggs on wax paper, as well as one male and one female parasitoid, freshly emerged, were put into a cage consisting of a small 90 ml clear plastic tub with snap-on lid. A small drop of honey was used as a food source for the parasitoids. The honey was placed on the inside of the lid, and covered with a 5 mm² piece of paper towelling to prevent parasitoids from sticking to the honey. Twenty such cages were prepared. The cages were incubated at 26°C. Seventy-two hours later the cages were cooled down to 9°C to immobilize any live parasitoids. The cages were opened, the FCM eggs carefully removed to prevent parasitoids from being removed as well, and replaced with a similar number of eggs. Counts of parasitoid mortality and egg parasitization were conducted 72 hours later.

To confirm the lifespan of the parasitoids, 150-200 freshly emerged parasitoids were placed into a 2 l plastic jar. Honey was supplied as food source. Two observations were made respectively 48 and 72 hours later.

Results and discussion

Because of the extremely small size of the parasitoids in the relatively large containers, it was impossible to conduct mortality counts 72 hours after the initial exposure. However, any parasitized FCM eggs in the second batch would have confirmed the presence of live parasitoids.

All parasitoids were dead 120 hours after initial exposure. Eggs of the second batch were parasitized in only one of the 20 containers, showing that a single female had lived for longer than 72 hours. In this experiment the parasitoids' lifespan was therefore not more than 72 hours.

Out of the 20 cages, no eggs were parasitized in five. A mean of 9,6 eggs were parasitized per female in the 15 remaining cages. In seven of the 15 cages, 1-10 eggs were parasitized per cage, while in the other eight, more than 10 eggs, and up to a maximum of 17 eggs, were parasitized.

In the 2 l jar all parasitoids were dead after 48 hours, with the exception of two that lived for 72 hours.

The relatively short lifespan of the parasitoids can be confirmed under other conditions. Until then, it must be accepted that they are short-lived and that when they are released artificially once a month, the suppression of FCM is based on the ability of the parasitoids to successfully reproduce after release.

The data also show that, to study the augmentative effect of the parasitoids under field cage conditions, fruit should only be exposed to moths in a field cage for, at most 72 hours. The FCM females live much longer than the parasitoids and if the fruit is left too long, many eggs will be deposited after the parasitoids have already died. Because there can be no overlap in parasitoid generations due to the single release envisaged for the field cage experiment, it also can't be left to succeeding generations to parasitize any such eggs.

3.4.5.14 False codling moth population fluctuations in proposed pilot project site

Opsomming

’n Proefperseel van 35 ha, wat alreeds in vorige jare vir proewe gebruik was, is weer eens gekies vir die uitvoer van ’n SIT-loodsprojek vir die kommersiële bestryding van VKM. Twee en twintig lokvalle en 110 databome is in die gebied versprei. Weeklikse tellings is uitgevoer om VKM se besmettingspatroon in die gebied te bestudeer met die oog op die loodsprojek wat in 2004-2005 uitgevoer moet word.

Die neigings ten opsigte van lokvalvangste en vrugverliese wat aangeteken is, het baie met dié van die vorige drie jaar ooreenstem. Daar kan dus met ’n redelike van sekerheid aangeneem word dat enige groot afwykings in lokvalvangste en oesverliese in die gebied nádat daar met die loslaat van gesteriliseerde motte begin is, aan die SI-loslatings toegeskryf sal kan word.

Introduction

It was planned to initiate a pilot project for the control of FCM with SIR, during the 2004-2005 season. An experimental site was selected that had been used for various large scale experiments in the past to evaluate, *inter alia*, the potential of certain mating disruption and attract&kill products for FCM control. A survey was therefore initiated to monitor FCM during the 2003-2004 season to compare population trends with preceding years in preparation for the pilot project.

Materials and methods

The experimental (release) site was 35 ha in size and consisted of 14 blocks of mature citrus trees arranged in a 7 x 2 pattern. The first 9 blocks were Washington navel orange orchards, the rest consisted of one Minneola tangelo and four Valencia orange orchards. Two Washington navel orange orchards, approximately 500 m from the experimental site, were used for controls.

Respectively 20 and two delta traps were distributed in the release site and the control orchards. Lorelei® synthetic pheromone was used as attractant and the dispensers were not replenished for the duration of the survey. Five adjoining data trees to monitor fruit drop, were selected next to each trap, excluding one of the control orchards, and the Minneola tangelo and Valencia orchards. Trap and fruit drop counts were conducted once a week from 29 December 2003 to harvest time starting on 31 May 2004. All trapped males were counted and removed, while all dropped fruit were collected, cut open and inspected for FCM infestation.

Results and discussion

The trends in the site were very similar to those of previous years (Fig. 3.4.5.28). Fruit drop that exceeded the fruit drop threshold (one fruit per tree per week) started relatively late during the season, as had become the pattern for the last approximately seven to eight years in the Citrusdal area. This increase in fruit drop occurred during mid March (2000-2001), early March (2001-2002), mid April (2002-2003) and end of April (2003-2004), respectively. In all cases the fruit drop was preceded by an increase in trap catches three to five weeks earlier.

It is planned to initiate FCM releases as early as August. It remains to be seen whether it will be necessary to start that early, or whether a delay until later in the season, which will have financial implications, will be possible. The available data on FCM for the site, which to a large extent coincides with the general FCM trends in the Citrusdal area on other farms, enables the initiation of the SIT project in the proposed site with a fair degree of confidence regarding FCM population patterns.

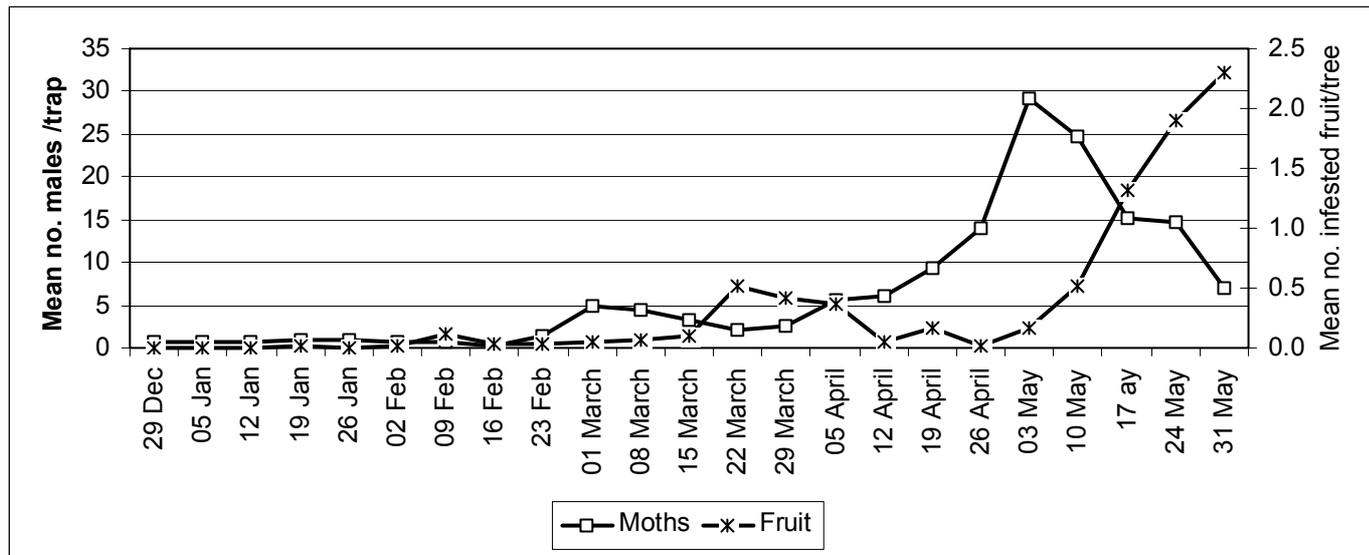


Fig. 3.4.5.28. False codling moth population fluctuations and infested fruit drop trends in a proposed SIT pilot project site on the farm Hexrivier, Citrusdal, during 2003-2004.

3.4.5.15 Comparison of the cold storage capabilities of different cool boxes

Opsomming

As gevolg van probleme met die vervoer van verkoelde VKM in koelkiste, is 'n ondersoek geloods na die doeltreffendheid van kommersieel-verkrygbare koelkiste. Drie tipes koelkiste, nl. 'n opvoubare tipe koelsak, 'n polistireenkoelkis en 'n "Coleman"-tipe koelkis is met mekaar en met 'n self-ontwerpte polistireenkoelkis, vergelyk. Die koelsak het die swakste isolering gebied, gevolg deur onderskeidelik die polistireenkis en die Coleman-tipe kis. Die selfvervaardigde polistireenkoelkis het die beste gevaar, waarskynlik te danke daaraan dat die wande van die koelkis van dubbeldikte polistireen vervaardig was.

Introduction

The problems associated with the transport of FCM in cool boxes were described in Section 3.4.5.9. Two further studies were conducted to compare the relative effectiveness of various cool boxes that have been used so far in the various SIT experiments. These experiments were conducted subsequent to the use of the double cool box system described in Section 3.4.5.9, and resulted in the development of a more effective system.

Materials and methods

Experiment 1: Three types of cool boxes were compared for their respective ability to maintain their interior temperatures, viz. a collapsible cool bag, a commercially obtainable cool box made from polystyrene foam sheet material (15 mm diameter) and a Coleman type cool box with hinged lid. All three containers had been stored next to each other in a storeroom for several days and all were at the same temperature. A Hobo datalogger was placed inside a Petri dish in each container. A fourth datalogger was placed next to the containers on a laboratory bench. A single freezer block (145 mm x 858 mm x 30 mm) was added to each

container. The containers were closed at 1030 and remained so for the duration of the evaluation. At 1215 the containers were moved outside the laboratory and placed on a table in solid shade in an open-sided carport. They remained there until 1300, when they were moved back to their original positions in the laboratory. The containers were opened to remove the dataloggers at 1530.

Experiment 2: Following the results of the first experiment, polystyrene cool boxes were specially manufactured for further evaluation. These cool boxes had appreciably thicker sides (30 mm diameter), and were compared to the same cool bag and Coleman type cool boxes as before. The same procedure was followed as above, except that the cool boxes were left in the air-cooled laboratory for the duration of the experiment.

Results and discussion

Experiment 1: The three types of containers differed appreciably in their ability to maintain interior temperatures. The Coleman type was most effective, followed respectively by the polystyrene box and the cool bag (Fig. 3.4.5.29).

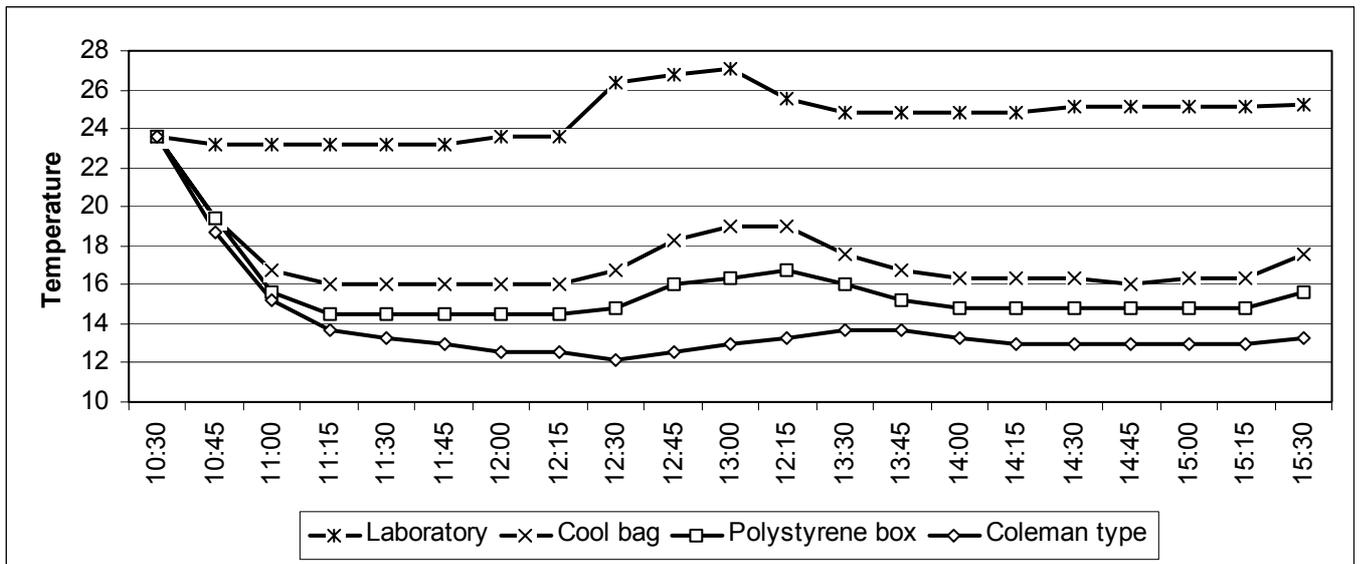


Fig. 3.4.5.29. Comparison of the ability of three types of cool boxes to maintain interior temperatures.

Experiment 2: Thicker sides improved the performance of the polystyrene box quite considerably and its ability to keep the interior cool, surpassed that of the previously best Coleman type cool box (Fig. 3.4.5.30).

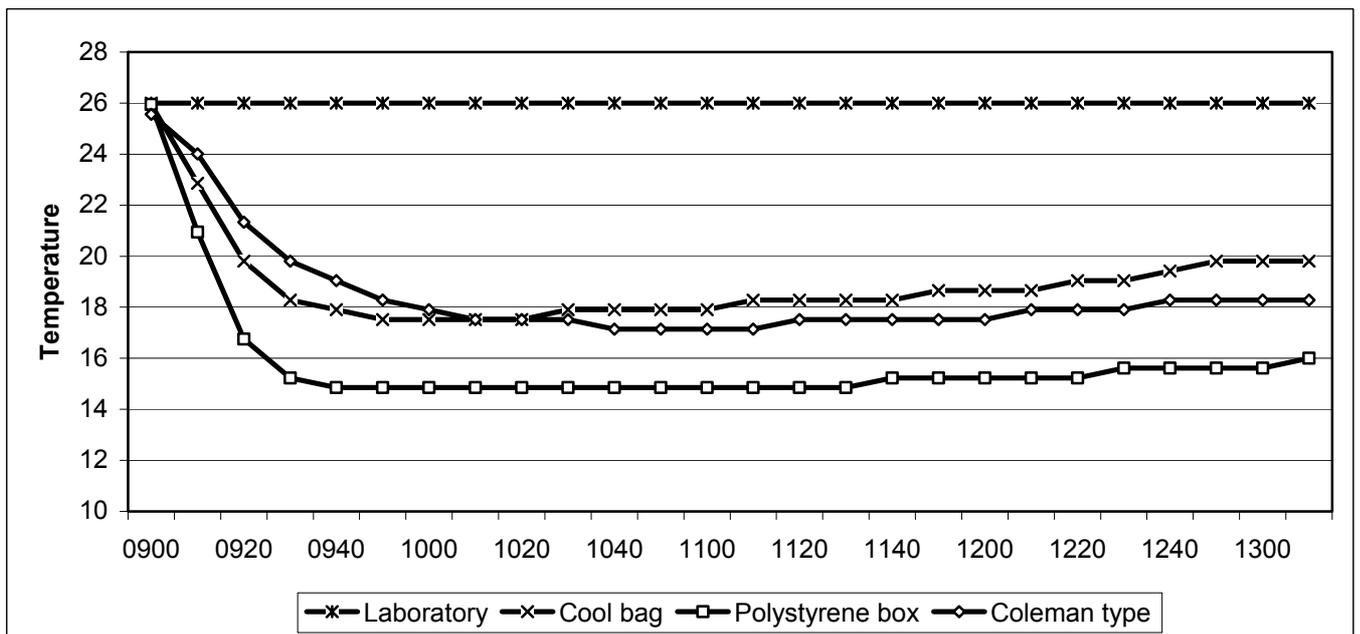


Fig. 3.4.5.30. Comparison of the ability of three types of cool boxes to maintain interior temperatures.

It was interesting to note the difference in performance when the Coleman type cool box was used for the transport of FCM and in the two experiments under discussion. During moth transport the cool box was wrapped inside a blanket every time and the interior temperature was reduced to below 10 °C for varying periods by the freezer block. In the above experiments at ambient temperatures, the freezer block wasn't able to reduce the interior temperature to below 10°C. This trend confirmed the decision to further develop the box within a box design which was used in Section 3.4.5.9.

3.4.5.16 Preparing the infrastructure necessary for a proposed Sterile Insect Release pilot project Experiment 662 by Hendrik and Marsheille Hofmeyr (CRI)

Opsomming

Vorbereidings vir 'n loodsprojek om VKM met behulp van SI-loslating te bestry, word beskryf. Die totale infrastruktuur om die teling, hantering, vervoer en loslaat van VKM te ondersteun, word bespreek. Studies is uitgevoer om probleme betreffende swam- en virusbesmettings te oorbrug, wat die begin van die massateling van VKM vertraag het. Verskeie diëte is geëvalueer en vordering is gemaak met die gebruik van verrykte dieet wat op mieliemeel en verskeie bymiddels soos melkpoeier, brouersgis, koringkiem en swamonderdrukkende produkte gebaseer is. Studies word voortgesit om behandelings te ontwikkel en te verfyn wat vir die oppervlakte-sterilisering van VKM-eiers gebruik kan word om veral virusse uit te roei voordat die eiers in teelflesse ingeënt word.

Perpekstrôe met deksels is ontwerp om papies in enkellaag-riffelkartonproppe te hou. Dié apparaat bewerkstellig die massaversameling van ongepaarde motte wat deur 'n insektarium verskaf word. 'n Lugsuiweringstelsel is ontwerp om die lug in 'n koelkamer waarin motte hanteer word, te reinig. 'n Dubbelisolering-koelkissstelsel is vervaardig om verkoelde motte vir bestraling 400 km ver met veiligheid te vervoer. 'n Vierwielaangedrewe motorfiets is aangeskaf waarop 'n outomatise motvystellingsapparaat, geskenk deur die USDA, gemonteer kan word.

Introduction

A study to investigate the radiation biology and induced F1 sterility in false codling moth (FCM), *Cryptophlebia leucotreta*, was initiated in July 2002. Excellent progress was made (Bloem *et al.*, 2003, Carpenter *et al.*, 2004) and in July 2004 preparations commenced to conduct a Sterile Insect Release (SIR) pilot study on a semi-commercial scale.

A site was allocated for the experiment and a local insectary was contracted to rear the required number of insects for release. Additionally, various items of equipment had to be purchased where necessary, or alternatively, designed and manufactured:

- 1 Ceder Biocontrol in Citrusdal was requested to supply 35 000 FCM twice a week for release in the experimental site.
- 2 Equipment had to be designed to transport pupae from the insectary to the CRI laboratory where they would eclose and the moths collected for further manipulation.
- 3 Equipment had to be designed to enable collection of the moths after emergence, but before they mated. In a similar SIT situation with Codling moth in Canada, more than 25% of the moths have already mated by the time they are collected for irradiation and release. This is not a complete loss, but mated females have to, at least, partially deplete their sperm through egg laying before they would mate again, and similarly, the males need a certain period of recovery before they would actively resume hunting for females. It is therefore better to release virgin moths. For the pilot project the stated intention was to collect unmated moths.
- 4 Moths have to be manipulated in the coldroom, *viz.* the required number of moths have to be measured off (by mass/volume, and not counted) and transferred to Petri dishes. The moths also have to be marked with fluorescent powder, for monitoring after release. Scales are copiously shed by the moths during such operations, and workers have to be protected against scale inhalation in the closed coldroom environment. An air extractor/purification system therefore had to be developed which would not vent contaminated air to the outside, and conversely, not introduce hot air from outside into the coldroom.
- 5 A cool box system had to be developed for moth transport on a round trip from Citrusdal to Stellenbosch for irradiation, and back to Citrusdal for release in the orchards (ca. 400 km).

- 6 A vehicle for the release of the moths had to be purchased.
- 7 Moth release equipment to automate the release of moths in the orchards, had to be tested and adapted.
- 8 Alternative diets for FCM had to be developed that would replace the original mould-susceptible maize flour diet and also replace the closed-jar system with an open container system which is easier to manage when mass rearing insects.
- 9 Other development would depend on any unexpected technical hitches occurring during any of the above operations.

Because of the developmental nature of the study conducted so far, the techniques involved and the results will be described concurrently.

Materials, methods and results

The experimental (release) site on the farm Hexrivier, Citrusdal, is 35 ha in size. It is isolated from other citrus plantings and consists of 14 blocks of mature citrus trees arranged in a 7 x 2 pattern. The first nine blocks from one end (4 x 2 + 1) are Washington navel orange orchards while the rest consist of one Minneola tangelo and four Valencia orange orchards. Two Washington navel orange orchards, approximately 500 m away, and isolated from the experimental site by natural bush, are intended as controls.

1 Rearing FCM for the pilot project: In the past, up to 200 FCM were routinely produced by Ceder Biocontrol from a single diet jar containing a standard diet of maize flour plus (inoculated) *Rhizopus* mould (Ripley *et al.*, 1939). To calculate the required production, the above number was reduced to a mean of 90 FCM per jar to provide for contingencies. It was therefore considered possible to collect the required 35 000 FCM from 400 diet jars, with ease. Moth collector equipment was subsequently designed with this number in mind (see Sub-section 3). Furthermore, the 35 000 moths had to be collected within 3 days, keeping in mind their restricted lifespan, and time limitations imposed by the moth collector equipment.

At the time that FCM rearing for SIR purposes was to commence, the insectary had already been suffering from fungal infections in their diet jars since December 2003. A range of fungi, including *Penicillium* sp. and *Aspergillus* spp., infected the diet jars, preventing larvae from developing normally and seriously impeding production. Nonetheless, 15 batches of FCM were consecutively reared and evaluated from mid-August to mid-November 2004. On average, only 14 000 moths could be produced per batch in the available time. The number of moths was calculated by *en masse* collection from emergence bottles in the insectary and weighing them on an analytical scale. The average mass of a single moth was 23,351 mg and was derived by weighing 10 female and 10 male moths individually from each of the first 5 batches. The number of moths collected was calculated from this figure.

The infections resisted all efforts at significant suppression and the rearing for SIR was stopped after the 15th batch. It also became clear that, whether the infection was suppressed or not, the possibility of recurring fungal problems in future could not be ignored, and it was decided to commence with the evaluation of an enriched diet developed by Moore & Richards (2001). A batch of FCM was reared on this diet in 25 diet jars, which produced a mean of 101 moths per jar during the obligatory 3 days (ca. 142 moths per jar in total). These results were promising enough to restrict further FCM rearing for SIR to that diet.

The production of 142 moths per jar was not sustained beyond the first batch and may have been due to an abnormally high number of eggs inoculated into the jars. From the second batch of FCM reared on the enriched diet, the production decreased to a mean of approximately 60 moths per jar. From previous experience (Sean Moore, pers. com., James E. Carpenter, pers. com.) it became clear that repeated rearing of successive generations was necessary for insects in general to adapt to a new diet before production would improve. FCM were therefore reared in consecutive batches of 28 diet jars each. To avoid having to remove pupae individually from cocoons for evaluation and data recording, the cotton wool stoppers on the diet jars were replaced with composite stoppers. Each stopper consisted of a cap and an SFK (single face corrugated; "C" flute) cardboard roll (Fig. 3.4.5.2). Each cap was made from a 25 mm wide segment of PVC pipe, one end of which was closed off by stainless steel mesh (30# x 0,27 mm wire diameter). A roll of SFK cardboard (35 mm wide) was pressed into the cap, the remaining 10 mm of SFK roll protruding from the PVC cap tightly fitted into the jar mouth. This system stopped larvae escaping from the diet jars by crawling through the SFK corrugations and offered ample space for pupation. After pupation, the SFK roll was removed, and the two layers of paper pulled apart, which split the cocoons and allowed the vast majority of pupae to drop out without further manipulation.

Production improved steadily in consecutive generations from averages of 50, 60, 73 and 142 to 183 moths respectively per jar. To ensure that the numbers of eggs inoculated per jar had not been increased unintentionally, egg paper squares were removed from 10 jars per generation and egg hatch counts were conducted. The ratio of hatched eggs to number of moths produced in the different generations decreased respectively from 4,9, 4,1, 3,5 and 2,3 to 2,0 hatched eggs per moth produced. This confirmed that the increase in production was due to adaptation of the larvae to the new diet, and not to an increase in the number of inoculated eggs. Production was immediately increased to two batches per week of approximately 500 jars each, to cater for SIR and the maintenance of the mother culture.

The enriched diet, combined with the surface sterilization of egg sheets with 0,2% Sporekill (eggs soaked for 15 minutes), provided mould-free rearing of FCM. However, in the initial stages of production increase, 2% to 3% of the jars had to be destroyed because of viral infection, probably the Crle-granulovirus. This infection rapidly increased and within the next 8 batches up to 60% of the production had to be destroyed (Table 3.4.5.10). The production was reduced to the size of the maintenance culture, and studies were initiated to solve the problem. In retrospect it was noticed that, although the insectary suffered from severe fungal infections, they were free from any noticeable viral problems. Upon enquiry, it was established that the insectary's rearing technique involved the use of 1% bleaching agent, sodium hypochlorite ("Jik"), as an egg sheet dip treatment 24 hours before inoculation into the diet jars. Additionally, the use of Formalin was alternated with Sporekill as an egg dip treatment to surface sterilize the pieces of egg sheets just before inoculation. Jik was subsequently tested, but was found to have little effect when used as an egg surface sterilant 24 hours before inoculation (Table 3.4.5.10, batches 8-14). Formalin was used as an egg dip treatment just before inoculation and was found to suppress the virus. A mixture of 0,2% Sporekill plus 1% Virkon S suppressed the virus, but was toxic to the eggs, resulting in very poor production (Table 3.4.5.10, batch 9). If treated eggs were rinsed with water, production increased, but fungal and viral suppression were forfeited (Table 3.4.5.10, batch 16).

Apart from the apparent excellent viral suppression conferred by Formalin, it was immediately noticed that moth production resulting from eggs that had been surface sterilized with Formalin, was very poor (Table 3.4.5.10, batch 9). The dose of Formalin used was progressively reduced from the original mixture of 40 ml Formalin plus 100 ml water to 20 ml Formalin plus 80 ml water. It was also established that a quick rinse with sterile water reduced the detrimental effect on the eggs, without apparently influencing the degree of viral suppression (Table 3.4.5.10, batches 17 and 18).

Table 3.4.5.10. Production of false codling moth using various treatments to surface sterilize the eggs before inoculation into jars containing enriched diet.

Batch no.	No. of diet jars	Surface sterilization of eggs just before inoculation, unless otherwise stated	Water rinse	% Jars with virus	Production ¹
1	60	0,2% Sporekill	No	38.3	Normal
2	263	0,2% Sporekill	No	17.9	Normal
3	363	0,2% Sporekill	No	27.3	Normal
4	437	0,2% Sporekill	No	51.7	Normal
5	485	0,2% Sporekill	No	21.9	Normal
6	402	0,2% Sporekill	No	31.1	Normal
7	198	0,2% Sporekill	No	29.3	Normal
8	28	1% Jik ² , 0,2% Sporekill, 0,4% Formalin ³ in diet;	No	0.0	Virtually no larval development
	26	1% Jik, 0,2% Sporekill, 0,2% Virkon S in diet	No	57.7	Normal
	28	1% Jik, 0,2% Sporekill	No	60.7	Normal
9	28	40 ml Formalin + 100 ml water	No	0.0	Very poor
	28	1% Jik, 40 ml Formalin + 100 ml water	No	0.0	Very poor
	28	0,2% Sporekill	No	39.3	Normal
	28	1% Jik, 0,2% Sporekill	No	32.1	Normal

	28	0,2% Sporekill + 1% Virkon S	No	0.0	Poor production; 2 jars with fungal infection
	28	1% Jik, 0,2% Sporekill + 1% Virkon S	No	0.0	Poor
10	196	1% Jik, 0,2% Sporekill	No	41.8	Normal
11	503	0,2% Sporekill	No	59.2	Normal
12	540	1% Jik, 0,2% Sporekill	No	0.2	Normal
13	56	1% Jik, 0,2% Sporekill	No	26.8	Normal
	56	1% Jik, 40 ml Formalin + 100 ml water	No	0.0	Poor
14	56	1% Jik, 0,2% Sporekill	No	16.1	Normal
	56	1% Jik, 40 ml Formalin + 100 ml water	No	0.0	Poor
15	56	0,2% Sporekill	No	16.1	Normal
	56	40 ml Formalin + 60 ml water	No	0.0	Very poor
16	56	40 ml Formalin + 60 ml water	No	0.0	Very poor
	112	25 ml Formalin + 75 ml water	No	0.0	Poor
	28	0,2% Sporekill + 1% Virkon S	No	0.0	Very poor
	28	0,2% Sporekill + 1% Virkon S	Yes	25.0	Slightly better than unrinsed; 2 jars with fungal infection
17	196	25 ml Formalin + 75 ml water	No	0.0	Poor
18	196	25 ml Formalin + 75 ml water	Yes	2.7	Normal
19	196	20 ml Formalin + 80 ml water	Yes	0.9	Normal
20	196	20 ml Formalin + 80 ml water	Yes	0.0	Normal
21	140	20 ml Formalin + 80 ml water	Yes	0.0	Normal

¹ "Very poor" = 1-10 moths per jar; "Poor" = 11 – 30 moths per jar; "Normal" = >60 moths per jar.

² Jik (3,5% a.i. product) was used throughout to surface sterilize eggs sheets 24 hours before inoculation.

³ Formalin = 35% a.i. product.

Two experiments were conducted which coincided with batch 17 above (Table 3.4.5.10) to further study the effect of Formalin and Jik bleach on FCM eggs. Five pieces of eggs sheet, similar in size and egg density to those used for inoculation, were used per treatment. The eggs were immersed in the appropriate sterilant for 3 to 4 seconds, removed, briefly rinsed in sterile water, and placed on wire mesh to dry at ambient temperatures. They were then placed into a Petri dish and incubated at 26°C until all viable eggs had hatched. Egg mortality counts were conducted by counting all eggs in a 7 mm x 7 mm square in the middle of each piece of egg sheet. Two hundred to 400 eggs were counted per treatment. The results were as follows (Table 3.4.5.11):

Table 3.4.5.11. Effect of Formalin and sodium hypochlorite bleach when used as a surface sterilant of false codling moth eggs.

Experiment	Treatment ¹	Unrinsed		Rinsed with sterile water	
		% Egg hatch	% mortality ²	% Egg hatch	% mortality ²
1	Control	73.8	-	69.0	-
	10% Formalin	45.3	38.6	70.1	-1.6
	15% Formalin	35.6	51.8	68.2	2.8
	20% Formalin	10.6	85.6	63.7	9.2
	25% Formalin	3.8	94.9	65.4	6.7
	40% Formalin	0.0	100.0	52.8	24.8
2	Control	75.4	-	66.4	-
	0,5% Jik	71.7	4.9	70.8	-6.6
	1% Jik	68.5	9.2	67.8	4.2
	2% Jik	69.6	7.7	74.8	-10.3

¹ Formalin = 35% a.i. product; Jik = 3,5% a.i. product.

² Adjusted for natural mortality with Abbott's formula.

The Formalin dip treatment was toxic and up to 100% of the treated eggs were killed depending on dosage. The water rinse markedly reduced the detrimental effect of all Formalin treatments. In contrast egg mortality after being treated with Jik bleach closely matched that of the untreated control even when not rinsed with water. It could therefore be regarded as non-toxic. The viral suppression of Jik bleach still has to be finalized before it can be considered a viable alternative to Formalin.

2 Equipment to transport moths from the insectary to the CRI laboratory: The insectary was to supply pupae in their cocoons that were produced in the cotton wool jar stoppers. Chipboard boxes (550 mm long x 550 mm wide x 600 mm high) were manufactured with five closed, and a single open side. Four slots (130 mm apart and 6 mm deep) were cut into the length of each of two opposing sides to hold the Perspex lids of the moth collector apparatus. Each box had space for four Perspex lids, held shelf-like, and containing 196 cotton wool stoppers in total.

3 Moth collector equipment: The equipment was designed to collect moths in large numbers before mating took place. Four Perspex troughs, 4 000 mm x 520 mm x 350 mm, and funnel-shaped in end-view, were manufactured (Fig. 3.4.5.31 and Fig. 3.4.5.32). Several prototypes were studied before the optimum slope for the sides, approximately 53° from the horizontal, were finalized. Two frames consisting of steel and aluminium were erected to each hold two moth troughs located one on top of the other. Four Perspex lids, each 520 mm long x 520 mm wide x 6 mm diameter, covered the top opening of each trough. Each lid contained 49 holes, 57 mm in diameter, drilled to accept one cotton wool stopper each (Fig. 3.4.5.33).



Fig. 3.4.5.31. Front view of two moth troughs, each with 10 containers at the bottom to collect emerging false codling moths.



Fig. 3.4.5.32. End view of two moth troughs designed to collect emerging false codling moths. The holes drilled to accept cotton wool stoppers containing false codling moth pupae, are visible in the lids of the bottom trough.



Fig. 3.4.5.33. Trough lids drilled to accept cotton wool stoppers containing false codling moth pupae.

Ten Petri dish-shaped containers, 210 mm diameter x 45 mm high, were located at the bottom of each trough to collect the moths (Fig. 3.4.5.34). The lid of each container was slotted, 210 mm x 27 mm, which was covered by a removable slot lid. The slot lid was removed when the container was in use, and replaced when the container was removed to collect the moths.



Fig. 3.4.5.34. Trough lids with pupae in cotton wool stoppers and Petri dish type containers below the trough to collect emerging false codling moths.

In the insectary, mature FCM larvae leave the diet, crawl upwards and pupate in the cotton wool stoppers inside the jar. The stoppers containing the pupae are removed from the jars, transported to the laboratory and placed into the trough lid holes with the cocoons to the inside. In practice, the moths would emerge from their pupal cases in the cotton wool, take flight within a short time, land on the sides of the trough and

slide down into the containers at the bottom. In a test run, it was discovered that many moths would cling to the sides of the trough. The trough sides were therefore lightly dusted with talcum powder, which prevented the moths from finding a foothold and causing them to slide down into the containers at the bottom. FCM do not mate for at least 12 hours after emergence from their pupal cases. Therefore, there is ample time to collect the freshly emerged, unmated moths for immobilization in a coldroom.

4 Air extractor and purifier: An air extractor was designed to purify the air inside the coldroom from moth scales shed during the various handling processes that the moths have to undergo. It consisted of a stainless steel cabinet, 800 mm wide x 750 mm deep x 600 mm high. The front side was partially cut away at a 55° angle and covered with a glass pane, to enable workers to see inside the cabinet while working (Fig. 3.4.5.35). A 200 mm high opening running the width of the cabinet, was left at the bottom through which work was conducted. 520 mm in from the front of the cabinet, a slot was cut to accept an air filter, 600 mm long x 600 mm wide x 48 mm diameter. The filter was slid in from the outside top and fitted snugly into place inside the cabinet in tailor-made channels at the sides and bottom. The filter was able to remove impurities from the air down to 5 µm in size, which was ample to clean the air from the smallest moth scales whose size averaged a minimum of 38 µm (Tim Grout, pers. com.).



Fig. 3.4.5.35. Front view of air extractor/purifier cabinet to remove moth scales from inside coldroom. The air filter is visible through the front glass pane, as is the slot in the top.

At the back of the cabinet, a hole was cut and fitted with a flange to which an in-line air extractor fan (190 l/s at static pressure of 200 Pa) was attached (Fig. 3.4.5.36). Air was drawn into the cabinet through the front opening, purified by the filter, exhausted through the fan and returned back into the coldroom.



Fig. 3.4.5.36. Rear view of air extractor/purifier cabinet to show attached extractor fan.

5 Cool box system for the long-distance transport of FCM

Following the experiments conducted to investigate various cool boxes commercially available, it was clear that a cool box system would have to be designed to satisfy the needs of the moth transport operation. For the pilot project, 120 Petri dishes filled with moths, needed to be transported. Three small, lidded cool boxes, each 320 mm long x 320 mm wide x 310 mm high, made from polystyrene sheets, 30 mm thick, were constructed. Each box was able to hold 40 Petri dishes (4 stacks of 10 each). These cool boxes fitted inside a larger container, 1 300 mm long x 600 mm wide x 450 mm high and made from polystyrene sheets, 100 mm thick. Enough space was allowed to enable freezer blocks to be placed between the small cool boxes and the inside walls of the large container. At the time of writing this report, this system still has to be finally evaluated.

6 Moth release vehicle: The site was too big to release moths by hand. Planting distances in the site and the uneven terrain also precluded the use of a light commercial vehicle. It was therefore decided to use a quad bike. A Yamaha Bruin 4x4 quad, water/oil cooled, and able to function under the summer conditions endured in Citrusdal, was acquired.

7 Moth release apparatus: The SIT facility in Osoyoos, British Columbia, Canada, uses 30 quad bikes each fitted with automated equipment to release irradiated Codling Moth commercially. Such a release apparatus was donated by USDA-ARS for test purposes. This equipment is not necessarily ideally suited for South African conditions, and will therefore have to be adapted where necessary.

8 Alternative diets for mass rearing FCM: While attempts were made to rear the required number of moths on the standard diet, several alternative diet ingredients were tested on a small scale, *viz.* 2 jars per diet. The purpose was (i) to determine whether the texture of the diet could be improved by adding coarse ingredients, (ii) to find an alternative to maize flour that would be unsusceptible or tolerant to the range of moulds infecting diets containing maize flour, (iii) to test some of these mixtures in an open tray system, as an alternative to the honey jar type system that has been exclusively used in insectaries for FCM rearing until now.

New ingredients such as coarse maize flour, soy flour, wheat bran, nutty wheat and oats were used in various diets in addition to the ingredients, excluding maize flour, included in the enriched diet (Table 3.4.5.12 and Table 3.4.5.13). The jars with diet were sterilized in an autoclave, and FCM eggs were inoculated following a dip treatment with 2% Sporekill. FCM eggs were inoculated into the diet and incubated. FCM developed in all of the various diet mixtures, with varying success:

Table 3.4.5.12. Alternate diet mixtures tested for the artificial rearing of false codling moth larvae. Diet mixtures **were sterilized** by autoclaving before inoculation with eggs.

Diet ingredients per jar: 5 g Wheat germ, 0,9 g Milk powder, 2,5 g Brewer's yeast, 0,5 g Methyl paraben, 0,2 g Sorbic acid plus one of the following:	Mean no. of moths per diet jar
50 g Nutty wheat	56
50 g Maize flour plus 10 g wheat bran	79
50 g Maize flour plus 10 g oats	31
50 g Oats	52

Table 3.4.5.13. Alternate diet mixtures tested for the artificial rearing of false codling moth larvae. Diet mixtures **were not sterilized** before inoculation with eggs.

Diet ingredients per jar	Comments
50 g Soy meal 5 g Wheat germ 0,9 g Milk powder 2,5 g Brewer's yeast 0,5 g Methyl paraben 0,2 g Sorbic acid The above <u>with and without 25 g wheat bran.</u> 85 ml Boiling water added	<u>With wheat bran:</u> 100% larval mortality. <u>Without wheat bran:</u> Three larvae developed to pupal stage.
50 g Nutty wheat. 85 ml Boiling water added.	Fermentation occurred, no larval development.
50 g Nutty wheat 2,5 g Brewer's yeast 0,5 g Methyl paraben 0,2 g Sorbic acid The above <u>with and without 5 g wheat germ</u> plus 0,9 g milk powder. 85 ml Boiling water added.	100% mortality of ca. 2nd instar larvae with and without wheat germ.
50 g Maize flour + 10 g bran. 85 ml Boiling water added.	100% mortality of larvae of variable ages
50 g Maize flour 10 g Bran 2,5 g Brewer's yeast 0,5 g Methyl paraben 0,2 g Sorbic acid. The above with and without 5 g wheat germ plus 0,9 g milk powder. 85 ml Boiling water added.	No larval development whatsoever.
50 g Coarse maize meal 5,0 g Wheat germ 0,9 g Milk powder 2,5 g Brewer's yeast 0,5 g Methyl paraben 0,2 g Sorbic acid 85 ml Boiling water added.	No larval development whatsoever.
50 g Oats 5,0 g Wheat germ 0,9 g Milk powder 2,5 g Brewer's yeast 0,5 g Methyl paraben 0,2 g Sorbic acid 85 ml Boiling water added.	A small number of larvae developing to 5th instar, but no pupation

It is obvious even from the relatively meagre data (Table 3.4.5.13), that sterilization is an extremely important aspect of the various diets used, as there was invariably little or no development in the non-sterilized mixtures. Because of the poor production it is impossible to draw any conclusions concerning the various diets or the value of an open-topped rearing system.

Most of the above diet mixtures were also evaluated in plastic containers, 210 mm long x 170 mm wide x 45 mm high. The plastic containers could not be sterilized in an autoclave, and the separate ingredients were therefore mixed dry and sterilized in a microwave oven for 10 minutes on a "high" setting. Three hundred grams of each diet mixture were then blended with 80 ml boiling water, and evenly spread out in a 15 mm-deep layer in a container. FCM eggs were placed on top of the diet, and each container was sealed into a brown paper bag, which mainly served to delay moisture loss. Production from all of the diets evaluated, was extremely poor. The diet texture was maintained in a fair, tending to dry, condition, which was promising. However, little larval development occurred, even in diets that performed better in the diet jars (Table 3.4.5.12) because of suspected viral infection. With the knowledge gained from the development study described above (Table 3.4.5.10), it is advisable that some of these diet mixtures be tested again. Much more attention also needs to be given to the development of the open-topped diet container rearing system as an alternative to the honey jar system commonly used.

These studies were conducted while the initial unsuccessful insectary rearing of FCM for SIR was in progress. At that time the importance of rearing consecutive generations to adapt to a new diet, was not realized, and the results were regarded to be too inferior to merit continued investigation (Table 3.4.5.12). It was therefore additionally impossible to evaluate the effect, if any, that any beneficial products such as bran might have had as texture modifying agents. Similarly, the question of a replacement for maize flour with more mould-tolerant qualities remains unanswered. Under current circumstances it may be advisable to repeat the investigation on a larger scale to investigate the possible advantages, if any, of basing a FCM diet on food sources such as nutty wheat, bran and oats.

9 Adapted trough system for the collection of FCM: During the rearing of FCM for adaptation to the new diet, it was noticed that the production from diet jars with SFK stoppers seemed to be better than from cotton wool stoppers. An experiment was conducted to study this phenomenon. Sixth generation FCM was used which had already adapted to the enriched diet. Thirty diet jars were prepared as follows:

- 10 jars with cotton wool stoppers.
- 10 jars with cotton wool stoppers and double the amount of diet normally used.
- 10 jars with SFK cardboard stoppers.

The average production from the three treatments was 151, 77 and 183 moths per stopper respectively. Although doubling the amount of diet per jar was fairly successful, it was relatively unproductive with regard to the amount of diet used. The SFK stoppers outclassed the cotton wool stoppers completely. It was subsequently noticed, and confirmed (Wayne Kirkman, pers. com.), that larvae crawled up to the cotton wool, but returned to the diet if a number of cocoons had already been formed in the stoppers. They then pupated in the top layer of the diet, which meant that they were not lost for production purposes in an insectary set-up where stoppers and jars were placed into emergence boxes. However, it was impossible to utilize the insects in the diet jars for SIT purposes, as the moth trough equipment could not be adapted to collect moths from the jars as well.

It was therefore decided to modify the moth troughs to accept SFK stoppers. The original Perspex lids (with holes for the cotton wool stoppers) were replaced with solid Perspex lids from which the SFK stoppers could be suspended. Self-tapping screws were screwed into the lids from the outside, and the SFK rolls were simply pressed onto the screws (Fig. 3.4.5.37).

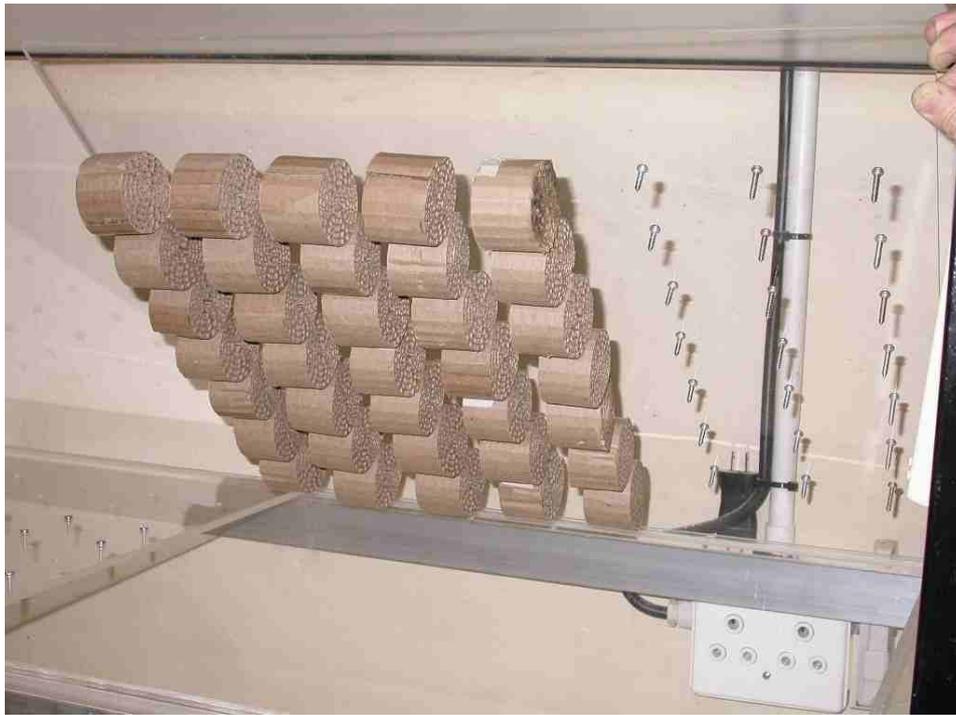


Fig. 3.4.5.37. Adapted lid for Perspex moth collector trough with SFK cardboard rolls suspended from screws (lid tilted to show details).

FCM larvae often pupate in both sides of the SFK flutes, leaving the centres of the flutes mainly uninhabited. Larvae that pupate in the middle of the flutes (mainly under conditions of extreme population density), with pupae on either side, would be hemmed in and unable to leave the stopper. Pupae killed by the screws would therefore be irrelevant.

Future research

Fungal and viral infections will be a continuous problem in the absence of sterile rearing facilities similar to the Codling Moth SIR facility at Osoyoos, British Columbia, Canada. Treatments will therefore have to be developed that can be used (and alternated) to suppress such infections successfully over the longer term.

The delay caused by the lack of sufficient FCM for release, will prevent results during the 2004-2005 season that will be significant from a commercial point of view. However, data have been generated, and equipment designed, that should be adequate for a successful restart of the project during 2005-2006.

The development of infrastructure to create facilities that can be used in the second phase of FCM SIR, viz. the semi-commercial application of FCM SIR in 200-300 ha of citrus, have to be considered a priority. This will involve a gamma irradiation facility, and a vastly bigger and improved insectary that will be able to provide the required numbers of moths.

3.4.5.17 Influence of temperature on recapture rate and lifespan of released false codling moths in orchards

Experiment 662 by Hendrik and Marsheille Hofmeyr (CRI)

Opsomming

Alle inligting in VKM merk-en-loslaatproewe, wat in dié verslag bespreek was, is saamgevat om 'n verklaring te vind vir die wisseling in die hervangspersentasie en die duur van die hervangstydperk. Die faktore het klaarblyklik met die tyd van die jaar te doen gehad, maar het nie die wisselinge in sekere gevalle verklaar nie. 'n Vergelyking van die minimum nagtemperature het getoon dat vangste toeneem wanneer dit warmer as 16°C raak. Die hervangstydperk neem egter af. Dit is waarskynlik as gevolg van dagtemperature wat saam met die nagtemperature in die somer styg. Dit is onbekend of dié afname in hervangstydperk te wyte is aan 'n korter leeftyd, of aan 'n groter drang van die mannetjies om vinniger en wyer te versprei as die gebied waarin die lokvalle geplaas is.

Introduction

Various mark&release experiments were conducted from 2003 to 2004. In the course of these studies, it was noticed that the recapture rate and the duration of recapture of irradiated and unirradiated FCM males varied from one experiment to the next. This variation was present even when experiments were conducted in the same time of the season, but not necessarily during the same year. All available data was therefore added together to find the reason for the variability as the reasons may impact upon any decisions taken with regard to the interval between releases in an SIR program.

Materials and methods

Data with regard to the number of males recaptured, the number of days during which they were recaptured, the mean minimum temperature during the experiment, and the time of the year, was recorded.

Temperatures varied during the course of any single experiment. The average minimum temperatures during the nightly period of moth activity, viz. 1800 to 0100, for the duration of each experiment, were therefore calculated. The average number of males recaptured per treatment per experiment were also calculated, disregarding low recapture rates caused by obvious moth mortality (invariably due to cold immobilization problems during moth transport) or treatment influences.

Results and discussion

When comparing the data it was clear that the main cause of variable catches could not be ascribed solely to the time of year during which the experiment was conducted (Fig. 3.4.5.38). Of course, temperatures are clearly related to the time of season, *i.e.* cold during winter and hot during summer. However, it is not unambiguous, as there was, for instance, a big difference in the recapture rate and duration of catches in two experiments which were both conducted in October.

A tendency could be noted for males to be trapped over longer periods during the colder times of the year. The recapture rate dropped markedly during the winter months, and increased during the warmer months.

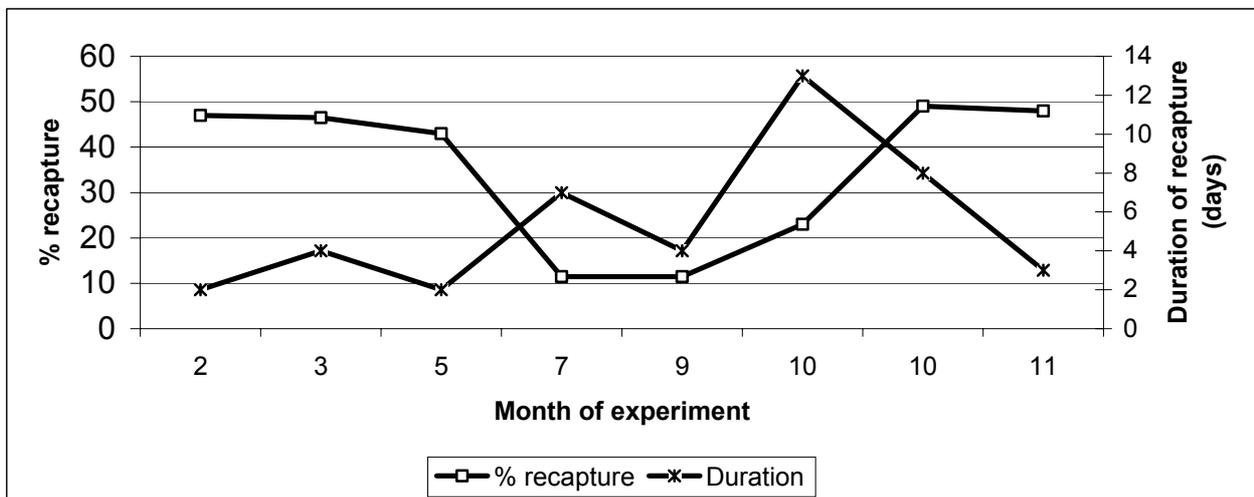


Fig. 3.4.5.38. Influence of the time of year on the recapture rate and lifespan of released false codling moth in orchards.

When the influence of temperature is studied, two clear patterns emerge (Fig. 3.4.5.39). The recapture rate increases, and less males are trapped as the mean minimum temperature rises. This explains the reason for the apparent discrepancy in the two October experiments referred to above, as the mean minimum temperatures in the experiments were respectively 12,5°C and 18°C.

In previous experiments reference was often made to the cessation of moth activity when the temperature decreases to below what was regarded as the lower threshold for moth activity. This cut-off can be clearly seen when the recapture rate suddenly increases from above 16°C (Fig. 3.4.5.39). The decrease in catches, presently attributed to a shorter lifespan, is probably the result of the harmful effect of high temperatures. The last-mentioned is obviously related to the increase in the mean minimum temperatures recorded.

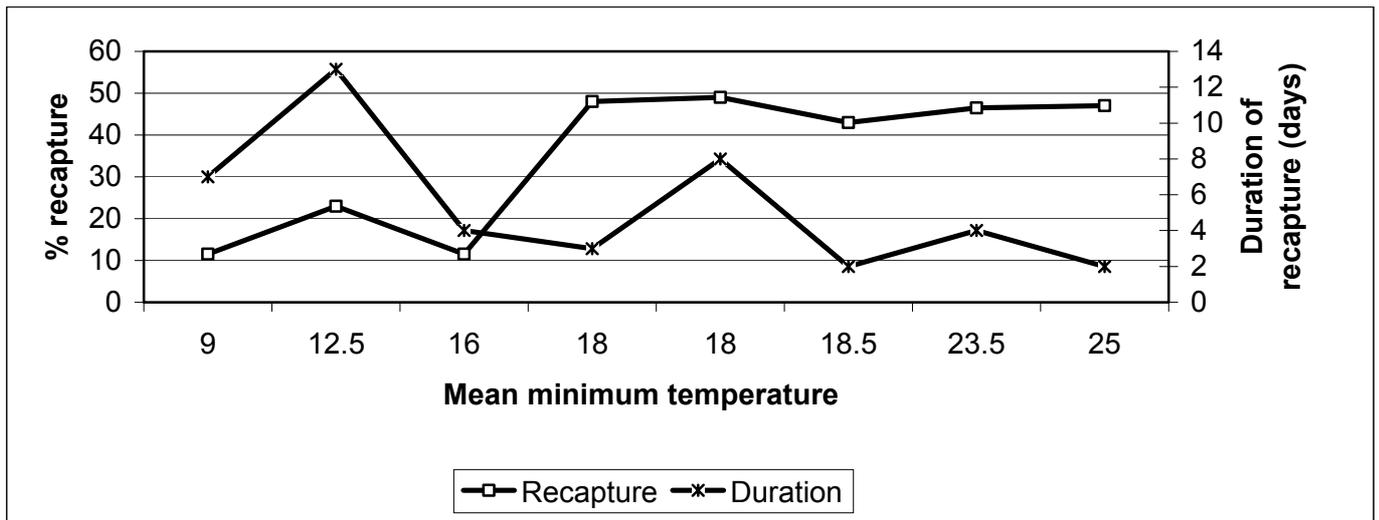


Fig. 3.4.5.39. Effect of mean minimum temperature recorded during the main activity period of false codling moth (1800 – 0100) in several mark&release experiments.

In general, it consequently seems that ambient temperatures prevent major changes with regard to the release of irradiated FCM during different times of the season. When nights are cold, the moths will live longer (or distribute slower), but their activity will be curtailed. When hot, they will be more active, but their usefulness will be relatively brief. Whether this is due to a short lifespan, or perhaps, a vastly increased drive to distribute (and in doing so, escaping the traps which are distributed in a relatively small area), is unknown.

3.4.5.18 Parasitization of Codling Moth, *Cydia pomonella*, eggs by the egg parasitoid, *Trichogrammatoidea cryptophlebiae*

Experiment 662 by Hendrik and Marsheille Hofmeyr (CRI)

Opsomming

Die vermoë van die VKM-eierparasitoïed, *Trichogrammatoidea cryptophlebiae*, om eiers van 'n ander belangrike vrugteplaag, die Kodlingmot (KM), *Cydia pomonella*, te parasiteer, is in 'n laboratoriumproef ondersoek. Daar is vasgestel dat die parasitoïed wel KM-eiers kan parasiteer en 'n lewendige nageslag daaruit kan produseer. Daar ontwikkel ook gemiddeld heelwat meer eierparasitoïede in KM-eiers as in VKM-eiers.

Introduction

It is to the advantage of any insectary to diversify their production of natural enemies as much as possible to spread the risk of the market for a particular biological agent collapsing for any reason whatsoever. It is equally important to be able to rear a natural enemy that is able to utilize more than one host species. The egg parasitoid, *Trichogrammatoidea cryptophlebiae*, is a well-known parasitoid which is used for the biological control of FCM. This parasitoid's ability to suppress FCM is well known and has also been discussed in the context of augmentative control in an SIT program (Section 3.4.5.10). It has been demonstrated that *Trichogramma platneri* can reduce crop loss by Codling Moth (CM) in field cages (Bloem *et al.*, 1998). SIT research in South Africa is being investigated alongside that of FCM, and it was therefore of interest to know whether *T. cryptophlebiae* will be able to parasitize Codling Moth eggs.

Materials and methods

Five small (75 ml) plastic cages were used. Three freshly-emerged egg parasitoids, two females and one male, were placed into each cage, together with 12 FCM and 12 CM eggs, in both cases less than 24 hours old. They were left *in situ* for 24 hours and were then removed. The cages were incubated at 22°C for the whole of the experiment. Counts were conducted 10 days later to investigate the degree of egg parasitism.

Results and discussion

T. cryptophlebiae was able to parasitize CM eggs successfully even when offered a choice between FCM and CM eggs (Table 3.4.5.14). Many egg shells of both species showed holes where the F1 parasitoids had emerged. An interesting aspect is that the number of offspring produced seems to be closely host egg size related, i.e. the bigger the host egg, the more parasitoids are produced per host. FCM eggs are, on average, approximately one mm in diameter (0,8 mm² surface area), whereas CM eggs are much bigger at 1,2 mm (1,13 mm² surface area). It is normal for FCM eggs to produce two parasitoids per egg, occasionally up to three parasitoid emergence holes can be noticed. In this study, , up to four parasitoid emergence holes were noticed per CM host egg. Additionally, seven fully developed parasitoids have been counted before emergence in a CM egg collected from an apple orchard (Tom Blomefield, pers. com.).

Table 3.4.5.14. The ability of *Trichogrammatoidea cryptophlebiae* to parasitize Codling Moth eggs

Total number of Codling Moth eggs parasitized	40 from 55*
% CM egg parasitism	72.7
Total number of False Codling Moth eggs parasitized	37 from 58*
% FCM egg parasitism	63.8

*Respectively five and two eggs were undeveloped and probably unavailable for parasitism by *T. cryptophlebiae*.

This study shows that *T. cryptophlebiae* is able to parasitize CM eggs under laboratory conditions. From observations by Blomefield it appears that *T. cryptophlebiae* can be field collected from naturally parasitized eggs on apples. It is therefore recommended that this study be continued by concerned parties to investigate the potential contribution that *T. cryptophlebiae* can make – with regard to both the augmentation of SIT efficacy and as stand-alone biological control for CM in the deciduous fruit industry.

Future research

This study is concluded.

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3.4.6 Development of a technique for mass rearing of FCM for SIT purposes

Experiment 689 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

'n Belangrike voorwaarde om sukses met die steriele insektegniek (SIT) te behaal, is die ontwikkeling van 'n stelsel vir die massateling van uiters groot getalle insekte. Die getalle VKM wat met huidige stelsels vir parasitoïedproduksie geteel kan word, is ontoereikend. In hierdie proefreeks is verskeie alternatiewe diëte en veranderings aan die verrykte dieet wat deur CRI gebruik word, ondersoek. Dit sluit in die teling van larwes in groot oop bakke in teenstelling met geseëldde heuningflesse. Ongelukkig is die meeste van die dieetproewe deur 'n hoë vlak van viruskontaminasie in die laboratorium-larwekultuur gekniehalter. Om dié rede was dit soms onmoontlik om betroubare resultate in proewe te kry en was daar min rede om die data te ontleed. Goedkoper bestanddele vir die verrykte dieet is wel gevind. Deur die gebruik van hierdie bestanddele is die koste van die dieet van R1.56 per teelfles tot R0.11 verminder. Die betekenisvolste deurbraak is waarskynlik die ontdekking dat oppervlak-sterilisasie van VKM-eiers met formalien 'n baie doeltreffende manier is om virusbesmettings in larwes te verhoed. Baie van die dieetproewe moet daarom herhaal word. Sterk klem sal op die teel van VKM-larwes in dieet in oop bakke geplaas word.

Introduction

Researchers in the southern African citrus industry, the deciduous fruit industry, the USDA (United States Department of Agriculture) and the IAEA (International Atomic Energy Agency) are collaborating on the investigation and implementation of the sterile insect technique (SIT) for control of false codling moth (FCM), codling moth and fruit flies in the Western Cape. SIT has a history of success in the control of insect pests in various parts of the world. It is important that this system also be tested against FCM on citrus in southern Africa, starting in the Western Cape. The IAEA has agreed to sponsor this project by funding expert advice, overseas visits and the purchase of certain capital equipment. This funding has been pledged until 2005. An important prerequisite to achieving success with SIT is to develop a system for mass rearing extraordinarily large numbers of insects. The numbers of FCM that can be produced with currently known systems, used for parasitoid production, are inadequate. Improvements in FCM mass rearing techniques have already been made (see exp. 402; Moore & Richards, 2000 & 2001). However, these changes were not made specifically with SIT in mind. Improvements geared towards SIT specifically, were only initiated more recently (Moore *et al.*, 2002). Changes are aimed at increasing production while keeping labour inputs and costs as low as possible.

Materials and methods

Diet Improvement

An artificial diet for rearing FCM larvae was developed by CRI a few years ago (Moore & Richards, 2000; Moore, 2002), as a replacement for the old *Rhizopus* sp. fungus inoculated diet (Ripley *et al.*, 1939; Theron, 1948). This is the diet now commonly used by CRI and two commercial insectaries (River Bioscience and Du Roi IPM) for mass rearing FCM larvae. In this report we refer to this diet as the "enriched" diet.

Trial 1:

The enriched diet for FCM larvae was usually prepared by first placing the measured volume of dry ingredients into a glass jar and then adding distilled water. At times, patches of dry diet are observed in the bottles after autoclaving. In an attempt to produce diet of a more consistent nature, 10 bottles were prepared by pre-mixing the diet and water (500g dry ingredients with 500 ml distilled water) and then dispensing the correct volume into each jar. A further 10 jars of the enriched diet were prepared in the conventional manner for comparison. Larval development and the incidence of viral infection were observed and recorded.

Trial 2:

Ten jars of the enriched diet were prepared in the conventional manner, but with the addition of 2 g paper pulp per jar. The paper pulp was added as a bulking agent and in order to provide some separation between penetrating neonate larvae so as to reduce any cannibalism. A further 10 jars were prepared without paper pulp. Development of larvae to pupation was observed.

Trial 3:

A trial was conducted to test the suitability of the codling moth diet used by the Canadian Sterile Insect Release (SIR) facility (Table 3.4.6.1), for rearing FCM larvae. The canola meal was substituted with soy meal, as canola meal was unobtainable. The paper pulp was soaked in distilled water for 2 hours. The ingredients in group 1 (Table 3.4.6.1) were placed in a pot and brought to boil. The ingredients in group 2

were then added and the mixture was boiled for seven minutes. Once the mixture was cooled to 65°C, the third group of ingredients were added and mixed in. The ingredients were then dispensed into four plastic trays (140 mm x 120 mm x 60 mm) and the surface was scarified with a fork. Once the diet had cooled, egg sheets with approximately 1500 eggs each were suspended over each tray. Two trays were covered with glass lids and two were kept uncovered. Dishes were kept at 27°C and approximately 30% RH. The development of the larvae and desiccation of the diet were monitored and noted.

Table 3.4.6.1. Diet for rearing codling moth at SIR facility in Canada.

Group	Ingredient	Quantity
1	Water	7330 ml
	Pulp	75 g
	Saw dust	625 g
2	Soy meal	780 g
	Whole wheat flower	400 g
	Sugar	156 g
	Wheat germ	52 g
	Gluten	30 g
	Fumaric acid	47 g
	Choline chloride	12 g
	Salt mixture	32 g
	Canola oil	15 ml
3	Nipagin (methyl paraben)	9 g
	Vitamin mixture	33 g
	Formaldehyde soln (24 ml of 37% in 600 ml water)	3 ml

Trial 4:

This was a repeat of Trial 3. However, some ingredients were omitted due to their high cost or unavailability in South Africa. The ingredients omitted were: Paper pulp, fumaric acid, choline chloride, salt mixture, nipagin and vitamin mixture (Fumaric acid, choline chloride, salt mixture and vitamin mixture had previously been imported). The trial was conducted twice.

Trial 5:

In another trial, four different variations of the Canadian SIR diet were prepared (Table 3.4.6.2). One tray of each was prepared. Tray 1 was the standard Canadian SIR diet. In Tray 2 the ingredients were the same as in Trial 4 except that nipagin was added to try and prevent fungal contamination of the diet. In Tray 3 the water, paper pulp and sawdust were prepared the same way, but then the dry ingredients of the enriched diet (with increased nipagin and sorbic acid) were added instead of the Canadian ingredients. Tray 4 differed from Tray 3 only in that soy meal was used instead of maize meal.

Table 3.4.6.2. Four variations to the diet used for rearing codling moth at SIR facility in Canada.

Ingredient	Tray 1	Tray 2	Tray 3	Tray 4
Water	458.1 ml	458.1 ml	540 ml	458.1 ml
Pulp	4.7 g	4.7 g	4.7 g	4.7 g
Saw dust	39.1 g	39.1 g	39.1 g	39.1 g
Soy meal	48.8 g	48.8 g		
Whole wheat flower	25 g	25 g	Enriched diet, except that nipagin and sorbic acid were used at 1.5 x the normal rate; total dry ingredients = 98 g.	Enriched diet, except that soy meal was used instead of maize meal and nipagin and sorbic acid were used at 1.5 x the normal rate; total dry ingredients = 98 g
Sugar	9.8 g	9.8 g		
Wheat germ	3.3 g	3.3 g		
Gluten	1.9 g	1.9 g		
Fumaric acid	2.9 g			
Choline chloride	0.8 g			
Salt mixture	2 g			
Canola oil	0.9 ml	0.9 ml		
Nipagin	0.6 g	0.6 g		
Vitamin mixture	2.1 g			

Formaldehyde soln (24 ml of 37% in 600 ml water)	0.2 ml	0.2 ml		
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Development and infection of larvae and contamination and texture of the diet were observed and recorded.

Trial 6:

In this trial, 20 jars were prepared normally with the ingredients of the enriched diet. However, they were cooked in an oven at 180°C for 20 minutes, instead of being autoclaved. Again, nature of the diet and development of larvae were noted.

Trial 7:

This trial was conducted to test various combinations of dietary ingredients and different jar lids with a view to improving larval penetration and development and reducing viral and fungal contamination (Table 3.4.6.3). As it was observed that penetration in diet with sorbic acid appeared to be worse than when it was excluded, treatments were included to test this assertion. Ten bottles of each treatment were prepared.

Table 3.4.6.3. Variations of the enriched diet on which FCM larval development was compared.

	Description
1	Enriched diet and cotton wool stoppers
2	Maize meal (50 g) plus distilled water (50 ml)
3	Dry ingredients (70g) plus distilled water (70 ml) (instead of 50 g + 50 ml, i.e. enriched diet)
4	Dry ingredients (50 g) + distilled water (70 ml)
5	Enriched diet, using lids with a denim septum in jar lids instead of cotton wool stoppers
6	Enriched diet with tap water instead of distilled water
7	Enriched diet with sorbic acid excluded
8	Enriched diet with sorbic acid excluded but nipagin at 1.5 x the normal rate

Nature of the diet and development of larvae were recorded.

Trial 8:

A cosmopolitan insect diet, proprietary product, no. 1 (PP1), developed by Tony Ware, was tested for FCM larvae. The diet was supplied in one fraction as a desiccated powder. The diet was prepared in two honey jars and was bulked up with paper pulp – 2 g paper pulp added to 40 g powder. Boiling distilled water was added, and stirred until a thick paste was formed. The diet was allowed to cool, and approximately 300 FCM eggs were placed onto the diet surface in each jar.

This trial was repeated with another four jars being prepared in the same way as above, except that the paper pulp was added to boiling distilled water and agitated with a Kenwood food processor until it looked like fine wet cotton wool. This was an emulation of the protocol practiced in the Canadian codling moth SIR facility. In two jars the egg sheets were pasted onto the inner side of the glass jar and in the other two, the egg sheets were placed on the diet surface.

Another similar diet, proprietary product no. 2 (PP2), was supplied by Tony Ware. This and the PP1 diet, were tested in open trays (160 mm x 140 mm x 60 mm). Trays were left uncovered. The two diets were mixed as in the previous experiment, with paper pulp and distilled water. The mixed diet was then transferred into open trays and egg sheets with approximately 1000 eggs each were suspended over the trays on wire frames.

Observations were recorded.

Diet cost

The ingredients used in the enriched diet can be quite expensive (Table 3.4.6.4). The cheapest sources for these ingredients were sought and tested as replacements for the originally used ingredients (Table 3.4.6.4). A series of experiments were conducted. In each trial all the original ingredients in the diet were used except for one – a cheaper replacement. In each trial, 6 – 12 jars of diet were prepared with the enriched diet and the same number with the modified diet. One to four replicates were used with each ingredient. Visual observations were made, and the percentage of jars in which larvae developed to pupation without any viral contamination was recorded. Notes were made on penetration density and any other obvious differences between the trials and the control.

Table 3.4.6.4. Cost of originally used ingredients in the enriched diet.

Ingredient	Make	Cost/kg	Quantity/jar	Cost/jar
Distilled water	-	-	50 ml	
Maize meal	Impala Special	R2.20	40 g	8.80c
Wheat germ	Vital	R37.47	4.0 g	14.99c
Brewers Yeast	Vital	R69.90	2.0 g	13.98c
Full Cream Milk Powder	Nespray	R72.48	0.73 g	5.29c
Nipagin	Sigma	R741.00	0.3 g	22.23c
Sorbic acid	Minima	R912.00	0.13 g	91.20c
TOTAL				156.49c

Virus control in diet

Trial 1:

It was noticed in the larval room that jars to the front of the shelf, which were directly exposed to the artificial light, seemed to be better developed and have less viral contamination. A trial was done to test this observation. Twelve jars of diet were placed towards the front of a shelf, directly in the light, while another 12 jars of diet were shielded from the direct light by a piece of cardboard. Viral infection of larvae was monitored and recorded.

Trial 2:

As it is well documented that ultra-violet light rapidly degrades virus particles (Shapiro, 1995), including CrleGV (Moore, 2002), artificial UV light should be tested as a virus control measure in the laboratory. However, there is a possibility that UV light could have a detrimental effect on the survival of larvae. This was tested by exposing FCM larvae, in diet, at three different stages of development (1st instar, 2nd instar and 4th instar) for 1 h each to UV-irradiation. Four jars of each of the three instars of larvae were used. The source of UV-irradiation was a standard germicidal lamp (UV-C; approximately 260 nm wavelength). Development of larvae to adulthood was then observed and compared with that of non-irradiated larvae.

Trial 3:

Three natural anti-microbial agents were tested for their effect on virus contamination in larvae. Five jars of diet were prepared with each of three different additives. These were olive leaf extract, *Echinacea* root extract and Golden Seal, *Hydrastis canadensis*. A total of 2 ml of each anti-microbial agent was added to each 50 ml volume of distilled water which was added to the dry diet ingredients. Larval development and viral infection were recorded.

Trial 4:

Three comparative trials were conducted, each with a different chemical anti-microbial agent in the diet, in an attempt to control viral contamination of larvae. These were formaldehyde (40% concentration); formalin (37% concentration); and Virkon. Each of these were mixed into the 50 ml of distilled water which was added to a jar of dry ingredients of enriched diet: 50 µl of formaldehyde, 150 µl of formalin and 0.05 g Virkon. Diet was then autoclaved for 20 min at 121°C, as per normal. Ten jars were prepared with each treatment and a further 10 jars with the enriched diet and no additive, for comparison. A further comparison was conducted by overspraying the diet surface in 10 jars with Virkon (0.1%) (once it had cooled after autoclaving) and comparing these with the enriched diet without spraying with Virkon. Jars with and without viral contamination were quantified.

Surface sterilisation of eggs

Ceder Biocontrol experienced severe problems with fungal contamination of diet, while CRI experienced severe problems with viral infection of larvae. Consequently, a trial was conducted to test different concentrations of Sporekill as egg surface sterilisation treatments. Four sheets, with approximately 50 eggs each, were used for each treatment: two being rinsed in distilled water after sterilisation and the other two being left unrinsed. Firstly, only the effect of the different concentrations on egg mortality were tested.

A subsequent trial was conducted in which the effect of two Sporekill concentrations on viral infection, was measured. 0.15% Sporekill and 0.5% Sporekill were again used, without rinsing egg sheets after dipping. This time egg sheets were placed into jars of diet; 10 jars per treatment. Observations were made for symptoms of viral infection in larvae and development of larvae.

In another trial, 10% formalin (i.e. 25% of a 40% concentration), 0.2% Sporekill and 0.17% sodium hypochlorite were compared as egg surface sterilisation treatments. Both symptomatic viral infection and larval development were recorded.

Results and discussion

Diet improvement

Trial 1:

Of the 10 jars in which the diet was mixed in the standard way, six showed good development of larvae without viral contamination. Of the 10 jars in which the dry ingredients and the water were pre-mixed, only four showed good development of larvae without viral infection. This difference was probably not significant. In the good jars, there was no noticeable difference in penetration and development of the larvae between the two treatments.

Trial 2:

The addition of paper pulp to diet appeared to have a detrimental effect on larval development. Of the 10 jars in which paper pulp was added to the diet only two showed any development of larvae to pupation. However, in the enriched diet without paper pulp, six out of 10 jars showed development of larvae to pupation. Previous use of paper pulp in non-autoclaved diet did not show any detrimental effect on larvae (Moore & Kirkman, 2003). Autoclaving of the paper pulp must therefore have caused the emission of some substance which was detrimental to larvae.

Trial 3:

In the first trial conducted with the Canadian codling moth diet, where trays were covered (with glass sheets) moisture in trays was too high, creating anaerobic conditions. There was therefore very little penetration of larvae. In the uncovered trays, the texture of the diet looked very good and good penetration of larvae was observed. Desiccation of the diet did not occur too rapidly and development of the larvae appeared satisfactory up to about the fourth instar larval stage. At this stage a high level of virus infection of larvae occurred. Some pupation and moth eclosion did take place, indicating that the diet has good potential.

Trial 4:

In both replicates of this second trial with the modified Canadian diet, the diet desiccated and cracked quite rapidly. This was an indication of the importance of adding paper pulp as a bonding agent. As could be expected, penetration of the diet by larvae was poor. A level of fungal contamination of the diet was also recorded. This was probably due to the omission of nipagin.

Trial 5:

In the third Canadian diet trial, the standard Canadian diet (Tray 1) showed good development of FCM larvae, but as before, a severe outbreak of virus occurred, obscuring any potentially good results. In Tray 2, where fumaric acid, choline chloride and the salt mixture were excluded, a high level of fungal contamination was recorded. This was surprising as nipagin was expected to control this. It is possible that the omission of fumaric acid might have made the diet too alkaline, therefore being more conducive to fungal growth. The diet in Tray 3 (using the enriched diet dry ingredients instead of the Canadian diet dry ingredients) and Tray 4 (as Tray 3 but replacing maize meal with soy meal) was too moist. This probably created anaerobic conditions for larvae. Consequently, many larvae died before penetration took place. There was no sign of fungal contamination in these trays, which is promising. Further work will be done to get the right moisture level in the diet. However, it is possible that maize meal will not be suitable unless autoclaved or boiled for a fairly lengthy period. Results from previous work seem to indicate such (Moore & Richards, 2002).

Trial 6:

Baking of diet in the oven seemed to result in a similar consistency of diet to autoclaved diet. Unfortunately, once again, a high level of viral contamination of larvae occurred in several of the jars of diet, prohibiting any evaluation of development to adulthood. This virus contamination was consistent with the contamination level in the culture as a whole, at the time.

Trial 7:

There was little difference in the development and health of larvae in four out of the eight different diets (Table 3.4.6.5). In the pure maize meal diet, development was substantially poorer than the other treatments. Larvae also took longer to develop. It was also ascertained that tap water was not a suitable alternative for distilled water (although this might differ from area to area) and that it was essential to add sorbic acid to the diet. There was a high level of virus contamination throughout (even in the good treatments), causing none of the treatments to show better than 60% of jars with full development of larvae.

Once again, this was most likely due to high levels of virus in the culture at the time. Different densities of denim cloth should be tested as jar covers to attempt to get the diet to dry out at an optimal rate, as it does with cotton wool stoppers. If such a material proves to be suitable, it will nevertheless not provide a pupation substrate for larvae. An alternative pupation substrate will therefore have to be created or cloth lids could be used on jars from which larvae will be used for virus production.

Table 3.4.6.5. Comparison of FCM larval development and contamination on different variations of the enriched diet.

Description	% Jars with full development of larvae without viral and fungal contamination	<u>Comments</u>
Enriched preparation (50g dry ingredients plus 50 ml distilled water)	60	-
50g maize meal plus 50 ml distilled water	10	Bad fungal contamination, due to no sorbic acid or nipagin
70g distilled water plus 70g dry diet ingredients (instead of 50g of each)	60	Penetration and development comparable to standard jars
70g distilled water plus 50g dry diet ingredients (instead of 50g of each)	50	Diet slightly 'runny', less penetration occurred.
Using lids with a denim septum in place of cotton wool stoppers	50	Penetration was comparable to normal, but development was slightly less, as the diet dried more rapidly
Enriched diet with tap water instead of distilled water	0	Initial larval penetration was good, but all the trial bottles developed viral contamination.
Enriched diet with sorbic acid excluded	10	Initial penetration appeared better than in enriched diet, but green fungus developed in all the jars, with only one showing development to pupation.
Enriched diet with sorbic acid excluded but nipagin at 1.5 x the normal rate	0	All jars developed fungal contamination.

Trial 8:

In the first replicate with the PP1 diet, a high level of egg mortality was recorded, possibly due to the mixture being too moist. However, the larvae which did hatch and penetrated into the diet, developed well and appeared to develop slightly faster than larvae in the enriched diet.

In the second replicate with the PP1 diet, penetration was still poor even where eggs had not been placed in direct contact with the diet. A positive point was that there were no signs of viral or fungal infection.

In the open-tray trial, the PP1 diet was too soggy and must therefore have provided somewhat anaerobic conditions for the larvae. Therefore, larval penetration was poor and no development of larvae took place. The PP2 diet was extremely hygroscopic and formed a thick, peanut-butter-like paste. Initial penetration was good but the diet shrunk markedly. Good development did take place until just before pupation, when viral contamination of the larvae occurred. The tray was put into an eclosion box, and approximately 25% of the larvae survived the virus outbreak to develop to adulthood. This trial will be repeated when the virus levels in the culture are brought under control.

Diet cost

As the enriched diet was being prepared on a fairly small scale, primarily for research purposes, cost of ingredients was not of paramount importance. Now that it has been ascertained that this diet is suitable and effective for rearing FCM larvae, commercial insectaries can use it. Cost now becomes a more important issue. Several potential replacements for the original, more expensive, ingredients were identified (Table 3.4.6.6).

Table 3.4.6.6. FCM larval development on the enriched diet where original ingredients were replaced with cheaper alternatives.

Ingredient	Alternative make	Control (enriched diet): Jars to full development (%)	Alternative: Jars to full development (%)	<u>Comments (comparison to enriched diet with original ingredients)</u>
Maize meal	Bokomo Purity	60	70	Comparable penetration and development
	Bokomo Sifted	83	66	Comparable penetration and development
	Bokomo Special	83	50	Comparable penetration and development
	White Star Super	66	50	Penetration less and development retarded in trial bottles
	Blue Star Unfortified	40	30	Comparable penetration and development
Wheat germ	Sasko	70	40	Penetration was weaker, possibly due to higher oil content.
	Sasko	50	60	Comparable penetration and development. Much better results than when first tested.
	Sasko	70	60	This trial was repeated due to conflicting results in earlier trials. In this trial, there was comparable penetration and development
	Sasko	75	75	Comparable penetration and development
	Roller	75	67	Comparable penetration and development
	Pouyoukas	60	60	Comparable penetration and development
Brewers yeast	Anchor	70	60	Comparable penetration and development
	Anchor Nutrivin	50	0	Bottles blackened when autoclaved, virtually no penetration or development
	SAB	67	77	Comparable penetration and development
Full Cream Milk Powder	Clover	60	60	Penetration and development better in the trial bottles.
Nipagin	Chempro	66	75	Better development
Sorbic acid	Protea	70	80	Comparable penetration and development

The cost of producing the enriched diet has been dramatically reduced (Table 3.4.6.7). It is now far more affordable for a commercial insectary to use. This adds to the existing benefit of a substantial reduction in contamination (fungal and bacterial) of the diet (Moore & Richards, 2000; Moore, 2002).

Table 3.4.6.7. Comparative cost of alternative ingredients and original ingredients in the enriched FCM diet.

Ingredient	Original ingredient cost/jar	Alternative make	Alternative make cost/kg	Alternative make cost/jar
Distilled water	-	-	-	-
Maize meal	8.80c	Bokomo Sifted	R1.40	5.60c
Wheat germ	14.99c	Sasko	R3.00	1.20c
Brewers Yeast	13.98c	SAB	R3.76	0.75c
Full Cream Milk Powder	5.29c	Clover	R25.85	1.89c
Nipagin	22.23c	Chempro	R39.50	1.18c

Sorbic acid	91.20c	Protea	R36.00	0.47c
TOTAL	156.49c			10.99c

Virus control in diet

Trial 1:

Viral contamination developed in the larvae in seven out of 12 jars (58%) which were shielded from light, while larvae in only two out of the 12 jars (17%) which were directly exposed to light developed viral contamination before pupation (no fungal contamination occurred in either of the treatments). This result could be due to the effect of UV rays in the artificial light having an effect on virus in the jars. Jars of diet should therefore be kept directly exposed to light whenever possible.

Trial 2:

The UV exposure did not appear to have any effect on the larvae, but also did not appear to control virus. Larvae developed at the same rate in all jars and there was a consistent level of viral contamination throughout (Table 3.4.6.8). The lack of control of viral infection was not necessarily an indication that the UV-light was ineffective against virus, but possibly that virus infection of larvae took place very early on in the life cycle. The introduction of UV-light, even at the first instar stage, might have been too late as larvae might already have been infected, albeit not yet symptomatically.

Table 3.4.6.8. The effect of UV-irradiation on viral contamination of larvae.

Larval instar	Number of jars (out of 4) with virus infected larvae	
	Jars exposed to UV light	Jars not exposed to UV light
1	1	2
2	2	2
4	2	1

Trial 3:

Larval penetration in the diet with olive leaf extract looked particularly good. Unfortunately, a high level of viral contamination of larvae ensued, indicating the ineffectiveness of all three of the additives.

Trial 4:

Adding 50 µl of formaldehyde to the diet in each jar before autoclaving did not appear to reduce viral contamination. Larvae in 35% of the control jars (i.e. without formaldehyde) completed development without signs of viral infection. Larvae in 40% of the jars, with formaldehyde added to the diet, completed development without signs of viral infection.

Adding 150 µl of formalin per diet jar before autoclaving did appear to eradicate viral contamination, but led to reduced larval penetration relative to the enriched diet without formalin. Larvae in 83% of the control jars (i.e. without formalin) completed development without signs of viral infection. Larvae in 100% of the jars, with formalin added to the diet, completed development without signs of viral infection. However, it is not clear whether this difference was significant. This trial should be repeated.

Neither the addition of Virkon to the diet or the overspraying with Virkon appeared to reduce viral contamination of larvae. With the former treatment, larvae in 60% of both treated and untreated jars completed development without signs of viral infection. With the overspraying treatment, larvae in 50% of jars developed uncontaminated, compared to 60% of untreated jars.

Surface sterilisation of eggs

Rinsing of egg sheets after dipping in Sporekill, reduced the mortality of eggs caused by the Sporekill treatment (Table 3.4.6.8). Without rinsing, mortality of eggs reached an unacceptable level after treatment with 1% Sporekill (a 15 times higher concentration than the standard usage (Moore, 2002)), although egg mortality was not significantly higher than that of untreated eggs (Table 3.4.6.9).

Table 3.4.6.9. The effect of various Sporekill treatments on FCM egg mortality.

	Sporekill concentration	% Eggs hatched	
		Unrinsed	Rinsed
1	0%	81.4a	87.0a
2	0.2%	86.5a	86.8a
3	0.5%	78.5ab	83.6a
4	1%	73.9ab	80.5ab
5	3%	69.6ab	77.8ab
6	5%	50.0b	73.6ab

*Values in the last two columns followed by the same letter are not significantly different ($P>0.05$; Bonferonni LSD multiple range test).

In a subsequent trial, larvae in 30% of diet jars in which the egg sheets were sterilised in 0.15% Sporekill developed viral infection, while 70% of bottles in which the egg sheets were sterilised in 0.5% Sporekill developed viral infection. This vast difference might have been purely by chance, unless the stress caused by the higher concentration of Sporekill led to a lowering in the immunity of the larvae. Nevertheless, this trial indicated that Sporekill was not effective for controlling virus, confirming what was regularly observed in the laboratory culture of FCM, where Sporekill dipping was the standard egg treatment.

In the next trial, larvae in 100% of bottles in which egg sheets were surface sterilised with formalin, completed development without viral contamination. Surface sterilisation of eggs with 0.2% Sporekill and 0.17% sodium hypochlorite resulted in 67% and 0% completion of larval development without viral infection, respectively. Sodium hypochlorite was shown by Ludewig (2003) to be an effective virucide and by Moore (2002) to be an effective fungicide. However, in both cases the concentrations required were highly detrimental to egg survival. Unquestionably, the most important finding of all was made in this last trial: formalin egg treatment appeared to hold the key to dramatically reducing viral contamination in the FCM culture. It is ironical that when CRI began work on FCM rearing, formalin was the standard surface sterilisation treatment for eggs (Moore & Fourie, 1999). Formalin was soon discarded as it was found to inadequately control fungal contamination and caused a level of egg mortality (Moore, 2002). At that time, fungal control was very important, as the septic *Rhizopus* sp. inoculated diet was still being used. Viral contamination was not an issue then, as the formalin was probably inadvertently controlling this. However, formalin was tested again following an investigation into the observed absence of viral contamination in Ceder Biocontrol insectary (Hendrik Hofmeyr, pers. com., also see Table 3.4.5.10).

Conclusion

A consistent problem which undermined most of the diet trials was a high level of virus contamination in the laboratory culture of FCM larvae. For this reason, results of trials were obscured and proper analysis of trials became superfluous. What was ascertained, was that there were cheaper alternatives for all ingredients in the enriched diet. By using these, the cost of the diet was reduced from R1.56 per jar of diet to 11c per jar. Probably the most significant breakthrough in this experiment was the discovery that surface sterilisation of FCM eggs with formalin was an extremely effective means of preventing a virus outbreak in larvae.

Future research

Due mainly to the high level of virus contamination in the culture, many of the trials conducted (particularly the diet trials) will have to be repeated. A strong emphasis will be placed on testing larval diets in open trays.

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3.4.7 Understanding and improving biological control of false codling moth larvae Experiment 690 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

'n Weeklikse opname van parasiete van VKM-larwes is gedurende Desember 2003 tot Mei 2004 in 'n nawellemoenboord in Sondagsriviervallei uitgevoer. *Agathis bishopi* is die enigste parasitoïed wat van VKM-larwes verkry is. Oor die volle proeftydperk is bietjie meer as 11% larwes geparasiteer. Parasitisme het in Desember en April onderskeidelik pieke van 40% en 37.5% bereik. Die hoogste vlak van parasitisme is in derde instar stadium larwes gekry wat uit vrugte versamel is. Dit dui aan dat meeste geparasiteerde larwes in die derde instar stadium of jonger aangeval word. 'n Regressie-analise het gewys dat daar nie 'n statisties-betekenisvolle verhouding tussen vlak van vrugbesmetting en vlak van larweparasitisme in die seisoen is nie. As data egter op 'n grafiek aangetoon word, is dit duidelik dat die twee faktore mekaar kon beïnvloed het.

In Desember 2004 is 'n aantal parasitoïede versamel om hulle in die laboratorium te probeer teel. Van die larwes wat uit nawellemoene versamel is, was gemiddeld 12.2% deur *A. bishopi* geparasiteer. 42.9% van eerste instar stadium larwes wat versamel is, was geparasiteer. Die data het weer bevestig dat parasitisme vroeg in die lewensiklus van die larwe plaasvind. Ongelukkig is daar nie in die laboratorium 'n tweede generasie van *A. bishopi* gekry nie. *A. Bishopi* sal weer versamel word om 'n laboratoriumkultuur te begin. As hierdie poging suksesvol is, sal vrylatings en translokasieproewe met *A. bishopi* uitgevoer word.

Introduction

Much emphasis has been placed on studying and exploiting the egg parasitoid of FCM, *Trichogrammatoidea cryptophlebiae*. The next step in advancing the biological control of FCM is to examine the potential for improvement of control of the larval stage. A total of nine larval or egg-larval parasitoids have been identified from FCM on citrus in southern Africa. Six of these species occur in South Africa. Larval parasitoids of FCM have been discussed by Ulyett (1939), CIBC (1984) and Prinsloo (1984). They speculated that, perhaps due to the inaccessibility of the host, they do not seem to be important mortality factors. Ulyett (1939) found that many of the larval parasitoids were poorly distributed and suggested the exchange of parasitoids between the different provinces of South Africa. However, the distribution, seasonal occurrence and effectiveness of these parasitoids is not sufficiently clear. Knowledge of the natural enemies of a pest species and the control they exert is important when considering commercial control measures. Such a survey may lead to the translocation of one or more species of parasitoid from one area to another or a parasitoid augmentation programme.

A preliminary survey on FCM larval parasitoids was conducted in the Eastern Cape, Western Cape and Mpumalanga from December 2001 to May 2002 (Sishuba *et al.*, 2002; Sishuba, 2003). Two parasitoids were reared from FCM larvae in this study: *Agathis bishopi* and *Apophua leucotretae*. *Agathis bishopi* was the more abundant of the two and appeared to be a valuable parasitoid of FCM on citrus, but was only found in the Eastern Cape Province. *Agathis bishopi* and *T. cryptophlebiae* seemed to compliment each other. *Agathis bishopi* exhibited high parasitism rates early in the season, at a time when *T. cryptophlebiae* was either absent or at very low levels. Egg parasitism increased in the latter part of the season when the larval parasitoid was at low levels. It is interesting, therefore, to speculate on the effect of releasing large numbers of the larval parasitoid in the latter part of the season and the egg parasitoid in the early part of the season, when wild populations of the parasitoids are often low. Because these surveys were only conducted

monthly, during the 2003/04 season weekly surveys of larval parasitoids were conducted in an Eastern Cape navel orange orchard with consistently high levels of FCM activity. Results up to the end of 2003 were reported in the previous CRI annual report (Moore & Kirkman, 2003).

Materials and methods

An orchard of navel oranges in the Sundays River Valley (orchard 1, Carden Farm) with a reputation for FCM problems was selected for the trial. Ten trees were marked and fruit underneath the trees were removed on 26 November 2003. Weekly, from 3 December, fruit that had dropped from the 10 trees was collected and taken back to the laboratory, where they were dissected and inspected to determine the cause of drop. FCM infestation was recorded. Simultaneously approximately 100 apparently infested fruit were collected from within the same orchard. These fruit were dissected carefully so as not to damage any live FCM larvae. Larvae were removed from fruit and transferred to glass vials containing plugs of the artificial diet, used for rearing FCM (Moore, 2002; Moore & Richards, 2001). A tightly fitting cotton wool plug was inserted into the opening of each glass vial. The life stage of the larva in each vial was recorded on the vial. These were monitored daily for parasitoid emergence. Parasitoids were identified and the life stage from which the parasitoid emerged was recorded.

On 1 December 2004 an attempt was made to mass collect parasitoids for rearing. A few hundred navel oranges, which had dropped from trees in an FCM infested orchard on Carden Farm were collected. In the laboratory, fruit were dissected, larvae recorded and placed individually on artificial diet in glass vials, as previously described. Emerging parasitoids were recorded and placed in a gauze cage. Parasitoids were provided with honey droplets and water saturated cotton wool. FCM infested diet in jars was placed into these cages. Larvae in the diet were at the first or second instar stage.

Results and discussion

Although the December 2003 results are recorded in the previous report (Moore & Kirkman, 2003), they have also been included here for the sake of continuity. From December 2003 to May 2004 a total of 1158 fruit were inspected from the 10 data trees (Table 3.4.7.1). This represented a loss of 115.8 fruit per tree over a 25-week period. Over this period 23.5% of all fruit collected were infested with FCM. Infestation peaked during December and also reached high levels in mid-January, mid-February and mid-May. More than half of the number of larvae collected from fruit were in the third instar.

Table 3.4.7.1. FCM infestation of navel oranges collected from four study sites.

Collection date	Number of fruit collected from 10 data trees	Number of fruit infested	Number of each larval instar					Total number of larvae collected
			1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar	
03/12	295	32	0	1	8	2	1	12
10/12	220	22	0	7	5	0	0	12
17/12	127	37	1	2	5	2	3	13
23/12	94	12	0	1	2	1	0	4
30/12	49	10	1	1	0	1	1	4
06/01	33	7	1	0	0	3	0	4
13/01	39	18	0	0	1	2	0	3
20/01	18	9	0	0	1	2	0	3
27/01	22	8	1	0	0	0	1	2
03/02	7	3	0	0	0	0	0	0
10/02	14	5	0	1	2	0	0	3
17/02	36	22	0	0	2	2	4	8
24/02	25	15	0	0	0	0	1	1
02/03	20	8	0	0	2	3	3	8
09/03	12	4	0	0	1	0	0	1
16/03	12	6	0	0	0	2	3	5
24/03	13	5	1	0	1	0	0	2

30/03	16	6	0	0	6	0	0	6
06/04	16	4	0	0	1	0	3	4
13/04	16	8	0	0	8	0	0	8
21/04	14	4	0	0	4	0	0	4
28/04	6	4	0	0	0	2	2	4
05/05	19	5	0	0	5	0	0	5
12/05	25	14	0	0	14	0	0	14
19/05	10	4	0	0	4	0	0	4
Total	1158	272	5	13	72	22	22	134

Over the full trial period, just over 11% of larvae were found to be parasitised. It is possible that this figure could be an underestimate as many larvae died inexplicably. These larvae were not included in the calculation. However, a number of them could have been parasitised. Parasitism peaked at 40% in December and 37.5% in April (Table 3.4.7.2). This coincided with findings two seasons ago, when the highest levels of parasitism found in two separate surveys in the Sundays River Valley and the Gamtoos River Valley were 34% and 37% respectively (this was recorded in mid-December) (Sishuba, 2003; Sishuba *et al.*, 2002). All parasitoids were identified as *Agathis bishopi*. This was also the only species of larval parasitoid found attacking FCM in the Eastern Cape during the 2001/02 season (Sishuba, 2003; Sishuba *et al.*, 2002). The highest level of parasitism was recorded for larvae dissected from fruit in the third instar. This indicates that the majority of larvae are parasitised as third instars or younger. This supports a similar finding in laboratory trials (Sishuba, 2003; Sishuba *et al.*, 2002). All parasitoids emerged from either the FCM pupa or the final instar larva. Prinsloo (1984) refers to *A. bishopi* as a larval parasitoid. However, Sishuba (2003) disputes this and claims it to be a larval-pupal parasitoid. It appears that both researchers are correct.

Table 3.4.7.2. Parasitism of the different FCM larval instars and parasitoid sex ratios.

Collection date	% Larvae parasitised (excluding larvae which died)	Number of each larval instar parasitised				
		1st	2nd	3rd	4th	5th
03/12	28.6	0	0	2	0	0
10/12	40.0	0	1	1	0	0
17/12	12.5	1	0	0	0	0
23/12	33.3	0	0	0	1	0
30/12	0	0	0	0	0	0
06/01	5.9	0	0	0	1	0
13/01	0	0	0	0	0	0
20/01	11.1	0	0	1	0	0
27/01	11.1	0	0	0	0	1
03/02	10.0	0	1	0	0	0
10/02	11.1	0	0	1	0	0
17/02	0	0	0	0	0	0
24/02	12.5	0	0	1	0	0
02/03	22.2	0	0	1	0	1
09/03	0	0	0	0	0	0
16/03	11.1	0	0	0	1	0
24/03	22.2	0	0	2	0	0
30/03	25.0	0	0	3	0	0
06/04	11.1	0	0	1	0	0
13/04	37.5	0	1	1	1	0
21/04	12.5	0	0	0	0	1
28/04	0	0	0	0	0	0
05/05	0	0	0	0	0	0

12/05	0	0	0	0	0	0
19/05	18.2	0	1	1	0	0
Total (of 238 larvae)	27	1	4	15	4	3
% larvae parasitised	11.3	3.7	14.8	55.5	14.8	11.1

A regression analysis revealed no statistically significant relationship between the level of infestation of fruit and the level of larval parasitism throughout the season. However, by graphically displaying these results, it appears that there might be a relationship between these two factors (Fig. 3.4.7.1). Peaks in infestation seemed to be followed by peaks in parasitism and peaks in parasitism seemed to lead to dips in infestation levels. However, this is fairly speculative as other ecological factors are also at play, such as the occurrence of FCM generations throughout the year (six in total) (Newton, 1998) and the impact of other natural enemies, such as egg parasitoids and granulovirus (Moore *et al.*, 2002), on FCM.

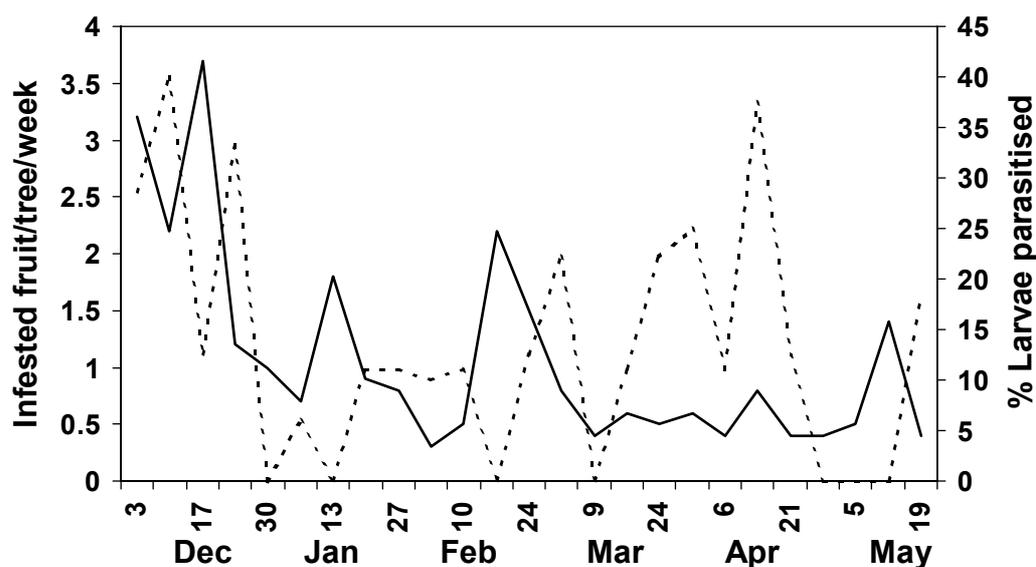


Fig. 3.4.7.1. Weekly FCM infestation of navel oranges (solid line) and parasitism by *A. bishopi* of FCM larvae (dotted line) on Carden Farm (SRV) from December 2003 to May 2004.

Of the larvae collected from navel oranges in December 2004, 12.2% were parasitised by *A. bishopi* (Table 3.4.7.3). However, 42.9% of larvae collected as first instars were parasitised. Again confirming that parasitism of larvae takes place fairly early on in the larval cycle. This makes sense, as even though *A. bishopi* has a relatively long ovipositor, it will not be able to reach a larva once it has penetrated further than a centimetre or two into the fruit.

Table 3.4.7.3. Parasitism of FCM larvae collected from navel oranges on Carden Farm in Sundays River Valley in December 2004.

FCM larval instar	Number of larvae collected	Number of larvae parasitised with <i>A. bishopi</i> males	Number of larvae parasitised with <i>A. bishopi</i> females	% Larvae parasitised by <i>A. bishopi</i>
1	7	3	0	42.9
2	32	2	2	12.5
3	48	3	3	12.5
4	19	0	2	10.5
5	17	0	0	0
Total	123	8	7	12.2

Unfortunately, no second generation of *A. bishopi* was obtained in the laboratory. Parasitoids lived for between one and three days. Many of the larvae in the diet introduced into the parasitoid cages developed virus infections. Therefore, even if they had been parasitised it would not have been detected.

Conclusion

During the second season that a survey of larval parasitoids of FCM was conducted in the Eastern Cape, *Agathis bishopi* was once again the only parasitoid found. Parasitism peaked at 40% of larvae at one time (in December) with most parasitism occurring in the first three larval instars. Graphically displayed data indicates that there might be a relationship between the level of FCM infestation of fruit and the level of larval parasitism by *A. bishopi*. However, this is speculative. An attempt to start a laboratory culture of *A. bishopi* was not successful.

Future research

Further collections of *A. bishopi* will be made with a view to starting a laboratory culture of the parasitoid. If this is successful, then augmentation and translocation experiments will be conducted with *A. bishopi*.

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3.4.8 Investigation of alternative hosts for FCM

Experiment 743 by Sean Moore and Wayne Kirkman (CRI)

Opsomming

Dit is onseker watter invloed alternatiewe gasheerplante aangrensend aan sitrusboorde op VKM-bevolkings in daardie boorde het. Opnames is op 'n gereelde basis in drie streke in die Oos-Kaap uitgevoer. Vrugte, galle en vlesige gedeeltes van moontlike alternatiewe gasheer vir VKM is versamel. Monsters van elk is vir tekens van VKM ondersoek. Ander monsters is aan dagoue VKM-larwes blootgestel en twee weke later vir tekens van besmetting, indringing en larwale ontwikkeling ondersoek. VKM-larwes is in *Ricinus communis* en *Crassula ovata* gevind, terwyl 'n verdagte VKM-larwe in *Opuntia ficus-indica* aangetref is. Omdat *Cryptophlebia peltastica* larwes in die sade van *Schotia afra* bome gekry is, word die moontlikheid dat hierdie plant ook 'n gasheer vir VKM is, verder ondersoek. Sulke bevindinge kan implikasies inhou vir die bestuur van plante in en om sitrusboorde. Die opname word voortgesit.

Introduction

Much emphasis is placed on the importance of orchard sanitation to keep FCM under control. However, little is known of alternative hosts for FCM. In the presence of alternative hosts, even diligent orchard sanitation might only have a limited impact on reducing FCM levels.

A recent survey in the Western Cape revealed very few alternative hosts (Stephan Honiball, section 3.4.10). This might not be the case in the Eastern Cape, as FCM infestation appears to peak a lot earlier in the season than is the case in the Western Cape, indicating a build-up on other hosts before citrus fruits are available in meaningful quantities. The discovery of alternative hosts could lead to better management of these hosts, where they occur within or adjacent to citrus producing areas. Stofberg (1939) and Schwartz (1981) have reported the following cultivated hosts (other than citrus) and wild hosts for FCM (Table 3.4.8.1):

Table 3.4.8.1. Literature recorded cultivated hosts (other than citrus) and wild hosts of FCM Stofberg (1939) & Schwartz (1981).

Cultivated hosts	Wild hosts
<i>Quercus robur</i>	<i>Ziziphus mucronata</i>
<i>Prunophora armeniaca</i>	<i>Diospyros lycoides</i>
<i>Persia americana</i>	<i>Schotia brachypetala</i>
<i>Punica granatum</i>	<i>Podocarpus latifolius</i>
<i>Gossypium hirsutum</i>	<i>Diospyros mespiliformis</i>
<i>Psidium guajava</i>	<i>Dovyalis caffra</i>
<i>Litchi chinensis</i>	<i>Psuedolachnostylis maprouneifolia</i>
<i>Macadamia ternifolia</i>	<i>Mimusops zeyheri</i>
<i>Mangifera indica</i>	<i>Sclerocarya caffra</i>
<i>Juglans sieboldiana</i>	<i>Combretum apiculatum</i>
<i>Olea europeae</i>	<i>Ximenia caffra</i>
<i>Diospyros virginiana</i>	<i>Bequaertiodendron magalisonatum</i>
<i>Amygdalus persica</i>	<i>Syzygium cordatum</i>
<i>Prunophora domestica</i>	<i>Annona senegalensis</i>
<i>Sorghum halepense</i>	
<i>Annona cherimoya</i>	

Materials and methods

At regular intervals during the year, four surveys were conducted within areas of wild vegetation (both on and adjacent to citrus farms), at three sites in the Eastern Cape (Table 3.4.8.2). Fruiting, gall forming and succulent plants were noted. Plant parts bearing fruit and galls and fleshy parts were collected. A minimum of 10 such parts were collected for each plant species. Therefore, at least five of the fruits and galls were dissected to check for FCM infestation, while at least five other seemingly healthy fruits and galls were exposed to 4-6 neonate FCM larvae each. These fruits and galls were kept at approximately 27°C and then dissected two weeks after exposure and inspected for the presence of FCM larvae or pupae. If these were found, an attempt was made to rear them to adulthood for identification. Adult moths were sent to either the Plant Protection Research Institute or the Transvaal Museum for identification. Plant species, where possible, were identified with the use of the following references: Gledhill (1981), Shearing (1994), Urton (1993), Van Wyk et al (1998), Von Breitenbach (1985), G Kerley and M Landman (personal communication).

Table 3.4.8.2. Details of the three sites used for investigating alternative hosts for FCM.

Area	Farm	GPS Coordinates
Kirkwood, Sundays River Valley	Welgelegen	S 33° 25' 15.2" E 25° 24' 33.3"
Sunland, Sundays River Valley	Woodridge	S 33° 28' 43.4" E 25° 41' 38.1"
Uitenhage	Citrus Foundation Block	S 33° 46' 26.9" E 25° 19' 36.7"

Results and discussion

Table 3.4.8.3. Results of a survey of alternative hosts for FCM done on 09/06/04.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Citrus Foundation Block	<i>Acacia karroo</i>	Pods	No infestation or signs of penetration.	Some seeds in the pods were damaged; no larvae found
Citrus Foundation Block	<i>Solanum nigrum</i>	Fruit	No infestation or signs of penetration.	Found 3 larvae – apparently not FCM
Citrus Foundation Block	<i>Eucalyptus torelliana</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Sarcostemma viminale</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Opuntia ficus-indica</i>	Fruit	No infestation or signs of penetration.	Found 1 larva closely resembling a 4 th instar FCM larva; failed to develop to adulthood for identification.
Citrus Foundation Block	Unidentified	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Aloe speciosa</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Azima tetraacantha</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Mesembryanthemum sp</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Lycium afrum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Viscum rotundifolium</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Rhoicoccus tridentata</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Grewia robusta</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Cirsium vulgare</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Solanum tomentosum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Schotia afra</i>	Pods	No infestation	Signs of

			or signs of penetration.	penetration, but no larvae found
Woodridge	<i>Asclepias physocarpa</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Euphorbia bothae</i>	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Euphorbia ledienii</i>	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Acacia karroo</i>	Galls	Found several larvae; did not survive – no identification possible.	No infestation or signs of penetration.
Welgelegen	<i>Rhus longispina</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Aloe speciosa</i>	Hard flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Melia azedarach</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.

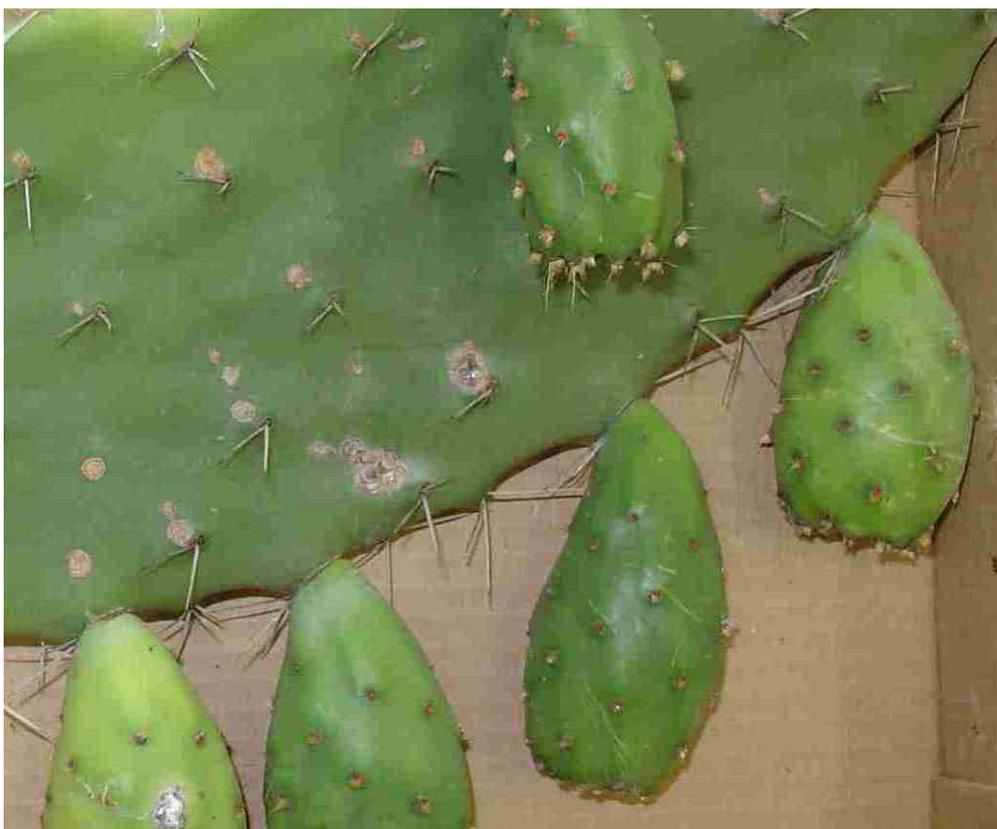


Fig 3.4.8.1. A larva resembling a fourth instar FCM larva was found in a fruit of *Opuntia ficus-indica*.

Table 3.4.8.4. Results of a survey of alternative hosts for FCM done on 18/08/04.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Woodridge	<i>Solanum tomentosum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Cirsium vulgare</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Casuarina sp.</i>	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Crassula sp.</i>	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Senecio radicans</i>	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	Unidentified	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	Unidentified	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Senecio sp</i>	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Eucalyptus torelliana</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Jacaranda mimosifolia</i>	Pod	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Aloe ferox</i>	Fruit	Found 2 larvae which could not be reared to adulthood.	No infestation or signs of penetration.
Woodridge	<i>Aloe africana</i>	Seeds	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Schotia afra</i>	Pod	No infestation or signs of penetration.	Pods penetrated, frass, but no larvae visible.
Citrus Foundation Block	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Schotia latifolia</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Crassula ovata</i>	Stem and	No infestation or	One 5 th instar

		leaves	signs of penetration.	larva found & reared. Moth identified as FCM.
Welgelegen	<i>Ricinus communis</i>	Seeds	No infestation or signs of penetration.	One larva found in hard seed kernel. Reared to moth and identified as FCM male



Fig 3.4.8.2. A larva was found in the stem of *Crassula ovata*, from which emerged an FCM female.



Fig, 3.4.8.3. A larva was found in a seed of *Ricinus communis*, which developed into an FCM male.

Table 3.4.8.5. Results of a survey of alternative hosts for FCM done on 30/09/04

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Citrus Foundation Block	<i>Ricinus communis</i>	Seeds	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Ficus capensis</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Passiflora sp</i>	Fruit	No infestation or signs of penetration.	One penetration mark, no larvae found
Citrus Foundation Block	<i>Lycium afrum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.

Table 3.4.8.6. Results of a survey of alternative hosts for FCM done on 15/11/04

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Welgelegen	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Crassula ovata</i>	Stem	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Schotia afra</i>	Pods	5 larvae found resembling FCM. 2 moths reared – similar to FCM but darker grey; identified as <i>Cryptophlebia peltastica</i> .	Four penetration marks, no larvae found.
Welgelegen	<i>Solanum retroflexum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Cynanchium sp</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Aloe africana</i>	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Aloe ciliaris</i>	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Scutia myrtina</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Datura ferox</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	Unidentified	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	Unidentified	Fruit	No infestation or	No infestation or

			signs of penetration.	signs of penetration.
Welgelegen	Unidentified	Seed	No infestation or signs of penetration.	No infestation or signs of penetration.

During these surveys, only two of the plant species collected were confirmed to host FCM. The jade plant, *Crassula ovata* (Table 3.4.8.4), occurs abundantly in the Addo area. However, FCM is known as a fruit and nut feeder. Therefore, the stem boring of this species could be viewed as aberrant behaviour. It is unlikely that this plant would host high numbers of FCM.

Ricinus communis (Table 3.4.8.4) has been reported as a host for FCM in Israel (Yael Argov, personal communication). This invasive weed is commonly known as the castor oil plant. It is very fast growing. This weed could contribute to FCM infestations in citrus orchards, as they are commonly found in areas where bush has been cleared and where there is an abundance of water. More attention should be paid by growers to eradicating this weed in and around their orchards.

Although the larva found in the fruit of the prickly pear, *Opuntia ficus-indica*, could not be reared to adulthood, it closely resembled a fourth instar FCM larva (Table 3.4.8.3). The prickly pear flowers during August and September, and fruit is set soon thereafter. Therefore it is possible that this plant could host FCM and help build up numbers leading to the typical peak in infestation in citrus during December. This plant occurs in abundance in the Uitenhage, Addo and Kirkwood areas. It is not yet conclusive that *Opuntia ficus-indica* does host FCM, but further work is being done to investigate this. If it were proven, then growers would be advised to clear as many of the plants in the areas adjacent to citrus orchards as possible, and consider the abundance of prickly pear plants in an area before deciding to develop citrus orchards there.

Cryptophlebia peltastica was identified attacking the pods of *Schotia afra* (Table 3.4.8.6). As FCM is very closely related to this moth and as FCM has previously been recorded attacking *Schotia brachypetala* (Schwartz, 1981) it is quite possible that FCM could also be found attacking *S. afra*. Surveys and trials to test this possibility will be continued.

Future research

The plant species which have been shown to host FCM should be investigated more thoroughly in order to determine the level of FCM infestation which they experience. High numbers of them should be dissected and searched for the presence of FCM. The search for other alternative hosts for FCM needs to continue. A possible improvement to the trial technique could be to study and identify species considered more likely to host FCM and to search areas adjacent to citrus orchards for these plants.

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- 3.4.9 **Some morphological features of the immature stages of the false codling moth, *Cryptophlebia leucotreta* (Meyrick), and comparison with related *Cryptophlebia* spp. (Lepidoptera: Tortricidae)**
 Experiment US-AT DNS by Alicia Timm (University of Stellenbosch)

Opsomming

Akkurate uitkenning van die eiers, larwes en papies van die valskodlingmot, *Cryptophlebia leucotreta*, is meesal moeilik ten spyte van die plaag se belang vir plaagbestuur- en uitvoerdoeleindes. Tot nou toe was dit nie moontlik om te onderskei tussen *C. leucotreta* and naby-verwante tortrisied-motte soos die lietsjiemot, *C. peltastica* en die makadamianeutboorder, *C. batrachopa* nie. Die eiers, finale instar larwes en papies van *C. leucotreta* is ondersoek om taksonomies-belangrike strukture vir juiste identifikasie vas te stel. Materiaal

is bestudeer deur gebruikmaking van digitale mikrofotografie en skandeer-elektronmikroskopie. Om identifikasie te vergemaklik, word lyntekeninge en mikrofoto's aangebied tesame met geskrewe beskrywings van taksonomies-belangrike strukture. Verder is vergelykings gemaak tussen die morfologieë van die onvolwasse stadia van *C. peltastica* en *C. bachatropa* en sleutels is opgestel om tussen hulle volgroeiende larwes te onderskei. Aangesien uitvoerbesendings van Suid-Afrikaanse vrugte dikwels op grond van die aanwesigheid van nie-identifiseerbare tortrisied-motte verwerp word, sal verdere navorsing fokus op die ondersoek en beskrywing van die morfologie van ander tortrisied-spesies van ekonomiese belang. Sodoende sal 'n databasis en inligtingsbron geskep word wat uitvoere vir die Suid-Afrikaanse vrugtebedryf sal bevorder.

Introduction

Accurate identification of the immature stages of *Cryptophlebia leucotreta* (Meyrick) is often problematic and in most cases identification at species level can only be made at the adult level. A thorough knowledge of the morphology of the immature stages is essential, especially for phytosanitary or quarantine purposes on fruit destined for the export market as well as for effective pest management. Thus far, the only attempt to study the morphology of *C. leucotreta* immatures was by Stofberg (1948), who described the basic morphology of the final instar larva. There are no published descriptions of *C. leucotreta* eggs or pupae. Therefore, the aim of this study was to provide detailed descriptions of *C. leucotreta* eggs and pupae and to supplement the aforementioned study of *C. leucotreta* final instar larvae, as advances in technology have allowed the study of various morphological structures in more detail with a greater degree of accuracy. In addition, a comparative study on the morphology of the litchi moth, *C. peltastica* (Meyrick), and the macadamia nut borer, *C. batrachopa* (Meyrick) is included. Line drawings or photomicrographs of these two species are also presented where appropriate in order to facilitate identification and comparison with *C. leucotreta*.

Material and methods

Immature stages of *C. leucotreta* were obtained from the CRI insectary at Citrusdal. Study material of *C. peltastica* was obtained by the establishment of a laboratory colony at Stellenbosch University and that of *C. batrachopa* by direct collection in the field. Larval material was prepared by the conventional treatment using 10% KOH to remove the gut contents, neutralizing material in 10% HCl and staining using acid fuchsin. Material was then dried progressively using an alcohol dehydration series and mounted permanently on microscope slides. Material was studied using digital photomicrography and scanning electron microscopy. Digital photomicrographs were taken of all structures using a Wild M8 stereomicroscope with a 5 mega pixel Nikon Coolpix camera and measurements taken using an ocular micrometer. Material for scanning electron microscopy (LEO 1430) was either sputter coated with gold particles or studied as such, using variable pressure at 50 Pascals or under low vacuum. Photomicrographs were taken, using a LEO1430 scanning electron microscope.

Results and discussion

- *Cryptophlebia leucotreta*

Egg: Eggs are almost circular in shape and scale-like, each egg measuring ca 900 µm in diameter (Fig. 3.4.9.1). The upper surface of the shell is convex whereas the underside of the eggshell, in contact with the substrate, is flat. The visible surface of the chorion is fairly rough and covered with tiny irregular depressions and elevations surrounded by minute ridges. The chorion is highly translucent and all stages of the developing embryo can be seen, especially if the egg is located on a transparent substrate. The darkened head of the first instar is easily visible a day or two before hatching (Fig. 3.4.9.2). Eggs are usually laid singly on fruit. However, eggs may sometimes be laid so that they overlap irregularly.

Final instar larva: The present study focuses on structures that can be used for diagnostic purposes. As the chaetotaxy and structures such as the mandibles of this stage were described by Stofberg (1948), these are presently not dealt with. Our studies, however, indicate that the shape of the head and position of the adfrontals, stemmata and other features of *C. leucotreta*, as reported by Stofberg (l.c.), are not in agreement with present findings.

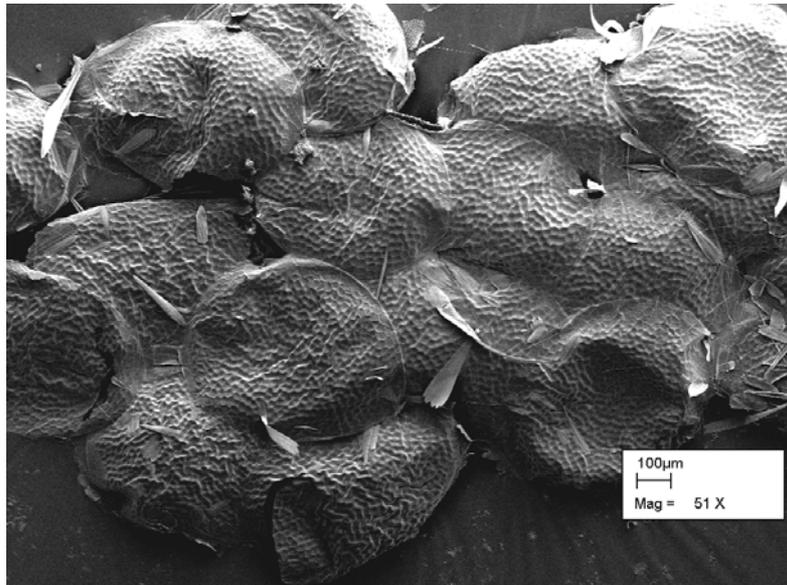


Fig. 3.4.9.1. Scanning electron micrograph showing the basic structure of *C. leucotreta* eggs.

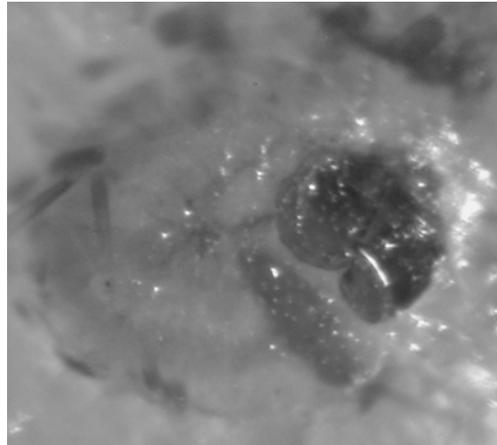


Fig. 3.4.9.2. Digital photomicrograph of the egg of *C. leucotreta* showing the developed larvae visible through the chorion.

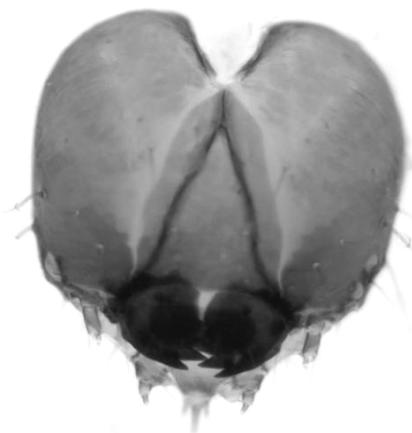


Fig. 3.4.9.3. Digital photomicrograph of the head (frontal view) of a final instar *C. leucotreta* larva.

Head: The head (Fig. 3.4.9.3) of the larva is hypognathous. The average width of the final instar head, measured prior to pupation, is 1.31 mm (range 1.127-1.460 mm, n = 40). The frons is well-developed, slightly longer than broad with distinct frontal sutures. The adfrontals are distinct and separated from the

lateral part of the head by light sclerotisations. The epicranial suture is short, one-fifth of the length of the frons. The stemmatal area consists of a semicircle formed by the six stemmata (Fig. 3.4.9.4, Fig. 3.4.9.5). All stemmata are irregularly rounded and approximately equal in size. Stemma IV is almost equidistant from III and VI, stemma I is closer to II than to III and stemma IV about equidistant from III and V. The antennae are elongate and three times longer than broad, with the scape being the largest segment (Fig. 3.4.9.6). Antennae are shorter, and less robust than labial palps with long terminal setae. The distal end of the spinneret is rounded and about eight times as long as wide (Fig. 3.4.9.7).

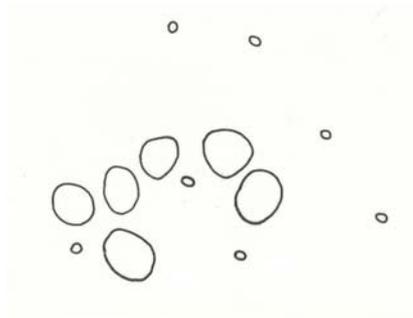


Fig. 3.4.9.4. Line drawing of stemmatal arrangement of *C. leucotreta* final instar larva.

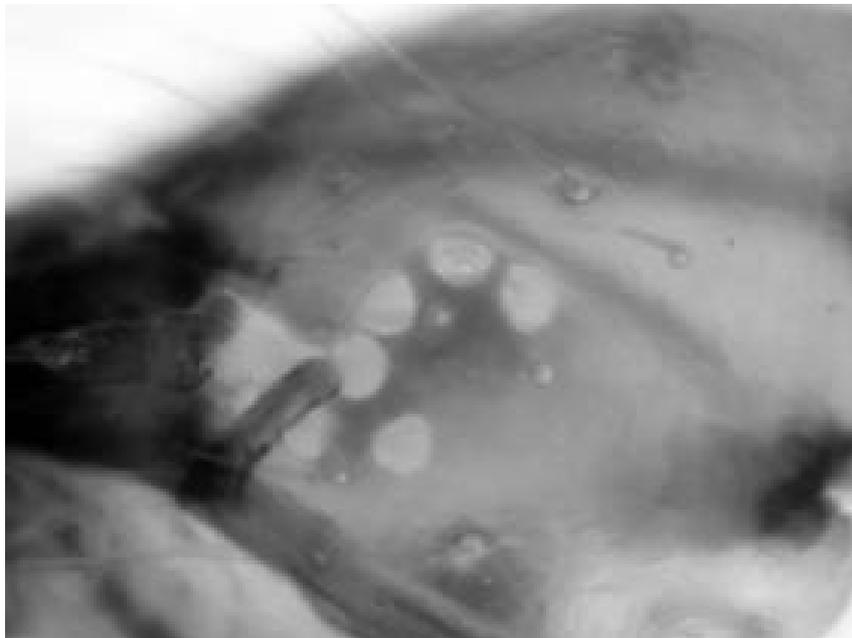


Fig. 3.4.9.5. Photomicrograph showing stemmata of *C. leucotreta*.

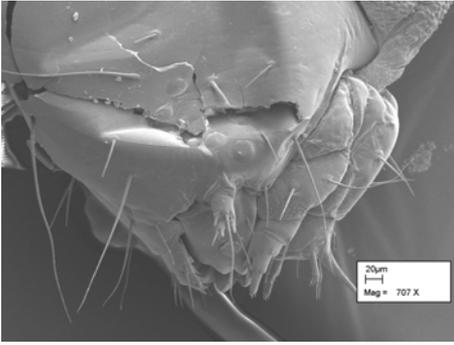


Fig. 3.4.9.6. Scanning electron micrograph showing the mouthparts (lateral view) of final instar *C. leucotreta* larva.

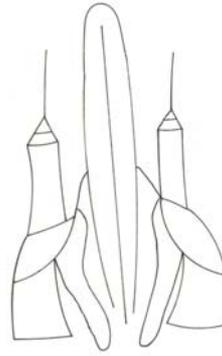


Fig. 3.4.9.7. Line drawing of the spinneret of final instar *C. leucotreta* larva.

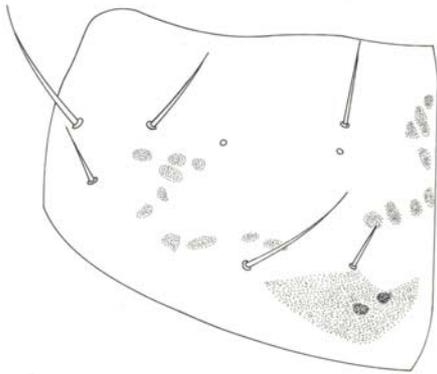


Fig. 3.4.9.8. Line drawing of the lateral half of prothoracic shield of final instar *C. leucotreta* larva.

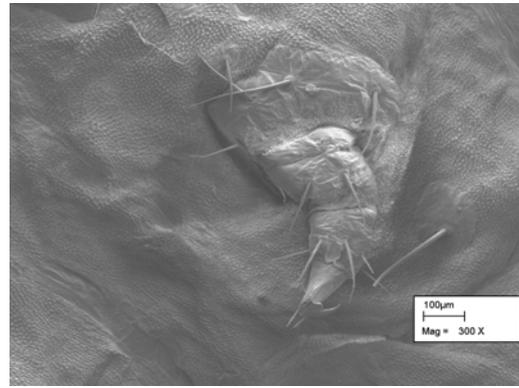


Fig. 3.4.9.9. Scanning electron micrograph of the thoracic leg of *C. leucotreta* final instar larva.

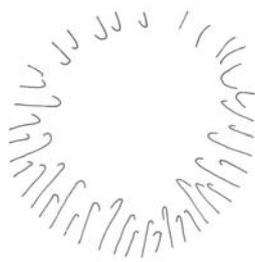


Fig. 3.4.9.10. Diagrammatic representation of crochets on the ventral proleg of final instar *C. leucotreta* larva.



Fig. 3.4.9.11. Diagrammatic representation of the crochets on the anal prolegs of final instar *C. leucotreta* larva.

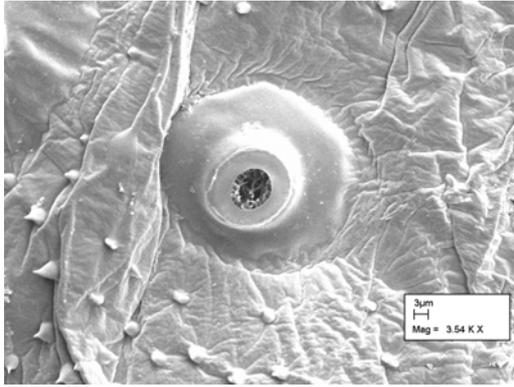


Fig. 3.4.9.12. Scanning electron micrograph of the spiracle of *C. leucotreta*.

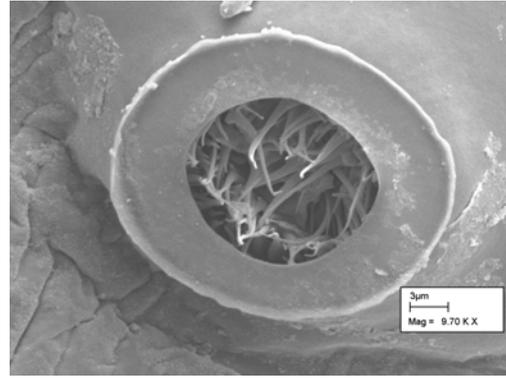


Fig. 3.4.9.13. Scanning electron micrograph of the spiracle of *C. leucotreta* on A8 showing detail of the filter apparatus.

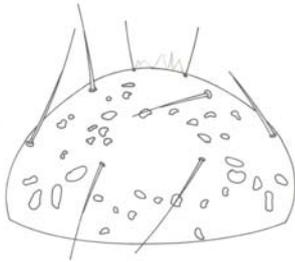


Fig. 3.4.9.14. Line drawing of the anal shield of *C. leucotreta*.

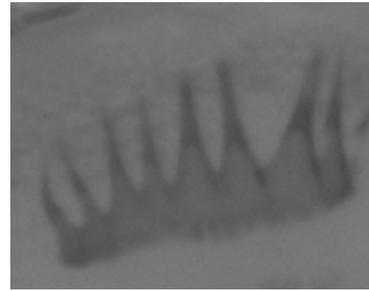


Fig. 3.4.9.15. Photomicrograph of the anal comb of *C. leucotreta*.

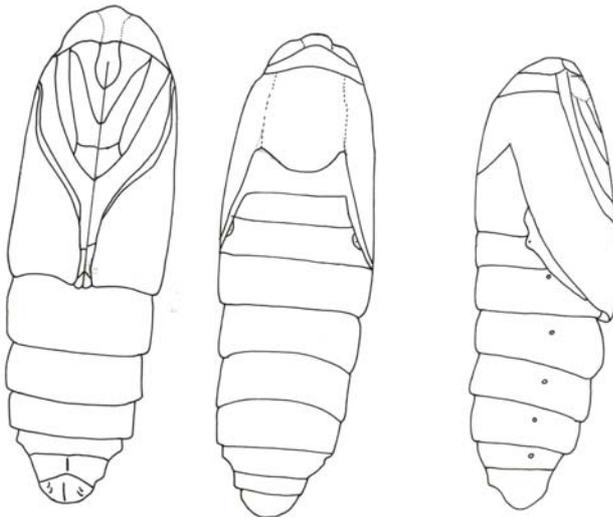


Fig. 3.4.9.16. Ventral, dorsal and lateral views of female pupae of *C. leucotreta*.

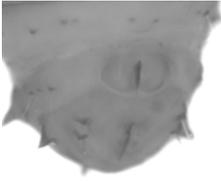
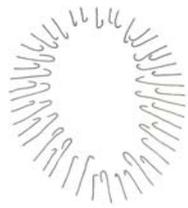


Fig. 3.4.9.17. Photomicrograph of the terminal segments of male *C. leucotreta* pupa

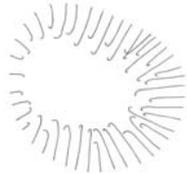


(a)



(b)

Fig. 3.4.9.18. Diagrammatic representation of the crochets on the ventral (a) and caudal prolegs (b) of *C. peltastica* final instar larva.



(a)



(b)

Fig. 3.4.9.19. Diagrammatic representation of the crochets on the ventral (a) and caudal prolegs (b) of *C. batrachopa* final instar larva.

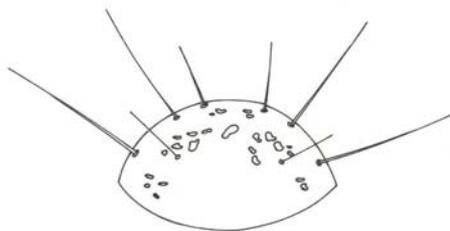


Fig. 3.4.9.20. Anal shield of *C. peltastica*.

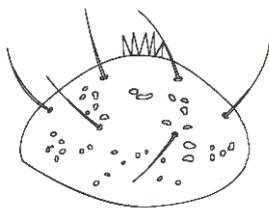


Fig. 3.4.9.21. Anal shield of *C. batrachopa*.



Fig. 3.4.9.22. Anal comb of *C. batrachopa* larvae.

Thorax: The distinct prothoracic shield of is medium brown with darker patches due to medium and extensive sclerotization (Fig. 3.4.9.8). The anterior lateral margin of the prothoracic shield is obtuse, slightly concave, curved at about one-third of its length and curving convexly towards the mid-line, lateral margin fairly straight and posterior margin evenly rounded towards mid-line. The setae on the prothoracic shield are as shown in Fig. 3.4.9.8. The sculpture of the integument has short, sharktooth-like projections. The prothoracic legs are medium brown. The thoracic claws are small, curved and shortish (Fig. 3.4.9.9) with the claw length almost one sixth the length of the leg. The spiracles are circular and well elevated on the spiracular base. The peritreme of the spiracle is well developed, with the width of peritreme almost half the diameter of the spiracular opening.

Abdomen: The sculpture of the integument has short, sharktooth-like projections. The crochets of the abdominal prolegs are irregularly triordinal and with an ovoid arrangement (Fig. 3.4.9.10). The ventral prolegs have 36-42 and anal prolegs 24-32 crochets (Fig. 3.4.9.11), respectively. Crochets of ventral prolegs are arranged triordinally on the lateral side, but reduced to irregularly spaced uniordinal crochets in the medial half. Anal prolegs have crochets arranged in a lateral semi-circle. Spiracles are oval in shape with a well-developed cuticular peritreme and a filter chamber, consisting of unbranched trichomes, in front of the atrium (Fig. 3.4.9.12, Fig. 3.4.9.13). The atrial filter chamber apparently prevents the entrance of particles or small parasites (Chapman 1969, Schmitz & Wasserthal 1999). The spiracles of the thorax and abdomen are similar in size. The anal shield (Fig. 3.4.9.14) is evenly rounded along posterior margin, lateral margin acute, angled anteriorly, anterior margin broadly curved. The anal comb is prominent and well developed and has between 2-10 bluntly dentate teeth (Fig. 3.4.9.15). The upper levels of the larger teeth medially are at about the same level. The basal part of each tooth strongly tapers dorsally, with the width of the base nearly one-quarter length of tooth.

Pupa: The pupa (Fig. 3.4.9.16) is almost always covered by a cocoon, constructed with debris and soil, but the cocoon is generally absent when pupation takes place directly in certain fruit, such as in Port Jackson galls and macadamia nuts. Pupae are dark brown and obdect, ranging from 15.8 - 21.2 mm (n = 20) in length. The proboscis and legs are shorter than the antennae and wing cases. The antenna and wing reach the posterior margin of the second abdominal segment. The spiracles are small and circular in shape. Two rows of spines, one anterior and one posterior, are present on the dorsum of abdominal segments 2 - 7. The eighth abdominal tergum has a single posterior row of spines. A distinct cremaster is absent. However, two pairs of hooks, one on either side of the anal pore, are present on the anal projections. Morphological differences between the sexes are apparent and there are distinct differences between the position of the genital orifice situated on the ninth abdominal segment in both sexes. In the female the eighth abdominal sternal region bears a distinct ventro-medial groove, the ninth sternum with a deep ventro-medial anal groove. Male pupae have a pair of small bulbous projections on the ventral surface of the ninth segment. Secondary sexual characteristics are also apparent. The inner margins of the metathoracic wings of the male are covered with short, heavy scales which cause a thickened appearance relative to that of the female (similar to *Laspeyresia caryana* [Teddars & Osburn, 1967]).

- **Comparison with the morphology of *C. peltastica* and *C. batrachopa***

The morphological features of the immature stages of *C. leucotreta*, *C. peltastica* and *C. batrachopa* are rather similar and to date no morphological characters or keys exist to distinguish with certainty between these three species. This problem is further complicated by the fact that the host range of the three species overlap on cultivated crops such as litchi, macadamias and occasionally also on citrus (Newton 1998, De Villiers 2001). This present study aims to identify and compare certain distinguishing morphological features of the three species. Since the ultimate aim is to facilitate identification in the field, only characters that are taxonomically significant, but that can be examined using a hand lens or simple microscope, are described.

Eggs: The eggs of *C. leucotreta*, *C. peltastica* and *C. batrachopa* can only be distinguished by means of scanning electron microscopy and are therefore not relevant in this regard.

Larvae: Fully-grown larvae of *C. leucotreta* and *C. peltastica* are usually pink to pinkish-red whereas those of *C. batrachopa* are greyish in colour. However, colour is not a reliable indicator as it may depend on or change with factors such as the type of diet and instar. Live fully-grown larvae of *C. peltastica* are longer than those of the other two species. The maximum width of the head of fully-grown *C. peltastica* is 2.05 mm whereas that of *C. batrachopa* has a maximum of 1.34 mm and that of *C. leucotreta* 1.46 mm.

The number and arrangement of the crochets of *C. leucotreta*, *C. peltastica* and *C. batrachopa* show morphological differences. Crochets on the ventral prolegs of *C. leucotreta* are unevenly triordinal (three

alternating lengths of crochets) whereas those of *C. peltastica* and *C. batrachopa* are biordinal (two alternating lengths of crochets) (Fig. 3.4.9.18, Fig. 3.4.9.19). Final instar *C. peltastica* larvae possess 50-58 crochets on the ventral prolegs and 46-54 on the caudal prolegs. *C. batrachopa* larvae possess 34-44 crochets on the ventral prolegs and 26-32 on the caudal prolegs. The anal shield of the three species differs, especially in relation to their size and arrangement of setae. In *C. leucotreta*, some setae are arranged along the posterior margin, with two pairs of setae situated mid-dorsally (Fig. 3.4.9.14). The anal shield of *C. peltastica* is smaller with distinct straight setae arranged along the posterior margin (Fig. 3.4.9.20). The anal shield of *C. batrachopa* has one pair of curved setae arranged laterally and two pairs of curved setae mid-dorsally (Fig. 3.4.9.21).

The easiest method to distinguish between *C. leucotreta*, *C. batrachopa* and *C. peltastica* larvae is to detect an anal comb. The larvae of *C. leucotreta* and *C. batrachopa* both possess an anal comb whereas those of *C. peltastica* do not. The anal comb of *C. batrachopa* has between 4 and 7 teeth, all teeth tapering to a medial point (Fig. 3.4.9.22).

A preliminary key to distinguish between fully-grown larvae of the three *Cryptophlebia* species:

1. Anal comb present2
 Anal comb absent; ventral prolegs with biordinal crochets, crochets ranging from 50-58 on ventral prolegs and 46 – 54 on anal prolegs, respectively (Fig. 3.4.9.18a,b); setae on anal shield straight and marginally arranged (Fig. 3.4.9.20)*C. peltastica*
2. Crochets on ventral prolegs triordinal in lateral half of arrangement (Fig. 3.4.9.10); anal comb with medial teeth of even length (Fig. 3.4.9.15).....*C. leucotreta*
 Crochets on ventral prolegs biordinal (Fig. 3.4.9.19a,b); anal comb with teeth merging into distinct medial structure (Fig. 3.4.9.22); anal shield with irregular curved setae (Fig. 3.4.9.21)*C. batrachopa*

Pupae: Comparative studies of the pupae of both sexes of all three species are presently being carried out.

Conclusion

Sufficient distinguishing characters of the immatures of *C. leucotreta* have been indicated. A comparison of the present studies with those published by Stoffberg (1948) indicates considerable inaccuracies in the latter's description of a mature larva of this species. Some similar characters of the related species *C. peltastica* and *C. batrachopa* have shown their usefulness towards specific and certain identification of the fully-grown larvae of the three species by means of a key using only four morphological characters.

Future research

Further detailed studies of the morphology of *C. leucotreta*, *C. peltastica* and *C. batrachopa* and some other tortricids, including the codling moth, oriental fruit moth and others, are needed for the compilation of keys to distinguish between the immature stages of the economically important species at professional as well as lay level. This will include the following morphological features: mandibles, setal maps, claws, prothoracic shields, stemmata and their arrangement, crochets, spiracles, heads and cremasters and other detail of their pupae (male and female) of *C. peltastica* and *C. batrachopa*. Ideally all larval stages will be covered.

Acknowledgement

The financial support of CRI, donation of insect cultures by the CRI Insectary, Citrusdal, and assistance with collection of field material and assistance with the establishment of breeding cultures by Mrs Tertia Grove of the Institute for Tropical and Subtropical Crops at Nelspruit is gratefully acknowledged.

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3.4.10 Opnames van gasheerplante vir valskodlingmot (*Cryptophlebia leucotreta*) in die Citrusdal-omgewing

Proef CB2004 deur Stephan J. Honiball (Ceder Biocontrol)

Summary

Surveys lasting for 19 months were conducted to find possible host plants for false codling moth in the Citrusdal area. Only four species, other than cultivated fruit trees, were found that supported FCM to a certain degree. None of the fruits of these species showed any signs of FCM larval damage. However, when exposed to newly hatched FCM larvae in the laboratory, larvae scarred fruit of the wild olive, *Olea europaea* subsp. *Africana*, but did not develop. FCM was reared successfully in fruit of the wild almond, *Brabejum stellatifolium*, and wild plum, *Harpephyllum caffrum*, while a few individuals were recovered from natural infestation in the galls of Port Jackson trees, *Acacia longifolia*. It is concluded that FCM will probably be unable to cause much commercial harm if dependent on wild host plants only.

Similar to a survey conducted previously, trap catches showed that FCM mainly occurs near or in citrus orchards and not further away.

Inleiding

Valskodlingmot (VKM) was 'n onbekende plaag in die Olifantsriviervallei voor 1974 (F. Honiball, pers. kom. 1999). Dit is dus onwaarskynlik, maar nie onmoontlik nie, dat VKM die natuurlike fynbos in die omgewing as 'n gasheer kan benut. Die doel van die studie was om te bepaal of daar alternatiewe gasheerplante van VKM in die natuurlike fynbos voorkom wat uiteindelik die sukses kan bepaal van 'n steriele insekloslatingsprogram.

Materiale en metodes

Opname van gasheerplante: Twee persele op die plase Modderfontein en Noordhoek is maandeliks besoek tussen Maart 2002 en November 2004. Die plase is in die Citrusdal-omgewing, ongeveer 20 km van mekaar, aan weerskante van die Olifantsrivier. Albei persele bestaan uit 'n opstal (met sier-/vrugteboom) en 'n bergfynbos komponent. 'n Voetpadroete van ongeveer 3 km is op albei persele gevolg. Alle vrugte, bessies, sade en galle wat teëgekomp is, is ondersoek vir vreet-/tonnelmerke, sowel as vir die teenwoordigheid van VKM-eiers. Vrugte is versamel in bruin kardoessakke en duidelik gemerk vir verdere ondersoek en identifikasie. Die vrugte is in die laboratorium blootgestel aan VKM-eiers is en by 25°C en 45% RH; 24H L gehou. Die vrugte is 'n maand later ondersoek vir die teenwoordigheid van VKM-larwes/motte.

Voorkoms van VKM volgens lokvalle in 'n landskapmosaïk: In 'n voortsetting van werk wat deur 'n student aan die US begin is, is 20 VKM feromoonvalle op verskillende plekke in die omgewing van 'n sitrusplaas, Floreat, uitgeplaas en weekliks ondersoek. Sommige valle is in boorde geplaas terwyl ander valle by eikebome of in die fynbos op verskillende afstande na die naaste sitrusboorde, geplaas is. Die aantal lokvalle is verminder tot 17 nadat bobbejane drie van die valle herhaaldelik verwoes het. Van die ander valle is ook van tyd tot tyd afgeruk/verwoes, maar dit het veel minder gebeur.

Resultate en bespreking

Opname van gasheerplante: Die volgende plante het tekens van vreeskade getoon of kon VKM-larwes onderhou:

Brabejum stellatifolium – wilde amandel
Olea europaea subsp. *africana* – wilde-olyf
Acacia longifolia – Port Jackson
Harpephyllum caffrum – kafferpruim

Die wilde amandel kom meestal in rivierlope voor en vorm gedurende Maart/April vrugte. Geeneen van die vrugte wat in die veld versamel is, het vreetmerke getoon nie, maar onder geforseerde omstandighede in die laboratorium het VKM wel daarop tot volwassenheid ontwikkel.

Die wilde-olyf word algemeen aangeplant as 'n geharde skaduboom, maar kom ook voor in klowe. Geen vrugte wat in die veld versamel is, het VKM-larwes bevat nie, maar die larwes het wel onder laboratoriumtoestande aan die vrugte gevreet. Die klein vruggies het vinnig in die laboratorium uitgedroog, wat moontlik verhoed het dat die larwes hul lewensiklus voltooi.

Die galle van die Port Jackson is versamel en dit het heelwat tunnels en vreetmerke gehad. Die meerderheid motte wat daaruit geteel is, was onbekende motte, maar enkeles is deur Dr. H. Geertesema (Universiteit Stellenbosch) as VKM geïdentifiseer. Die galle word deur 'n galwespe veroorsaak, wat ingevoer is om die Port Jackson biologies te beheer. Die galwespe, tesame met pogings van die regering se "Werk vir Water" inisiatief vir die bekamping van indringerplante, behoort geleidelik te lei tot 'n afname van die aantal Port Jackson-bome in die vallei.

Die kafferpruim word algemeen aangeplant in huistuine en langs strate in Citrusdal en omgewing. Alhoewel geeneen van die vrugte wat versamel is, VKM-skade getoon het nie, het VKM wel sy lewensiklus suksesvol daarop onder laboratorium toestande voltooi.

Die gasheerplante hierbo genoem, tesame met die bekende (verboede) gashere (makadamia, pekanneute, granate, steenvrugte, sitrus, akkerbome) sorg waarskynlik vir 'n aaneenlopende bron van VKM-herbesmetting in die vallei. Baie produsente in die vallei ondervind die hoogste VKM-aanwesigheid in boorde wat aan die fynbos grens, maar tydens hierdie opname is byna geen alternatiewe gashere in die fynbos gevind nie. Dit dui dus op 'n teenstrydigheid.

Windlanings word algemeen aan die rand van boorde geplant om windmerke op vrugte te voorkom. Dit is moontlik dat die windbrekers 'n gunstige mikroklimaat skep vir VKM om ongehinderd eiers te lê. VKM skade neem gewoonlik af hoe verder daar van die windlanings beweeg word.

Die fynbos wat rondom die sitrusboorde in die Olifantsriviervallei voorkom, word gekenmerk deur drie komponente:

- 'n Restoid-komponent, bestaande uit die Kaapse Rietfamilie.
- 'n Erikoid- of heide-komponent, bestaande uit die families Ericaceae, Asteraceae en Fabaceae.
- 'n Proteoid-komponent - die bekende Proteaceae-familie.

Gedurende die opname is relatief min vrugte, bessies en sade opgespoor. Alhoewel die Kaapse fynbos bekend is vir sy blomverskeidenheid, word daar selde opsigtelike bessies en saad geproduseer. Saad is dikwels klein en droog vir windverspreiding en huisves selde enige larwes. Wanneer bessies en sade wel opsigtelik en/of vlesig is, is dit meesal helderkleurig (rooi/oranje/pers) en giftig. Sover dit hierdie opname betref, kon slegs een moontlike gasheer (wilde amandel) van VKM in die natuurlike fynbos opgespoor word.

Voorkoms van VKM volgens lokvalle in 'n landskapmosaik: VKM-feromoonvalle op die plaas Floreat wat 50 m of verder vanaf die naaste sitrus in die fynbos geplaas is, het tussen September 2003 en Januarie 2005 deurgaans min VKM-mannetjies gevang (tussen 10 en 33 volgens Tabel 3.4.10). Die vangste in lokvalle wat binne-in valencia-boorde geplaas is, het heelwat gewissel (T18 met 39 mannetjies teenoor T13 met 172). Lokvalle wat in die omgewing van eikebome geplaas is, het heelwat motte gevang (T6 en T1 met 55 en 89 motte onderskeidelik). Die hoogste tellings is in navelboorde gevind (T10 met 150 en T11 met 251 motte). Die aantal motte wat tussen September 2003 en Januarie 2004 gevang was, is heelwat hoër as die ooreenstemmende tydperk 'n jaar later (ongepubliseerde data). Dit kan toegeskryf word aan die produsent wat gedurende April 2003 vir die eerste keer in 20 jaar 'n chitien groei-inhibeerder vir VKM-bestryding toegedien het (Norman, pers. kom. 2005). Die produsent het ook sukses behaal deur van akkers ontslae te raak deur sy eikebome met 'n speenmiddel (Corasil E) te spuit.

Tabel 3.4.10. Totale hoeveelheid mannetjiemotte gevang in geel deltavalle met Lorelei-lokmiddel vanaf September 2003 tot Januarie 2005 op die plaas Floreat. Resultate word nie chronologies weergegee nie, maar volgens toenemende vangste).

Lokval no.	Ligging	Totale aantal mannetjies gevang
T12	Pad tussen boorde 11 en 12, diep in fynbos (200-250 m van naaste boord)	10
T16	Olyfboom teen berg op; 250 m van naaste boord	13
T14	By die kantoor, verskillende plante bv. wilde olyf, suur-pruim	14
T15	Tussen boorde 11 en 12, by bloekomboom; 10 m van boorde	23
T7	Fynbos – 200 m vanaf boorde	29
T3	Tussen bloekombome en oop grasvlakte, naby spruit met riete; naaste boord 150 m	32
T19	Naby boord 12, in die fynbos; 150 m van naaste eikebome	33
T18	Boord 14 (oorwegend Valencia's); naby plaashuis	39
T5	Boord 1 nawels met bietjie Valencias, omring deur fynbos, riete, braambos	42
T4	In boord 6, ry 3-4	42
T17	Boord 13 (Valencia en navels)	51
T2	Oop grasvlakte, naby boord 6 (Valencia en navels); 100 m van naaste boord	54
T6	By eikeboom, naby boord 2 (navels); 10 m van naaste boord	55
T1	Op die rand van die eikebome, naby boord 7 (Valencia's); 15 m van naaste boord	89
T10	In boord 8, (navels)	150
T13	In boord 12 (Midknight Valencia's), ry 5-6	172
T11	Tussen boorde 9 en 11 (Robynnavels)	251

Toekomstige navorsing

Die opnames is afgesluit en geen verdere werk word beoog nie.

Bedankings

Mnr. Mark Norman van die plaas Floreat word bedank vir sy hulp met die weeklikse lees van feromoon valle op sy plaas.

3.4.11 **Gammabestraling van valskodlingmotlarwes vir die disinfestasië van verpakte uitvoervrugte**
 Proef 719 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

Two experiments were conducted to further investigate the influence of doses of 200 Gy to 400 Gy gamma irradiation on false codling moth larvae in diet jars. Irradiation doses of as little as 200 Gy completely prevented the development of 3rd or 4th instar larvae to adults.

Inleiding

Die agtergrond tot die beweegredes vir bestralingsproewe wat op die uitwissing van VKM-larwes in vrugte gemik is, is in 'n vorige verslag bespreek (2003 CRI Jaarverslag). Uitstekende resultate is verkry met bestralingsdosisse wat van 200 Gy tot 400 Gy gewissel het. Verskillende ouderdomme VKM-larwes is in dieetflesse bestraal en toegelaat om te ontwikkel. Geen larwe kon tot volwassenheid (motte) ontwikkel nie en die nagenoeg 5% van hulle in verskillende ouderdomsgroepe wat wel daarin kon slaag om te puppeer, het in die papiestadium gevrek.

Gammabestraling, selfs teen dosisse wat kommersieel gebruik word, se sterkte neem vinnig af met afstand én die tipe materiaal waardeur dit moet dring. Daar word gewoonlik bereken dat, indien 'n pallet volgestapel met kartonne vrugte bestraal word, die bestralingsdosis met 'n faktor van ongeveer 3:1 van die buitekant van die stapel na die binnekant sal afneem. Dit bring mee dat, indien 'n pallet byvoorbeeld met 'n minimum dosis van 100 Gy behandel moet word, sal 300 Gy toegedien moet word. Om dié rede is dit van groot belang dat die dosis vir die uitwissing van die larwes gebruik word, so laag as moontlik moet wees, sodat die totale dosis (3x hoër) nie vrugkwaliteit sal benadeel nie.

Twee verdere proewe is uitgevoer terwyl inligting vanaf die USDA ingewag is om hul vereistes ten opsigte van gammabestralingsbehandelings duidelik te maak. Die betrokke tegniek het in beide gevalle ooreengestem en die proewe word saam bespreek.

Materiale en metodes

Drie ouderdomme VKM-larwes is gebruik, naamlik sogenaamde “klein”, “medium” en “groot” larwes. Teelflesse is van Ceder Biocontrol se insektarium verkry. Daar is gepoog om dieeflesse uit te soek wat larwes van die vereiste grootte bevat het. Kopkapsule-metings is uitgevoer om vas te stel hoe ver die larwes in elke behandeling ontwikkel het. Eenhonderd larwes van elke behandeling is ewekansig vir dié doel uit bykomende dieeflesse verwyder.

'n Bestralingstudie is vooraf uitgevoer om te verseker dat die bestralingsdosisse akkuraat was vir die tipe blootstelling wat uitgevoer moes word. Fricke dosimeters is gebruik wat in die dieet van 'n verteenwoordigende aantal flesse ingedruk was. Die meters is na bestraling deur Dr Kobus Slabbert van iThemba Labs, Somerset-Wes, ontleed. Agt teelflesse is per ouderdom larwe vir bestralingsdoeleindes gebruik en bestralingsdosisse van 200 Gy tot 400 Gy, in inkremente van 50 Gy, is getoets.

Die flesse met larwes is na bestraling by 26°C gehou totdat geen tekens van lewe meer in die flesse opgemerk kon word nie. Weens die vertragende invloed wat gammabestraling op insekontwikkeling het, is larwes in die bestralingsbehandelings 11 dae langer tyd gegun om hul ontwikkeling te voltooi.

Die dieeflesse se watterproppe is met SFK-doppe vervang sodat alle larwes wat wou pupeer, maklik herwin kon word. Papies is verwyder en in 5 ml pilbotteltjies geplaas sodat hul kon ontpop. Daar is boekgehou van die aantal papies en motte wat in die verskillende behandelings ontwikkel het.

Resultate en bespreking

Dit is moeilik om dieeflesse te kies waarvan die oorgrote meerderheid larwes ooreenstem met die verlangde ouderdom. Daar was dus, veral in Proef 1, 'n redelike afwyking van die verlangde ouderdomme (Fig. 3.4.11.1). Die larwes was oor die algemeen almal ouer as wat verwag is en die meeste was in die 4'de of 5'de instar. Dit is nie 'n ernstige fout nie, aangesien die larwes se bestralingsgevoeligheid met ouderdom toeneem en die gedeeltelike verlies van data oor die jonger larwes is relatief onbelangrik. In die tweede proef was 70% van die “klein” larwes 3'de instar, terwyl daar 'n tekort van 4'de instar larwes (40%) in die “medium”-groep was (Fig. 3.4.11.2). Die “groot” larwes was in beide proewe goed verteenwoordig en meer as 90% van die larwes in dié groep was 5'de instar.

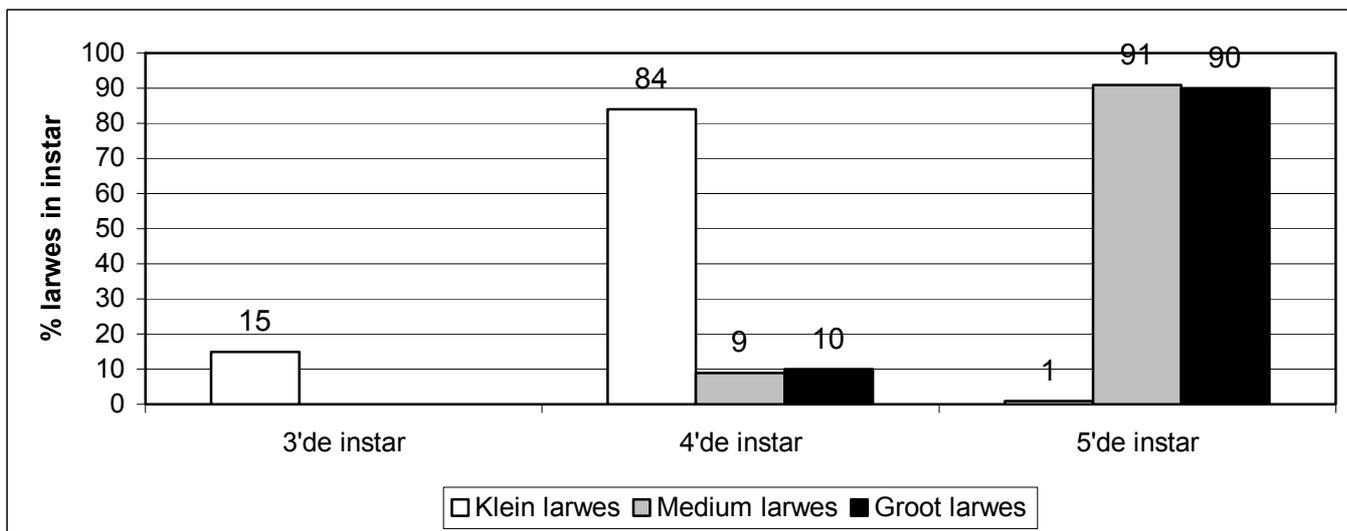


Fig. 3.4.11.1. Ouderdomsverspreiding van valskodlingmotlarwes in Proef 1.

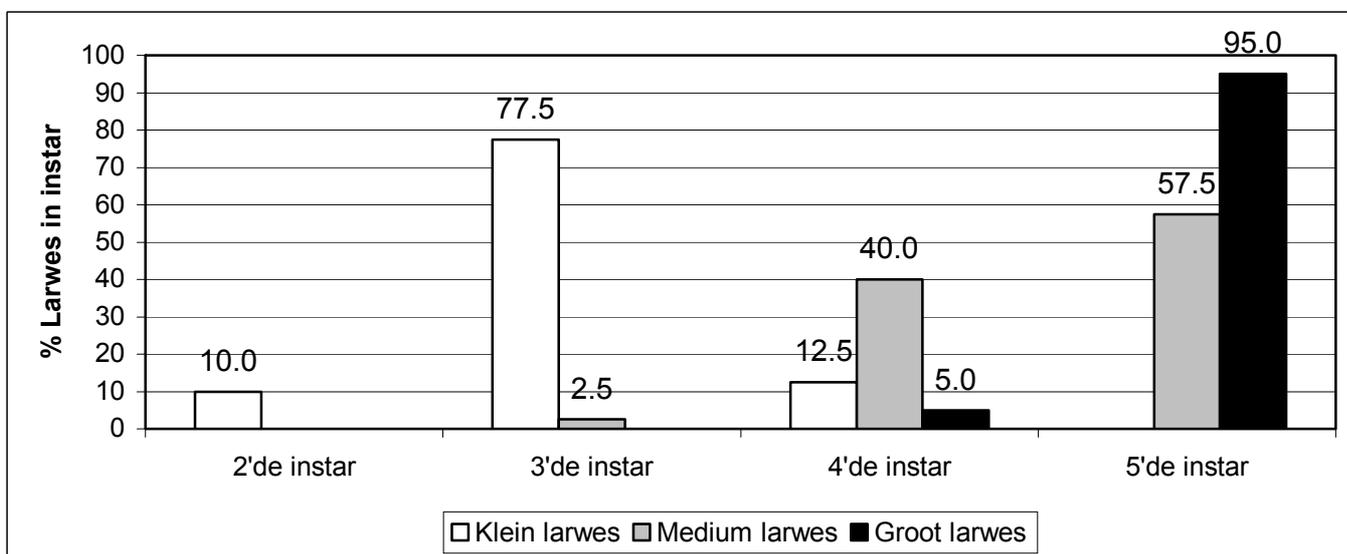


Fig. 3.4.11.2. Ouderdomsverspreiding van valskodlingmotlarwes in Proef 2.

Dieetflesse met die onderskeie ouderdomme larwes is ewekansig aan die verskillende behandelings toegeken. Daar kan dus aanvaar word dat dieselfde aantal larwes min of meer in elke behandeling bestraal was.

Proef 1: Gammabestraling het 'n ernstige uitwerking op VKM-larwes van alle ouderdomme gehad en slegs enkeles kon daarin slaag om tot papies te ontwikkel (Tabel 3.4.11.1). Omdat die drie groepe larwes oor die algemeen ouer was as wat verlang was, kan daar nie waarde geheg word aan die aantal sogenaamde "klein" en "medium" larwes wat wel daarin geslaag het om te verpop nie. Die larwes in dié groepe wat wel verpop het, kon bes moontlik ouer instar larwes gewees het.

Tabel 3.4.11.1. Invloed van gammabestraling op die ontwikkeling van valskodlingmotlarwes van verskillende ouderdomme.

Bestralings-dosis	Aantal larwes wat verpop het			Aantal papies wat ontpop het		
	Klein larwes	Medium larwes	Groot larwes	Klein larwes	Medium larwes	Groot larwes
0 Gy	1124	1450	1073	1064	1408	1061
200 Gy	3	40	41	0	0	0
250 Gy	0	0	2	0	0	0
300 Gy	0	0	1	0	0	0
350 Gy	0	0	0	0	0	0
400 GY	0	0	0	0	0	0

Proef 2: 'n Gemiddeld van 1 200 larwes is per behandeling gebruik. Geen larwe in enige van die behandelings kon daarin slaag om te verpop nie. Slegs enkele larwes het gedeeltelik verpop tot wanstaltige papies met eienskappe van beide larwes en papies.

Die resultate van beide proewe is weer eens, soos in 'n vorige proef, baie belowend. Dit is duidelik dat die VKM-larwes baie radiosensitief is vir die bestralingsdosisse wat getoets was en hul ontwikkeling erg gestrem word.

Daar is sterk aanduidings dat die VSA 'n bestralingsbehandeling sal aanvaar wat nie noodwendig die larwes in vrugte sal doodmaak nie, maar wat wel sal verseker dat die P1-generasie (die motte wat uit die bestraalde larwes ontwikkel het) heeltemal steriel is. Dit beteken dat die dosisse wat tot dusver getoets was, bes moontlik heelwat verlaag sal kan word, wat goed sal wees vir die behoud van vrugkwaliteit.

Voortgesette navorsing

Die invloed van laer bestralingsdosisse op VKM-larwes sal in toekomstige proewe ondersoek word. Die relatiewe verskil tussen die reaksie van bestraalde larwes in dieeflesse en in vrugte, moet ook vasgestel word, aangesien grootskaalse proewe baie makliker met larwes in dieeflesse as in vrugte uitgevoer kan word.

3.5 PROJECT FRUIT FLY

Project Coordinator: Tony Ware (CRI)

3.5.1 Project summary

This group of pests is receiving much attention lately because of its enhanced phytosanitary status and the barrier this imposes to markets, both new and traditional. On the pre-harvest aspect of the research, attention was given to the monitoring for exotic fruit flies along the Maputo Corridor between Maputo and Nelspruit (3.5.8). Fourteen species of indigenous fruit fly were recorded in the 80 weeks of monitoring. No exotic fruit fly species were recorded. However, there remains the danger of incursions, particularly as *Bactrocera cucurbitae* and an undescribed *Bactrocera* are present in East Africa, while the peach fruit fly (*Bactrocera zonata*) is known from Egypt, Mauritius and Reunion. The research has been terminated but the newly established Early Warning Unit within the Department of Agriculture is expected to assume responsibility for monitoring for, and eradication of, any exotic fruit fly species entering the country. In a mark-release-recapture exercise it was shown that current monitoring methods are less than 5% effective (3.5.7). Questlure was generally shown to be the superior monitoring tool for Natal fruit fly, particularly if female numbers need to be determined. With the worldwide trend to reduce the use of organophosphates, the currently used toxicants in fruit fly baits have a limited life. Cost-effective alternatives to these toxicants were investigated (3.5.3). Two products tested (formetanate and methomyl) showed promise but these will not be investigated further as the carbamate group is also under threat of being phased out of use. Mevinphos, tartar emetic, chlorfenapyr and fipronil were considered unsuitable. Further testing is to be conducted in the new research year. The M3 bait station underwent further modifications this year (3.5.9) with a new fungicide being investigated and the replacement of the sponge component with paper. On the post-harvest front, phase III (medium scale) disinfestation trials were done using lemons, Clementines, oranges and grapefruit (3.5.4). Further disinfestation research will be done but the exact nature thereof will be dependent on the outcome of negotiations between the Departments of Agriculture of South Africa and

Japan. Two contract disinfestation trials were undertaken. One was for the litchi industry (3.5.5) and the other for the persimmon growers (3.5.6).

Projekopsomming

Hierdie groep peste kry deesdae meer aandag as gevolg van hul verhoogde fitosanitêre status en die versperring wat dit vir nuwe en tradisionele markte meebring. Met die voor-oes aspek van die navorsing is aandag gegee aan die monitering van uitheemse vrugtevlieë langs die Maputo-Korridor, tussen Maputo en Nelspruit (3.5.8). Veertien inheemse vrugtevliespesies is aangeteken in die 80 weke van monitering. Geen uitheemse vrugtevliespesie is aangeteken nie. Die gevaar van instroming bestaan egter steeds, veral aangesien *Bactrocera cucurbitae* en 'n onbeskryfde *Bactrocera* in Oos-Afrika voorkom en die perskevrugtevliespesie, *Bactrocera zonata*, bekend is van Egipte, Mauritius en Reunion. Die navorsing is beëindig, maar die nuut-gevestigde Vroeë Waarskuwingseenheid binne die Departement van Landbou is veronderstel om verantwoordelikheid te aanvaar vir die monitering en vernietiging van enige uitheemse vrugtevliespesie wat die land mag binnekom. Merk-loslaat-herwinproef het getoon dat die huidige moniteringsmetodes minder as 5% effektief is (3.5.7). Questlure was oor die algemeen die beste moniteringsinstrument vir Natalse vrugtevliespesie, veral vir die bepaling van wyfiegetalle. Met die wêreldwye tendens om die gebruik van organofosfate te verminder, het die gifstowwe wat tans in vrugtevliespesie gebruik word, 'n beperkte lewensduur. Lonende alternatiewe vir hierdie doders is ondersoek (3.5.3). Twee middels wat getoets is (formetanate en methomyl), het belofte ingehou, maar sal nie verder ondersoek word nie aangesien die karbamaat groep ook die gevaar loop om uitfaseer te word. Mevinphos, braakwynsteen, chlorfenapyr en fipronil was nie geskik geag nie. Verdere toetse sal in die nuwe navorsingsjaar gedoen word. Die M3 lokaasstasie het verdere modifikasies ondergaan in die jaar (3.5.9) met 'n nuwe swamdoder wat ondersoek word en die vervanging van die sponsgedeelte met papier. Op die gebied van na-oes is fase III (medium skaal) ontsmettingsproewe gedoen met suurlemoene, Clementines, lemoene en pomelo's (3.5.4). Verdere ontsmettingsnavorsing sal gedoen word, alhoewel die presiese aard daarvan afhang van die uitslag van onderhandelings tussen die Departemente van Landbou van Suid Afrika en Japan. Twee kontrak-ontsmettingsproewe is gedoen, een vir die lietsjiedryf (3.5.5) en die ander vir tamatiepruimprodusente (3.5.6).

3.5.2 Rearing of fruit flies

Experiment 407 by Tony Ware, John-Henry Daneel and Rooikie Beck (CRI)

Opsomming

Vrugtevliespesie is van groot ekonomiese belang vir die suider-Afrikaanse vrugtebedryf. Wyfies beskadig die vrugte met eierlegging en die ontwikkelende larwes veroorsaak skade wanneer hulle aan die vrugte vreet. Vrugtevliespesie-beskadigde vrugte is baie vatbaar vir na-oesbederf. Daarbenewens is vrugtevliespesie van fitosanitêre belang en enige onderskepping deur 'n invoerende land kan lei tot die verwerping van daardie besending, met gevolglike verlies aan inkomste.

Daar is drie belangrike vrugtevliespesies in die bedryf, nl. *Ceratitis capitata* (Wiedemann) (Mediterreense vrugtevliespesie), *Ceratitis rosa* Karsch (Natalse vrugtevliespesie) en *Ceratitis cosyra* (Walker) (maroela vrugtevliespesie). Eersgenoemde twee spesies is van besondere belang vir die sitrusbedryf.

Om navorsing op enige geleedpotige te doen, is toegang tot genoegsame getalle noodsaaklik. Die doel van die navorsing was om 'n metode te ontwikkel om voldoende vlieë te produseer vir navorsingsbehoefte. Die doel is bereik en goeie kwaliteit vlieë is voorsien om aan die aanvraag te voldoen. Alle vlieë is geteel by die CRI fasiliteit in Nelspruit, die volwassene in hanghokke en larwes in Petribakkies en koekdose. Volwasse vlieë is 'n mengsel van suiker en torula-gis gevoer en larwes patentregtelike diëte wat deur CRI ontwikkel is. Vrugtevliespesie is gebruik in die volgende navorsing:

Proef 772. Ontwikkeling van 'n koue-sterilisasiëbehandeling by 0°C teen Mediterreense vrugtevliespesie vir sitrus. 'n Medium-groote proef met Clementines, lemoene, suurlemoene en pomelo's is uitgevoer. Ten minste twee herhalings van elke kultivar-tipe is ingesluit (3 000 000 vlieë verskaf). Lemoene is beskou as die geskikste gasheer, alhoewel daar nie statisties-betekenisvolle verskille in die resultate was nie.

Proef 773. Vrugtevliespesie lokaas – alternatiewe vir organofosfate. Hoktoetse is gedoen met laboratorium-geteelde Mediterreense vrugtevliespesie (1 500 vlieë verskaf). Die produkte wat getoets is, was methomyl, mevinphos, braakwynsteen, formetanate en fipronil. Slegs methomyl het potensiaal getoon as moontlike alternatiewe behandeling. Ander produkte sal ondersoek word.

Proef 774. Ontwikkeling van 'n vinnige diagnostiese toets om te onderskei tussen mediterreense vrugtevlieg larwes en ander vrugbewonende spesies. Larwes van al drie spesies (300 vlieë verskaf) is na Engeland verskep waar Central Science Laboratories die ontwikkeling van 'n toetsstel onderneem het. Voorlopige resultate lyk belowend.

Vrugtevlieë is ook verskaf aan 'n akademiese instansie (Universiteit van Stellenbosch) en ander verwante bedrywe (avokado, mango, lietsjie en tamatiepruim).

Summary

Fruit flies are of major economic importance to the southern African fruit industry. The flies damage the crop when the female stings the fruit while ovipositing and the developing larvae cause damage when eating the fruit. Fruit fly damaged fruit is highly prone to post-harvest waste. Furthermore fruit flies are of phytosanitary importance and any interception by an importing country can result in the rejection of that consignment and a resultant loss of income.

There are three fruit fly species of importance to the industry. These are *Ceratitis capitata* (Wiedemann)(Mediterranean fruit fly), *Ceratitis rosa* Karsch (Natal fruit fly) and *Ceratitis cosyra* (Walker)(marula fruit fly). *C. capitata* and *C. rosa* are two species of particular importance to the citrus industry.

In order to conduct research on any arthropod it is essential that one has access to adequate numbers. The research objective was to develop methods of producing enough flies for research needs. This was achieved and good quality flies were supplied as needed. All flies were reared at the CRI facility in Nelspruit using suspended cages for adults and petri dishes and cake boxes for larval life stages. Adult flies were fed a mixture of sugar and torula yeast while larval flies were reared on proprietary diets developed by CRI. Fruit flies were used in the following in-house research.

Experiment 772. Development of a Mediterranean fruit fly cold disinfestation treatment above 0°C for citrus. A medium scale trial using Clementines, oranges, lemons and grapefruit was undertaken. At least two replicates of each cultivar type were done (3 000 000 flies supplied). Oranges were considered the most suitable host although there were no statistical differences in the results.

Experiment 773. Fruit fly baits – alternatives to organophosphates. Cage tests were done using laboratory-reared Mediterranean fruit fly (1 500 flies supplied). Products tested were methomyl, mevinphos, tartar emetic, formetanate and fipronil. Only methomyl was considered to have some potential as an alternative treatment. Further products will be investigated.

Experiment 774. Development of a rapid diagnostic test to distinguish Mediterranean fruit fly larvae from other fruit-inhabiting species. Larvae of all three species (300 flies supplied) were shipped to England where Central Science Laboratories are undertaking the development of a test kit. Preliminary results appear to be good.

Fruit flies have also been supplied to an academic institution (Stellenbosch University) and sister industries (avocado, mango, litchi and persimmons).

3.5.3 Fruit Fly Bait Sprays – Alternatives to Organo phosphates

Experiment 773 by Tony Ware, Peter Stephen and Bruce Tate (CRI)

Opsomming

Die gebruik van organofosfate is bestem om in die toekoms uifaseer te word. Ses produkte is ondersoek in hoktoetse as lonende plaasvervangers vir organofosfate wat tradisioneel in lokase gebruik is vir vrugtevliegbeheer in die sitrusbedryf. Mevinphos, braakwynsteen, chlorfenapyr en fipronil het nie lonende beheer gegee nie. Twee produkte, methomyl en formetanate, het belofte ingehou, maar aangesien hulle tot die karbamaat-groep behoort wat ook gevaar loop om uifaseer te word, sal hulle nie verder ondersoek word nie. Navorsing op ander middels gaan voort.

Introduction

There is a worldwide trend to phase out the use of organophosphates. This will place pressure on the citrus industry where two organophosphates (mercaptotion and trichlorfon) are being used in baits for the control of fruit fly. As fruit flies are not only of economic importance but are also of phytosanitary significance, it is

imperative that the industry has alternatives when the traditional compounds are withdrawn. There are two registered non-organophosphate treatments (GF 120 NF and the M3 bait station) but the industry considers these not to be cost-effective alternatives. CRI has, therefore, undertaken to investigate alternatives that satisfy this criterion.

Materials and methods

One seedling of approximately 1.2 m in height was treated with exactly 1 ml of bait mixture of any one of the concentrations tested. One 10 µl drop was placed on each of 100 leaves using a micropipette. The bait was allowed to dry for 3 hours after which the trees (one per cage) were moved into gauze cages (1.1 X 0.6 X 1.85 m (l X b X h)). The product rates used are shown in Table 3.5.3.1. In general the highest rate tested was twice that registered for use on citrus (Nel *et al.*, 2002). Mevinphos (150 EC) is registered at 100 ml/100 l for bollworm (*Helicoverpa amigera* (Hübner), methomyl (200 g/l SL) at 450 ml/100 l for red scale (*Aonidiella aurantii* (Maskell)) and for thrips (*Scirtothrips aurantii* Faure), formetanate (500 g/kg SP) at 25 g/100 l, tartar emetic (995 g/kg SP) at 400 g/100 l, chlorfenapyr (360 g/l SC) at 30 ml/100 l and fipronil (200 g/l SC) at 10 ml/100 l. The traditional bait mercaptothion (500 g/l EC) was used as a standard at 175 ml/100 l.

For six days after their emergence, adult laboratory-reared Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) were fed granulated sugar and water *ad lib.* (i.e. were protein starved). Approximately 100 flies (\pm equal numbers of each sex) were released into each cage shortly after the trees were positioned. Exactly 24 hours later all the dead flies were removed and counted. The total numbers of flies in the cages were assessed and the percentage mortality determined. POLO-PC (LeOra Software) was used to determine LD₅₀ and LD₉₀ data.

Results and discussion

The mortality of the flies exposed to the various compounds is shown in Table 3.5.3.1. Based on the LD levels, mevinphos, tartar emetic, chlorfenapyr and fipronil did not show potential as cost-effective alternatives to the organophosphates (Table 3.5.3.2) and no further testing will be done on these products. Methomyl and formetanate showed potential but, as they belong to the carbamate group which may also be phased out, no further testing will be carried out.

Table 3.5.3.1. The percentage mortality of Mediterranean fruit fly after 24 hours exposure to various concentrations of candidate fruit fly baits

Mercaptothion	ml/100 l	0	0.175	0.875	3.5	17.5	52.5	175
	Mortality (%)	12.4	12.4	24.2	72	84.5	92	97
Mevinphos	ml/100 l	0	0.5	1	10	25	100	250
	Mortality (%)	3.0	9.4	10.4	14.4	17.9	39	31
Methomyl	ml/100 l	0	2	10	30	100	225	450
	Mortality (%)	17.9	30	67	86	97	97	100
Formetanate	g/100 l	0	2	10	25	75	200	-
	Mortality (%)	9.6	11.8	52	80	95	94	-
Tartar emetic	g/100 l	0	10	30	100	200	400	800
	Mortality (%)	3.9	4.5	14	12	15	18	37
Chlorfenapyr	ml/100 l	0	0.3	1	3	10	30	60
	Mortality (%)	10.3	2.3	1.1	17.9	29	32	37
Fipronil	ml/100 l	0	0.1	0.3	1	3	10	30
	Mortality (%)	8.6	9.4	8.5	40	34	59	50

Table 3.5.3.2. The LD₅₀ and LD₉₀ levels (% ai) after 24 hours exposure of Mediterranean fruit flies to various candidate fruit fly baits.

Product	LD ₅₀		LD ₉₀	
	mℓ/100 ℓ	% ai	mℓ/100 ℓ	%ai
Mercaptothion	3.12	0.002	34.4	0.017
Mevinphos	1644	0.247	682666	102.4
Methomyl	7.6	0.002	46	0.0092
Formetanate	11.25	0.006	47	0.024
Tartar emetic	42950	42.38	164090	163.27
Chlorfenapyr	115	0.041	5698	2.05

Fipronil	15.5	0.003	1146	0.229
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Conclusion

None of the non-organophosphate/carbamate products tested shows potential to be developed into fruit fly bait sprays as replacements for Malathion and trichlorfon.

Further research

It is recommended that abamectin, imidacloprid, acetamiprid, cypermethrin, permethrin and tau-fluvalinate be screened to determine their effectiveness as fruit fly baits.

Reference cited

Nel, A., Krause, M. and Khelawanlall, N. 2002. A Guide for the Control of Plant Pests. Department of Agriculture (39th Edition).

3.5.4 Sensitivity of Mediterranean Fruit Fly larvae in mandarins, lemons, grapefruit and oranges to cold treatment of 1°C

Experiment 772 by Tony Ware, Bruce Tate, Peter Stephen, John-Henry Daneel and Rooikie Beck (CRI)

Opsomming

Ontsmettingsnavorsing vereis tradisioneel dat 'n groot aantal individue behandel moet word om aan internasionale fitosanitêre sekuriteitsvereiste te voldoen. In hierdie navorsing word verslag gedoen oor mediumskaal proewe, wat 'n voorvereiste is vir toekomstige grootskaalse Probit vlak 9 navorsing. Vier *Citrus* spesies (*C. limon* Burm. F. "Eureka suurlemoen", *C. paradisi* Macfady "Marsh pomelo", *C. sinensis* (L.) Osbeck "Valencia lemoen" en *C. reticulata* Blanco "Clementine mandaryn") is besmet met Mediterreense vrugtevlieg (*Ceratitis capitata*) en blootgestel aan kouebehandeling van ongeveer 1°C vir verskeie tye. 'n Totaal van 41 940 larwes in 7 873 Clementine mandaryne, 108 516 in 8 063 Eureka suurlemoene, 143 251 in 20 278 Marsh pomelo's en 101 913 in 12 213 Valencia lemoene is behandel. Volgens Probit analises wil dit voorkom van larwes in Valencia lemoene die meeste bestand is teen koue, alhoewel daar nie statistiese regverdiging was om die spesie bo die ander te selekteer nie. Dit word aanbeveel dat Valencia lemoen in enige verdere navorsing gebruik word en dat die resultate op ander *Citrus* van toepassing sal wees. Resultate het verder aangedui dat kouebehandeling van 16 dae by 1°C sal voldoen aan die Probit 9 internasionale fitosanitêre standaard.

Introduction

The inter-regional movement of fresh produce can be severely restricted if it has the potential to harbour pests and diseases that do not occur in the country to which they are destined for export. One such group of pests are the fruit flies (Diptera; Tephritidae) and host commodities may be required to undergo some form of post-harvest disinfestation treatment before acceptance for importation. Of all the fruit fly species, arguably the most important is the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) (White and Elson-Harris, 1992). From its probable East African origin it has spread to most corners of the world with the notable exceptions of Far East Asia and North America (although periodic invasions do occur in California and Florida (Metcalf, 1994). One of the reasons for the species' success in its worldwide establishment is its large host range (White and Elson-Harris, 1992).

There are a number of post-harvest treatments that can be used to ensure phytosanitary security. These include fumigation, irradiation, hot water and heated air treatments, controlled atmosphere and the use of pesticides (Sharp and Hallman, 1994). However, it is cold treatment that has become the treatment of choice, mainly because of its ease of application and the fact that many fruit types can withstand low temperature exposure without undue deterioration of quality. Cold storage was originally used to inhibit decay and extend shelf life of fresh produce.

Baker (1939) recommended that a Probit 9 level of security be used as the phytosanitary standard. This requirement has been accepted by many countries including the United States and Japan and essentially implies that there be no survivors in 30 000 individuals treated at a 95% confidence level (Couey and Chew, 1986). In this research the effect of 1°C cold treatment on four species of Mediterranean fruit fly-infested citrus was investigated. These medium scale trials (also called Phase 3) are a prerequisite to large-scale

disinfestation trials (also called Phase 4) that are designed at demonstrating a Probit 9 level of phytosanitary security.

Materials and methods

Previous research (Ware, 1999) has shown that Mediterranean fruit fly larvae were most tolerant to cold treatment (Phase 2: Susceptibility of Mediterranean fruit fly life stages to cold treatment) when in their young phase of larval development (Phase 1: Development of life stages in mandarins). All research reported here was done using this life stage.

Test insects: Plums infested with Mediterranean fruit fly were collected from the Western Cape Province on December 6, 1998. These were transported to Nelspruit where they were used to initiate the laboratory colony. Annual additions of “wild” flies were made to this colony in order to maintain genetic variability.

The adult fruit flies were maintained in a constant environment room where the temperature was maintained at 26°C (\pm 2°C) with a relative humidity of approximately 60% and a day/night photoperiod of 14/10 hours. Colonies of approximately 15000 adults were housed in cylindrical cages made of gauze (length 140 cm; diameter 45 cm). The tops and bottoms of the cages consisted of plastic bowls. Yeast hydrolysate and white granulated sugar were supplied *ad lib*. The females oviposited through the gauze and eggs were collected in a water-filled bowl placed under the cage. In order to perpetuate the colony, eggs were collected at regular periods and allowed to hatch on an artificial medium made from bran, carrot powder, sugar and yeast. The larvae were maintained on this diet and allowed to pupate in sand.

The following data were obtained on the laboratory colony. Eggs (600) were placed on wet filter paper and 96% hatched. Pupae (500) were placed in a cage and the number of flies emerging determined. The sex ratio was almost equal with 50.7% of the flies emerging being female. These flies laid 113 375 eggs or an average of 487 eggs per individual. The duration of each life stage under the rearing conditions was 3 days for eggs, 8 days for larvae, 6 days for pupae, while the adults lived for approximately 30 days.

Test fruit. Clementine mandarins (*C. reticulata* Blanco) were sourced from Esser Boerdery near Burgersfort, the Eureka lemons (*C. limon* Burm. F.) were supplied from Bakgat farm situated in the Schoemanskloof Valley near Nelspruit, Valencia oranges (*C. sinensis* [L.] Os.) from Crocodile Valley Citrus Company near Nelspruit and most of the Marsh grapefruit (*C. paradisi* Macf.) were obtained from Neo-Nova Packhouse (Onderberg) (all in Mpumalanga Province). Grapefruit for the last replicate was sourced from Eros Packhouse in Kirkwood (Eastern Cape Province). All fruit was maintained in a cold room at approximately 4°C until required. Twenty-four hours before they were used they were removed from the cold room, allowed to come to ambient temperature and then treated with a fungicide (imazalil sulphate (0.5%) or guazatine (0.1%)).

Cold chamber. One cold chamber (room 1) was a custom-built unit constructed out of Isowall (polystyrene sandwiched between aluminium sheets) while the other (room 2) was constructed from polystyrene sandwiched between glassfibre sheeting. The two units were positioned side-by-side on a concrete floor in a warehouse. The dimensions of the rooms were: Room 1 - length 4.0 m, breadth 2.95 m and height 2.42 m; Room 2 - length 4.7 m, breadth 3.8 m and height 2.45 m. The chiller units in both the rooms were situated near the ceiling opposite the sliding doors. Heating coils were used to defrost the chiller units. These were employed every 8 hours for 15 minutes. During the defrost cycle the fans were switched off. The cold chamber temperatures were controlled using a Carel CR72 Universal electronic controller. Fruit core temperature variation during the defrost cycle was less than 0.2°C.

Three Grant 1200 Series (12 bit) Squirrel meter/Loggers were used to record data obtained from a set of 16 two-wired resistance thermocouples placed in each of two cold rooms. The accuracy of the thermoprobes was tested using the freezing-point method. This involved placing the thermoprobes on melting ice and measuring the resultant temperature. Approximately 20 readings were taken at 2-minute intervals before each experiment. These were summed and averaged and these averages were deemed to be the correction factors and are shown in Tables 3.5.4.1a and 3.5.4.1 b.

All but two thermoprobes were placed in untreated fruit and selectively placed within the treated fruit as shown in Figure 3.5.4.1. The remaining two probes were used to measure air temperature with probe 1 measuring the outlet temperature and probe 2 measuring the inlet temperature. Because some fruit was removed every second day from day three, the thermoprobes placed in the fruit were not randomly distributed but rather placed in the lug boxes destined to be removed last. This was a compromise so as not to disturb the thermoprobes unduly. Treatments were considered to have begun immediately after the transfer of the fruit into the cold chamber. The thermoprobe readings were downloaded hourly. Minor

temperature adjustments were made to the cold chamber throughout the experiment to ensure that the long-term average approximated 1°C.

Table 3.5.4.1a. Calibration factors for each experiment as calculated using the freezing-point method for Room 1.

Logger serial number	1025 KE-05002	1025 KE-05002	1025 KE-05002	1025 KE-05001	1025 KE-05001	1025 KE-05001
Species	Mandarins 1	Mandarins 2	Lemons 1	Oranges 1	Grapefruit 3	Grapefruit 5
Date	07/05/04	24/05/04	09/06/04	12/07/04	26/07/04	18/08/04
Sensor 1	0.01	0.02	0.00	0.13	0.19	0.16
Sensor 2	0.04	0.03	0.01	0.14	0.16	0.17
Sensor 3	0.08	0.06	0.03	0.09	0.13	0.08
Sensor 4	0.19	0.08	0.06	0.00	0.05	0.02
Sensor 5	0.02	0.05	-0.03	1.63	1.52	1.66
Sensor 6	0.05	0.07	-0.03	1.56	1.48	1.62
Sensor 7	0.07	0.08	-0.01	1.36	1.39	1.44
Sensor 8	0.12	0.11	0.01	1.21	1.17	1.29
Sensor 9	0.22	0.12	0.05	0.91	0.91	0.95
Sensor 10	0.34	0.24	0.11	0.77	0.80	0.91
Sensor 11	0.29	0.16	0.08	0.50	0.53	0.49
Sensor 12	0.32	0.18	0.15	0.16	0.25	0.19
Sensor 13	0.07	0.11	0.02	1.40	1.34	1.53
Sensor 14	0.03	0.09	0.04	1.05	1.03	1.15
Sensor 15	0.05	0.10	0.04	0.68	0.63	0.74
Sensor 16	0.11	0.10	0.13	0.39	0.39	0.45

Table 3.5.4.1b. Calibration factors for each experiment as calculated using the freezing-point method for Room 2.

Logger serial number	1F8 / KS0418001					
Species	Lemons 2	Oranges 2	Grapefruit 4	Grapefruit 6	Oranges 3	Grapefruit 7
Date	03/06/04	15/07/04	29/07/04	23/08/04	13/09/04	18/10/04
Sensor 1	0.02	0.02	-0.06	-0.01	0.08	0.07
Sensor 2	0.01	-0.05	-0.09	-0.02	-0.02	0.01
Sensor 3	0.19	0.04	-0.07	0.02	0.12	0.10
Sensor 4	0.11	0.01	-0.08	-0.07	0.04	0.05
Sensor 5	0.29	0.02	-0.04	0.05	0.07	0.01
Sensor 6	0.03	-0.13	-0.22	-0.23	-0.21	-0.03
Sensor 7	0.21	-0.02	-0.10	-0.06	-0.03	0.00
Sensor 8	0.03	-0.04	-0.12	-0.10	-0.06	-0.03
Sensor 9	0.02	-0.11	-0.22	-0.20	-0.21	-0.11
Sensor 10	0.00	-0.11	-0.20	-0.19	-0.18	-0.08
Sensor 11	-0.01	-0.11	-0.21	-0.19	-0.18	-0.10
Sensor 12	-0.02	-0.21	-0.29	-0.28	-0.28	-0.17
Sensor 13	0.04	0.01	-0.12	-0.07	-0.26	-0.04
Sensor 14	0.05	-0.13	-0.22	-0.18	-0.25	-0.14
Sensor 15	0.08	-0.12	-0.22	-0.19	-0.28	-0.16
Sensor 16	-0.06	-0.35	-0.42	-0.46	-0.31	-0.30

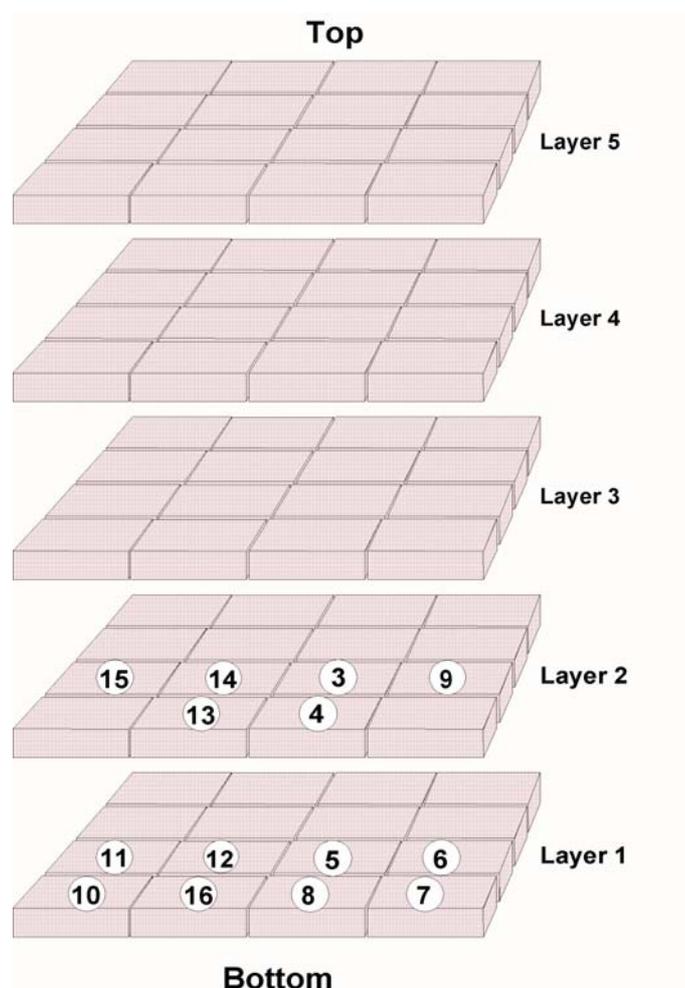


Fig. 3.5.4.1. Placement of thermoprobes in cold chambers.

Inoculation procedure. The fruit was prepared for inoculation with fruit fly eggs by drilling through the calyx end towards the centre using a 6 mm wood drill bit to a depth of approximately 2 cm. The drill bit was washed with fungicide after approximately every 10 fruit processed to ensure that fungal cross contamination was kept to a minimum.

An inoculum of eggs was prepared by gathering the eggs from beneath the cages within 12 hours of their oviposition. The eggs were then diluted in water until approximately 40 eggs were delivered into the fruit using an automatic pipette (Kartell Pluripet PL 200) (Table 3.5.4.2). The wound was then plugged with polystyrene or cotton wool covered with paraffin wax. Each fruit was then placed into a brown paper bag. The fruit were then randomly packed into plastic lug boxes that were then placed at 26°C for 6 to 7 days in order to ensure that all larvae were in the young larval development phase (Ware, 1999). All the fruit, except for 100 untreated controls, were then moved to the cold chamber. The control fruit were dissected and all larvae (alive and dead) counted. Approximately 650 fruit were removed from the cold chamber after 3, 5, 7, 9, 11 and 13 days cold treatment. This fruit was placed at 26°C for 24 hours before being dissected. Again all larvae (dead and alive) were recorded. A diary of events is presented in Table 3.5.4.2.

Table 3.5.4.2. Diary of events together with the amount of eggs inoculated into the fruit.

Species (replicate number)	Date				Mean number of eggs (range)
	Inoculated	Control dissected	Cold treatment		
			Started	Ended	
Mandarin (1)	4 May	12 May	10 May	23 May	43.7 (27-55)
Mandarin (2)	19 May	25 May	26 May	8 June	38.4 (22-57)
Lemon (1)	3 June	9 June	9 June	22 June	41.1 (27-63)
Lemon (2)	8 June	14 June	14 June	27 June	41.5 (26-54)
Grapefruit (1)	24 June	30 June	*	*	44.6 (37-65)
Grapefruit (2)	29 June	*	*	*	42.8 (26-59)
Orange (1)	6 July	12 July	12 July	25 July	44.6 (16-104)

Orange (2)	9 July	15 July	15 July	28 July	43.5 (32-58)
Grapefruit (3)	20 July	26 July	26 July	8 Aug	55.4 (34-68)
Grapefruit (4)	23 July	29 July	29 July	11 Aug	53.0 (38-84)
Grapefruit (5)	12 Aug	18 Aug	19 Aug	1 Sept	49.2 (38-65)
Grapefruit (6)	17 Aug	23 Aug	23 Aug	5 Sept	50.9 (38-62)
Orange (3)	7 Sept	13 Sept	13 Sept	26 Sept	52.9 (41-71)
Grapefruit (7)	12 Oct	18 Oct	19 Oct	31 Oct	38.2 (19-62)

* Experiments aborted

Statistical analysis. All data were subjected to Probit analysis (Finney, 1971) using POLO-PC software (LeOra Software). The fiducial limit of each replicate was calculated to a 90% confidence limit as some of the data varied excessively from the mathematical model for higher confidence reading to be obtained. Where possible the data for each citrus species were combined and a 99% fiducial limit confidence was used. The Probit analysis was done in two ways. The first was to use the number of eggs inoculated and the number of survivors in the control to estimate the number of individuals treated (indirect method). The second was to use the actual number of dead and live individuals in the analysis (direct method). The use of the two methods served as a check on technicians' accuracy.

Results

Cold Chamber. The corrected thermoprobe data are presented in Appendices 1 to 12. A summary of the cold chamber experimental conditions is shown in Table 3.5.4.3. Although every attempt was made to minimise the period from when the fruit was placed in the cold chamber and the fruit reaching the target temperature of 1°C, the time recorded for this to happen varied from 46 to 91 hours. The average temperature was maintained within 7% of the targeted temperature with the exception of the 5th grapefruit replicate. The hourly average maximum temperatures were within 1°C of the target temperature while the hourly average minimum temperatures were within 0.6°C of the desired temperature.

Table 3.5.4.3. Summary of thermoprobe data. The hourly average, hourly average maximum and hourly average minimum readings were calculated from the time seven of the thermoprobe readings were at 1°C or below. The trial was terminated immediately after the last fruit were removed from the cold room.

Species (replicate)	Time taken for average fruit probe temperature to reach 1°C (hours)	Average (°C)	High (°C)	Low (°C)
Mandarin (1)	56	0.998	1.490	0.544
Mandarin (2)	52	0.990	1.576	0.641
Lemon (1)	50	1.070	1.668	0.490
Lemon (2)	67	1.029	1.295	0.716
Grapefruit (3)	87	1.036	1.569	0.468
Grapefruit (4)	56	1.015	1.315	0.702
Grapefruit (5)	91	1.160	1.627	0.676
Grapefruit (6)	56	0.998	1.379	0.701
Grapefruit (7)	71	1.038	1.377	0.667
Orange (1)	57	1.007	1.504	0.604
Orange (2)	46	0.993	1.394	0.624
Orange (3)	51	1.031	1.395	0.663

Clementine mandarins. The results of the two replicates done using mandarins are shown in Table 3.5.4.4. An average of 43.7 eggs per fruit were inoculated in the first experiment from which 6.9 larvae/fruit emerged from the controls. In the second experiment 38.4 eggs were inoculated and this resulted in 6.23 larvae in each of the untreated control fruit. Only 1 larva survived the cold treatment for 11 days and none for 13 days.

Table 3.5.4.4. Mortality of larval Mediterranean fruit fly in Clementine mandarins after cold treatment of more than 0°C for various periods.

Rep-licate	Exposure (days)	Number of fruit inoculated	Estimated number of eggs inoculated	Estimated number of larvae treated ^a	Actual number of larvae treated	Number of survivors	Estimated mortality (%)	Actual mortality ^b (%)
1	0	100	4370		690	660		4.3
	3	620	27094	4092	2952	2558	37.5	13.3
	5	617	26963	4042	3308	1473	63.8	55.5
	7	618	27007	4079	3245	620	84.7	80.9
	9	625	27313	4125	3839	36	99.1	99.1
	11	631	27575	4165	4731	0	100	100
	13	681	29760	4195	4412	0	100	100
2	0	101	3878		623	612		1.8
	3	659	25306	3993	2774	2409	39.7	13.2
	5	646	24806	3915	2605	1684	57.0	35.4
	7	634	24346	3842	2632	591	84.6	77.6
	9	632	24269	3830	3474	13	99.7	99.6
	11	652	25037	3951	3236	1	99.97	99.96
	13	657	25229	3981	3419	0	100	100

^a This value was calculated by determining the average number of live larvae in each of the control fruit times the total number of fruit inoculated.

^b This value is based on the actual number of dead and live larvae counted.

The Probit analyses of the Clementine mandarin data are presented in Table 3.5.4.5. The two methods of calculating the values (using the estimation of the number of eggs treated and the number of larvae surviving or the actual number of dead and live larvae after treatment) gave similar results. The time to kill 99% of the larvae (combined data) was calculated to be 9.86 (fiducial limits 8.63 – 13.75) days using the egg data and 9.88 (fiducial limits 8.62 – 13.07) days using the larval data. The data indicates that any exposure of infested fruit exceeding 14 days will provide phytosanitary security for Clementine mandarins. The close correlation of the two sets of results to within 0.3% indicates the accuracy of the operators in assessing the numbers of larvae.

Table 3.5.4.5. Probit analyses of Clementine data (Table 3.5.4.4). The figures in parentheses indicate the confidence limits of the fiducial values.

Replicate		LT ₅₀	LT ₉₀	LT ₉₉
1 (egg)	Mean	5.34	7.59	10.12
	Lower limit (0.90)	3.50	6.61	8.76
	Higher limit (0.90)	6.24	8.78	14.72
2 (egg)	Mean	5.78	7.64	9.60
	Lower limit (0.90)	4.05	6.81	8.45
	Higher limit (0.90)	6.56	8.79	13.74
Combined data from replicate 1 and 2 (egg)	Mean	5.57	7.63	9.86
	Lower limit (0.99)	3.97	6.77	8.63
	Higher limit (0.99)	6.37	8.75	13.75
1 (larva)	Mean	5.06	7.43	10.14
	Lower limit (0.90)	4.16	6.82	8.95
	Higher limit (0.90)	5.66	8.27	12.87
2 (larva)	Mean	5.83	7.65	9.55
	Lower limit (0.90)	5.30	7.15	8.64
	Higher limit (0.90)	6.24	8.41	11.30
Combined data from replicate 1 and 2 (larva)	Mean	5.46	7.57	9.88
	Lower limit (0.99)	4.59	6.90	8.62
	Higher limit (0.99)	6.03	8.69	13.07

The graphs of the hourly average, hourly maximum and hourly minimum temperatures of the Clementine mandarin fruit cores are presented in Figures 3.5.4.2 and 3.5.4.3. No unusual trends were noted in either of these experiments.

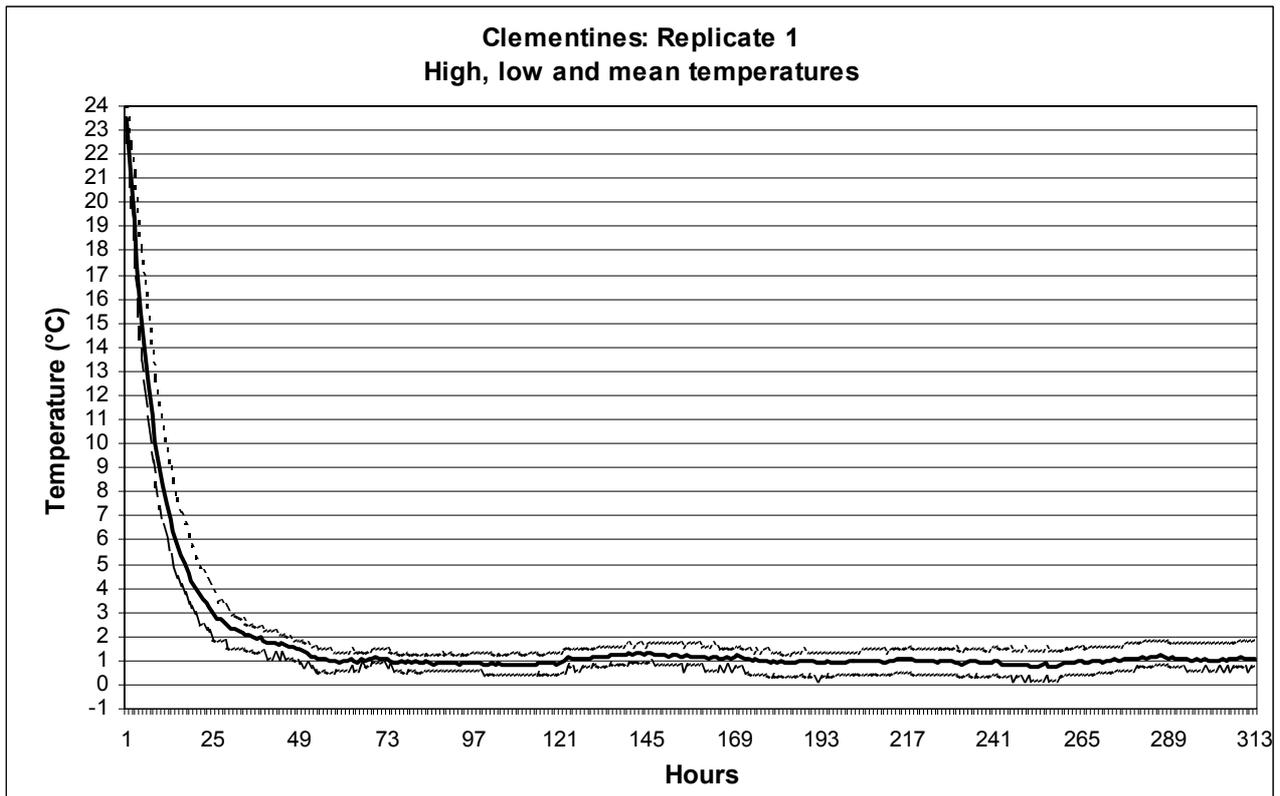


Fig. 3.5.4.2. Hourly mean, high and low temperatures in fruit cores of Clementine mandarin replicate 1

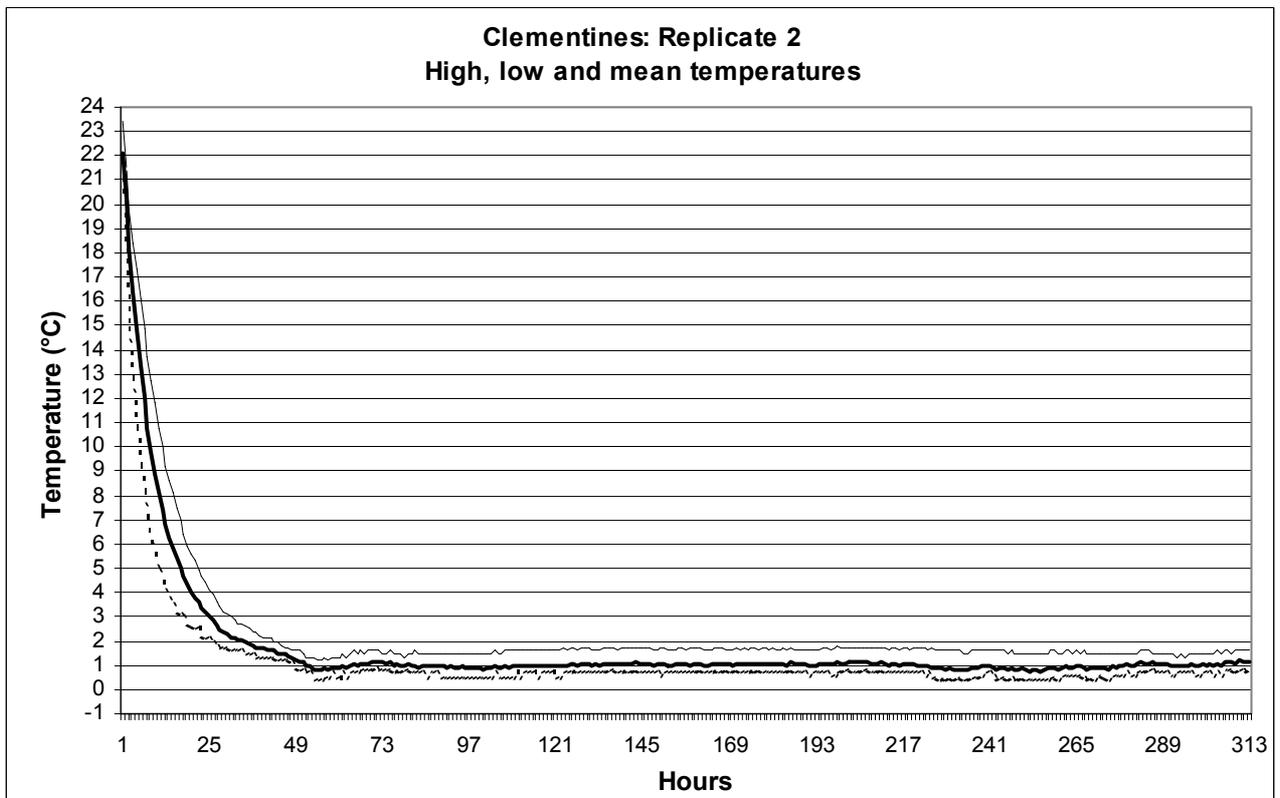


Fig. 3.5.4.3 Hourly mean, high and low temperatures in fruit cores of Clementine mandarin replicate 2

Eureka lemons. The results of the two replicates using Eureka lemons are shown in Table 3.5.4.6. In the first experiment an average of 41.1 eggs/fruit was inoculated from which 12.8 larvae/fruit emerged in the untreated control fruit while in the second experiment an average of 41.5 eggs was inoculated into each fruit

and this also resulted in an average of 12.8 larvae recorded (live and dead) from each fruit. In the two replicates only 2 larvae survived 11 days of cold treatment and none survived 13 days.

Table 3.5.4.6. Mortality of larval Mediterranean fruit fly in Eureka lemons after cold treatment of more than 0°C for various periods.

Rep- licate	Exposure (days)	Number of fruit inoculated	Estimated number of eggs inoculated	Estimated number of larvae treated ^a	Actual number of larvae treated	Number of survivors	Estimated mortality (%)	Actual mortality (%) ^b
1	0	109	4480		1393	1333		4.3
	3	645	26510	7888	8461	7545	4.4	10.8
	5	647	26592	7912	8997	5056	36.1	43.8
	7	641	26345	7839	9752	1417	81.9	85.5
	9	640	26304	7827	9246	43	99.5	99.5
	11	665	27332	8133	10609	2	99.98	99.98
	13	667	27414	8157	10497	0	100	100
2	0	102	4233		1303	1234		5.3
	3	647	26851	7828	8284	7232	7.6	12.1
	5	654	27141	7912	6922	3303	58.3	52.3
	7	658	27307	7961	7922	939	88.2	88.2
	9	651	27017	7876	8136	36	99.5	99.6
	11	666	27639	8057	8153	0	100	100
	13	671	27847	8118	8841	0	100	100

^a This value was calculated by determining the average number of live larvae in each of the control fruit times the total number of fruit inoculated.

^b This value is based on the actual number of dead and live larvae counted.

The Probit analyses of the Eureka lemon data are presented in Table 3.5.4.7. Again the two methods of calculating the values (using the estimation of the number of eggs treated and the number of larvae surviving or the actual number of dead and live larvae after treatment) gave similar results. The time to kill 99% of the larvae (combined data) was calculated to be 9.34 (fiducial limits 8.38 – 11.40) days using the egg data and 9.26 (fiducial limits 8.57 – 10.38) days using the larval data. The data indicate that any exposure of infested fruit exceeding 12 days will provide phytosanitary security for Eureka lemons. The close correlation of the results to within 0.1 of a day is indicative of the accuracy of the technicians' work.

Table 3.5.4.7. Probit analyses of Eureka lemon data (Table 3.5.4.6). The figures in parentheses indicate the confidence limits of the fiducial values.

Replicate		LT ₅₀	LT ₉₀	LT ₉₉
1 (egg)	Mean	5.64	7.44	9.34
	Lower limit (0.90)	5.24	7.14	8.79
	Higher limit (0.90)	5.95	7.78	10.15
2 (egg)	Mean	4.86	6.93	9.27
	Lower limit (0.90)	4.34	6.56	8.58
	Higher limit (0.90)	5.25	7.34	10.33
Combined data from replicate 1 and 2 (egg)	Mean	5.28	7.23	9.34
	Lower limit (0.99)	4.45	6.66	8.38
	Higher limit (0.99)	5.84	7.99	11.40
1 (larva)	Mean	5.43	7.30	9.30
	Lower limit (0.90)	5.12	7.00	8.70
	Higher limit (0.90)	5.64	7.66	10.17
2 (larva)	Mean	5.13	7.09	9.22
	Lower limit (0.90)	4.80	6.80	8.59
	Higher limit (0.90)	5.40	7.46	10.19
Combined data from replicate 1 and 2 (larva)	Mean	5.30	7.21	9.26
	Lower limit (0.99)	4.96	6.87	8.57
	Higher limit (0.99)	5.59	7.67	10.38

A three-hour power failure was recorded 217 hours into the experiment resulting in a temperature increase of nearly 1°C. It took 26 hours for the temperature to return to the desired 1°C (Figure 3.5.4.4). In the second lemon replicate the experiment was initiated but the logger was not functioning. A new one was bought and logging was initiated 49 hours into the experiment. The desired temperature of 1°C was reached at 67 hours (Figure 3.5.4.5).

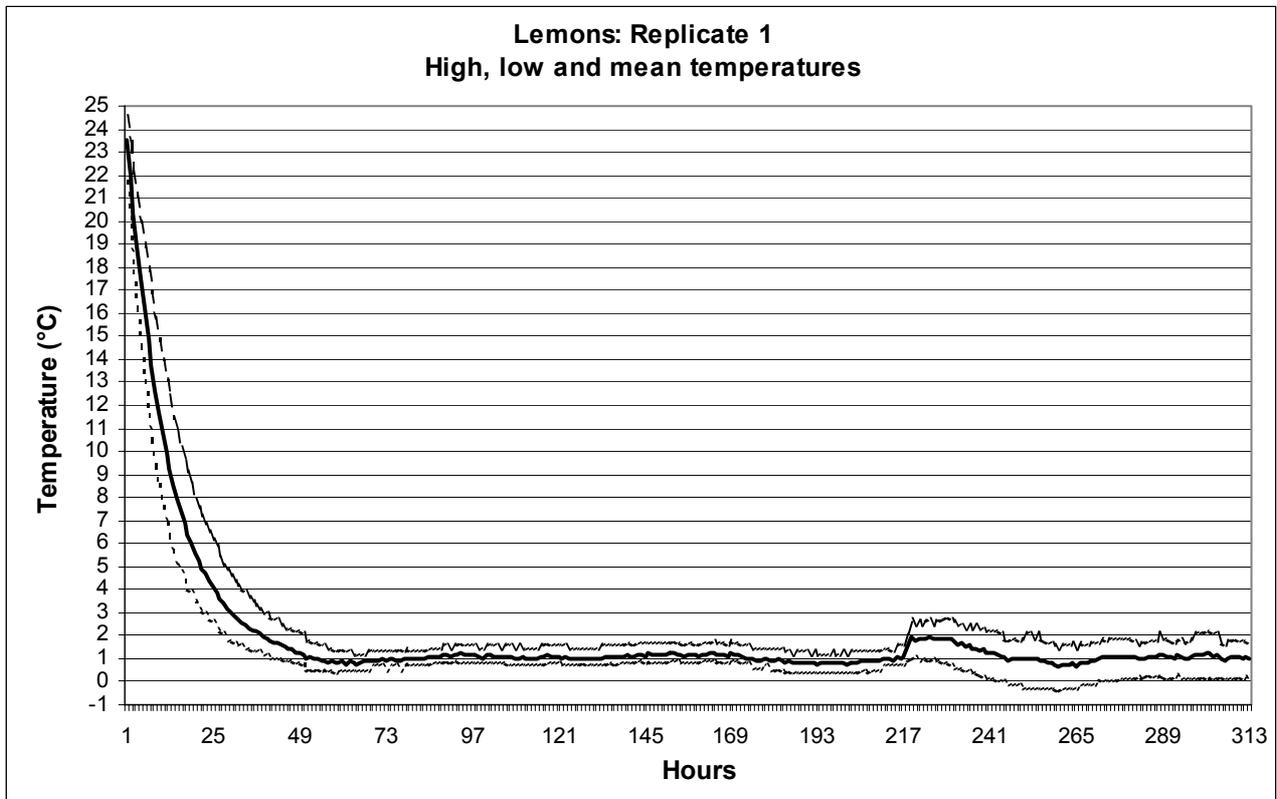


Fig. 3.5.4.4. The hourly mean, high and low temperatures in fruit cores of Eureka lemons replicate 1

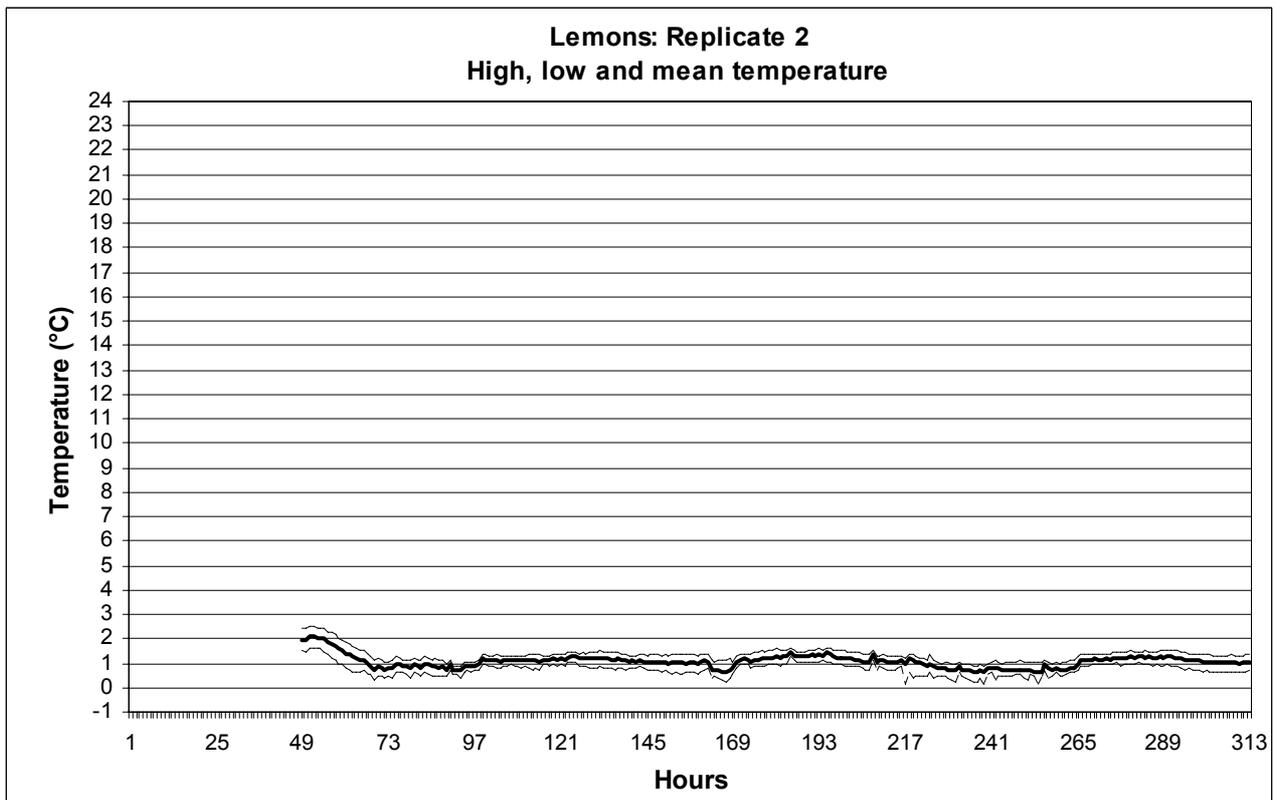


Fig. 3.5.4.5 The hourly mean, high and low temperatures in fruit cores of Eureka lemons replicate 2

Marsh grapefruit. Seven experiments were done on the grapefruit (Table 3.5.4.8). The first replicate was aborted after determining the control survival rate (3.2 larvae/fruit) was below the target of at least 5 larvae per fruit. The low number was ascribed to the fact that there was a high occurrence of sour rot in fruit. The second replicate was also aborted because of fruit condition and low larval survival. Although the third and fourth experiments also had low larval control survival (4.8 and 2.0 respectively) it was decided to complete these experiments. Sixty individuals from the third experiment and one individual from the fourth experiment survived 9 days of treatment. There were no survivors after 13 days exposure. The fifth experiment had a control larval survival rate of 7.2/fruit. Forty individuals survived 9 days of treatment. In the sixth experiment there was a control survival rate of 8.5 larvae/fruit from the 50.9 eggs inoculated. Six individuals survived 9 days treatment. In the final grapefruit experiment the control survival rate was 8.3 larvae/fruit and 86 individuals survived 9 days of treatment.

Table 3.5.4.8. Mortality of larval Mediterranean fruit fly in Marsh grapefruit after cold treatment of more than 0°C for various periods.

Rep- licate	Exposure (days)	Number of fruit inoculated	Estimated number of eggs inoculated	Estimated number of larvae treated ^a	Actual number of larvae treated	Number of survivors	Estimated mortality (%)	Actual mortality (%) ^b
1	0	153	6824		750	492		34.4
	3	Aborted						
2	Aborted							
3	0	103	5706		585	475		18.8
	3	651	36065	3002	3242	2331	22.4	28.1
	5	651	36065	3002	2641	1193	60.3	54.8
	7	654	36232	3016	2956	884	70.7	70.0
	9	658	36453	3035	3598	60	98.0	98.3
	11	654	36232	3016	2279	0	100	100
	13	653	36176	3012	3011	0	100	100
4	0	100	5300		289	203		29.8
	3	652	34556	1324	1193	486	63.3	59.3
	5	651	34503	1322	1039	252	80.9	75.8
	7	650	34450	1320	1528	21	98.4	98.6
	9	649	34397	1318	927	1	99.9	99.9
	11	643	34079	1305	1668	0	100	100
	13	650	34450	1320	1436	0	100	100
5	0	103	5068		828	740		10.6
	3	656	32275	4713	3907	3302	29.9	15.5
	5	656	32275	4713	4723	2008	57.4	57.5
	7	649	31931	4662	4502	1239	73.4	72.5
	9	659	32423	4734	4023	40	99.2	99.0
	11	653	32128	4691	4791	0	100	100
	13	675	33210	4849	5463	0	100	100
6	0	103	5243		1020	879		13.8
	3	664	33798	5666	5624	4437	21.7	21.1
	5	660	33594	5632	5178	796	85.9	84.6
	7	658	33492	5615	4917	64	98.9	98.7
	9	658	33492	5615	5768	6	99.9	99.9
	11	665	33849	5675	4982	0	100	100
	13	664	33798	5666	5077	0	100	100
7	0	106	4049		937	882		5.9
	3	656	25059	5459	8132	6881	72.5	15.4
	5	677	25861	5633	8091	2447	90.5	69.8
	7	672	25670	5592	10117	527	98.0	94.8
	9	677	25861	5633	10615	86	99.7	99.2
	11	668	25518	5559	9226	6	99.89	99.93
	13	680	25976	5658	8938	0	100	100

^a This value was calculated by determining the average number of live larvae in each of the control fruit times the total number of fruit inoculated.

^b This value is based on the actual number of dead and live larvae counted.

Probit analyses of the Marsh grapefruit data are presented in Table 3.5.4.9. Although the results of the third and fourth replicates are reported here the control survival rates were unacceptably low. It was interesting to note that the data derived from the number of eggs inoculated in experiment 6 varied from the mathematical model to such an extent as to invalidate the analysis although the data developed from the dead and live larvae allowed analysis to proceed. Based on the larval data set (replicates 6 and 7) a 10-day treatment is likely to provide phytosanitary security.

Table 3.5.4.9. Probit analysis of grapefruit data from Table 3.5.4.8. The figures in parentheses indicate the confidence limits of the fiducial values.

Replicate		LT ₅₀	LT ₉₀	LT ₉₉
3 (egg)	Mean	5.29	7.47	9.90
	Lower limit (0.90)	3.40	6.47	8.54
	Higher limit (0.90)	6.19	8.71	14.69
4 (egg)	Mean	3.06	5.49	8.85
	Lower limit (0.90)	0.70	2.95	6.77
	Higher limit (0.90)	4.55	7.15	14.58
5 (egg)	Mean	*	*	*
	Lower limit (0.90)	*	*	*
	Higher limit (0.90)	*	*	*
6 (egg)	Mean	*	*	*
	Lower limit (0.90)	*	*	*
	Higher limit (0.90)	*	*	*
7 (egg)	Mean	4.34	6.38	8.72
	Lower limit (0.90)	4.27	6.32	8.63
	Higher limit (0.90)	4.42	6.43	8.83
Replicates 6 and 7 combined (egg)	Mean	*	*	*
	Lower limit (0.99)	*	*	*
	Higher limit (0.99)	*	*	*
3 (larva)	Mean	5.60	8.36	10.95
	Lower limit (0.90)	3.84	7.30	9.24
	Higher limit (0.90)	6.94	10.49	19.60
4 (larva)	Mean	5.03	6.33	7.64
	Lower limit (0.90)	3.67	5.67	6.66
	Higher limit (0.90)	5.62	7.88	12.58
5 (larva)	Mean	*	*	*
	Lower limit (0.90)	*	*	*
	Higher limit (0.90)	*	*	*
6 (larva)	Mean	4.04	5.48	7.02
	Lower limit (0.90)	3.91	5.36	6.79
	Higher limit (0.90)	4.17	5.60	7.28
7 (larva)	Mean	4.35	6.38	8.71
	Lower limit (0.90)	4.32	6.34	8.62
	Higher limit (0.90)	4.40	6.42	8.74
Replicate 6 & 7 combined (larva)	Mean	4.24	6.11	8.24
	Lower limit (0.99)	3.68	5.64	7.40
	Higher limit (0.99)	4.67	6.71	9.71

* Probit analysis could not be done as data varied excessively from the mathematical model

The temperature profiles of the grapefruit trials are shown in Figures 3.5.4.6 through to 3.5.4.10. In the fifth grapefruit replicate the chiller unit developed a refrigerant leak that manifested itself 150 hours into the experiment and was repaired at 177 hours (Figure 3.5.4.8). It took approximately 31 hours for the temperature to return to “normal”.

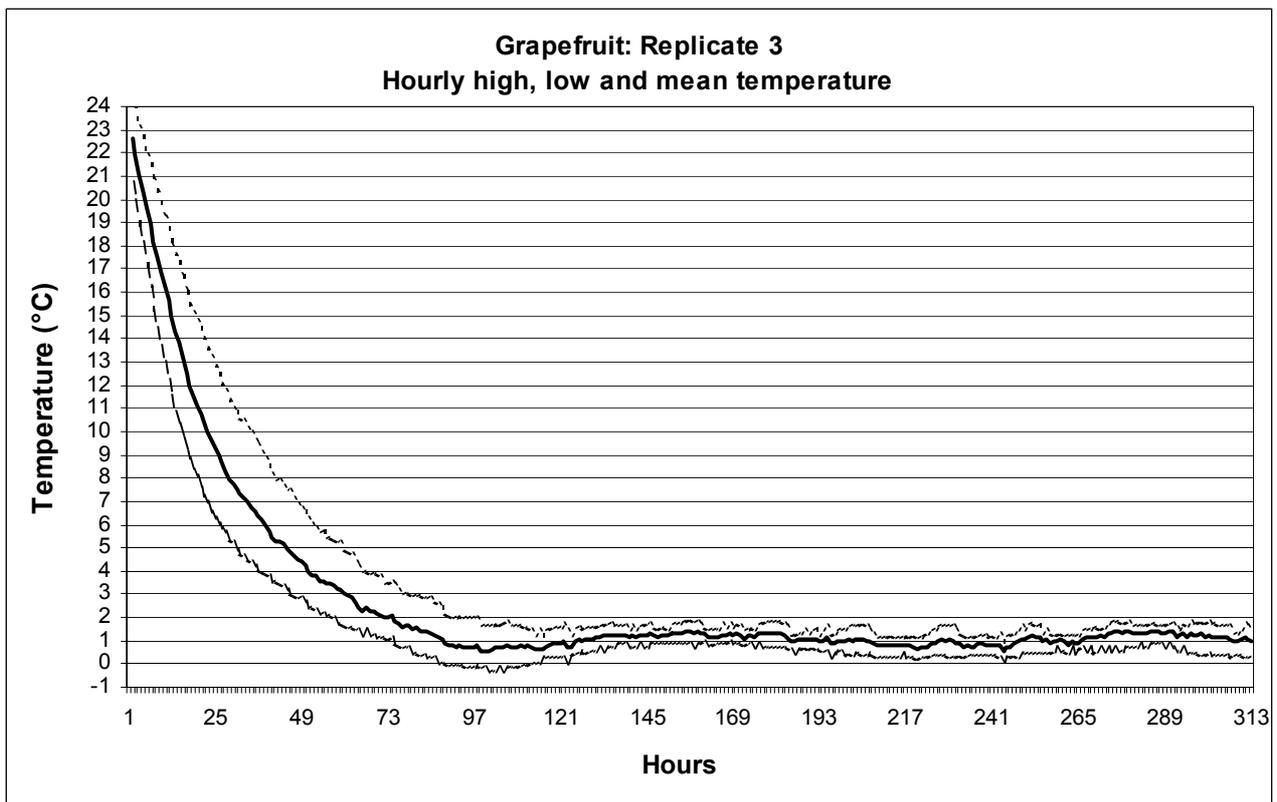


Fig. 3.5.4.6 The hourly mean, high and low temperatures in fruit cores of Marsh grapefruit replicate 3

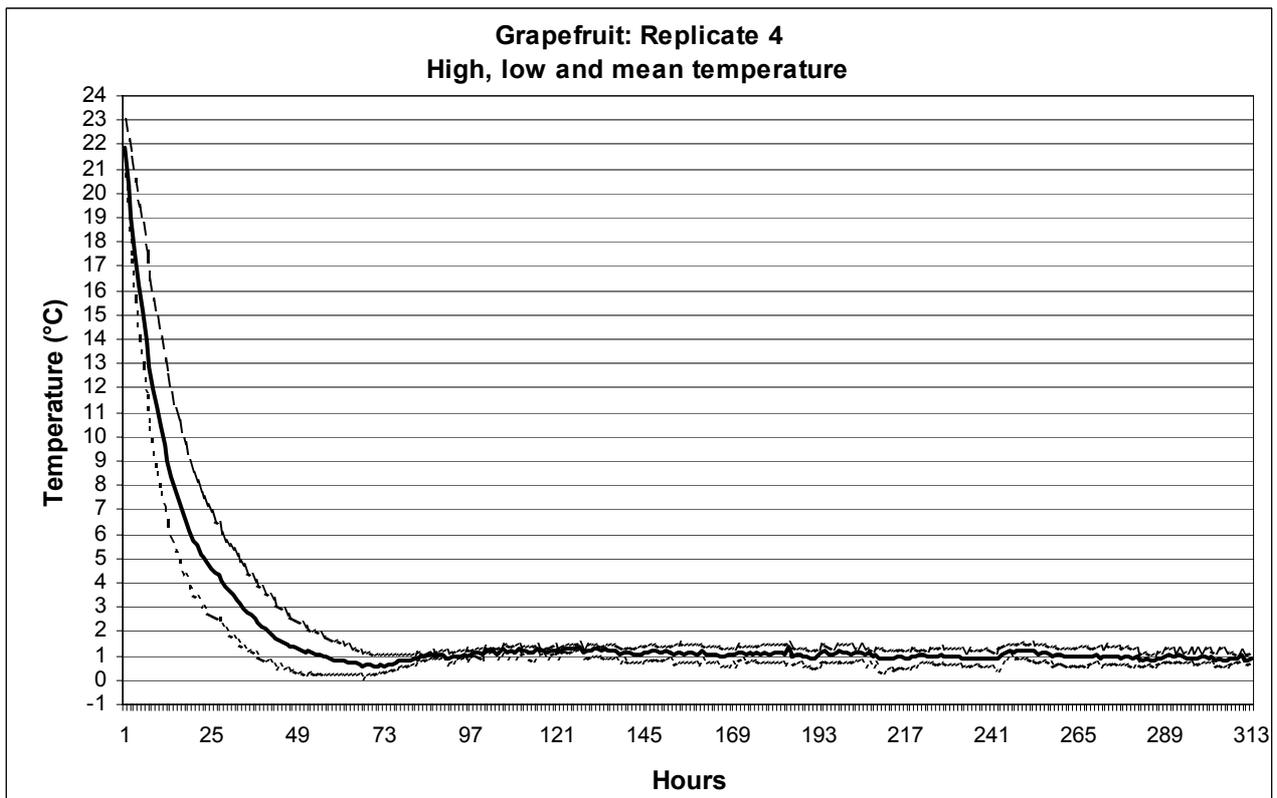


Fig. 3.5.4.7 The hourly mean, high and low temperatures in fruit cores of Marsh grapefruit replicate 4

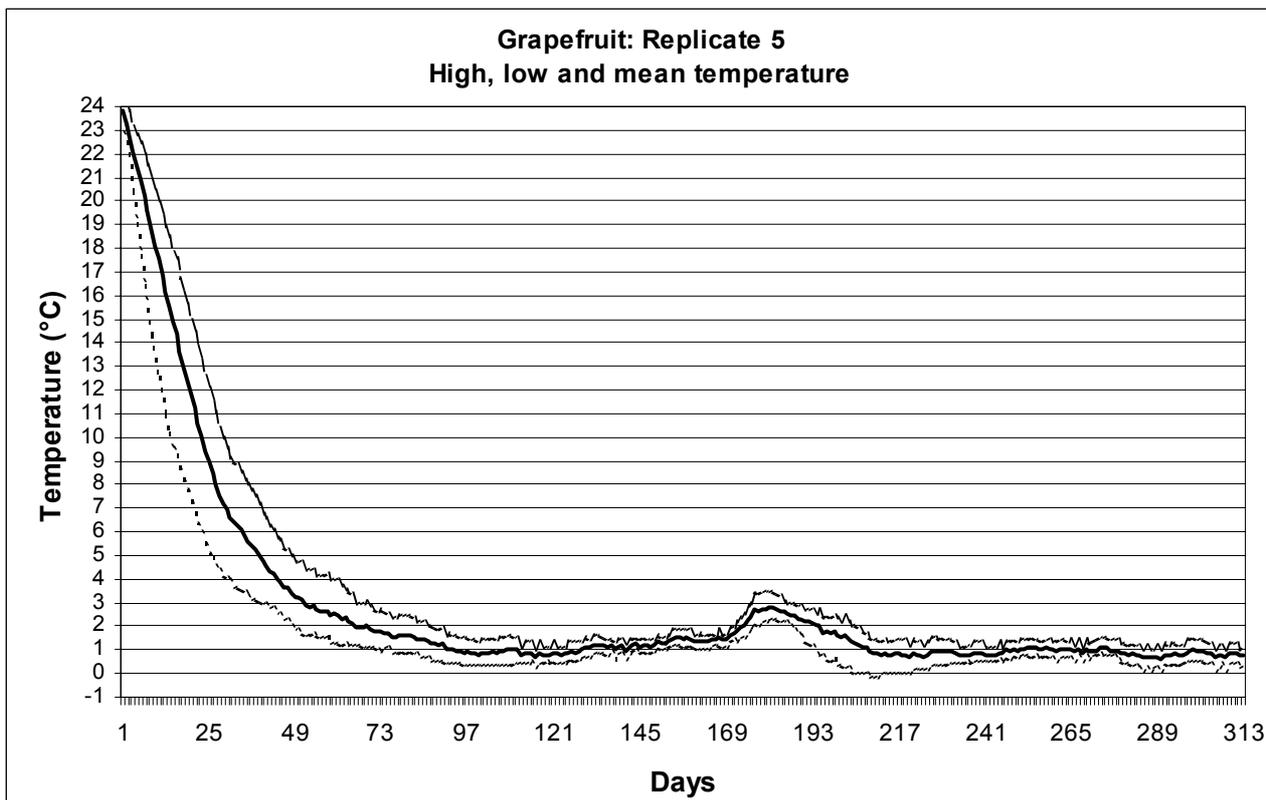


Fig. 3.5.4.8. The hourly mean, high and low temperatures in fruit cores of Marsh grapefruit replicate 5

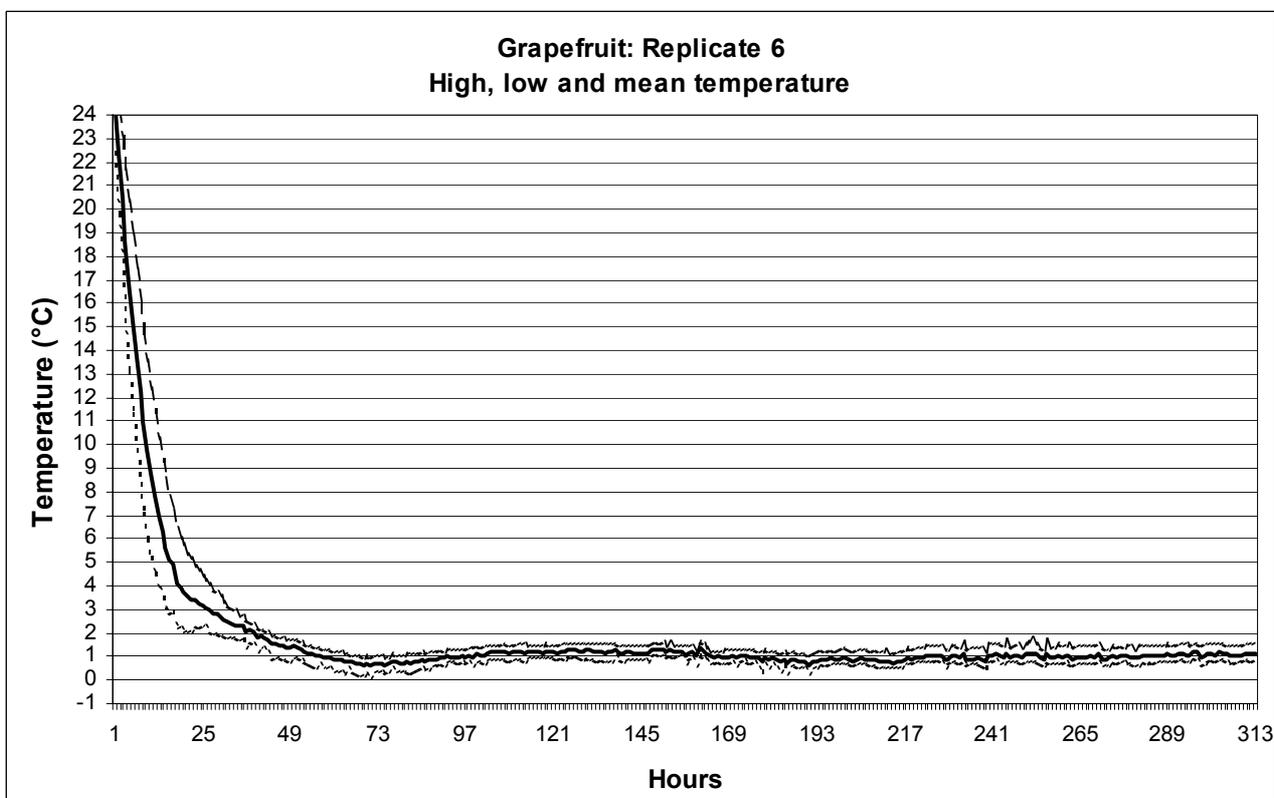


Fig. 3.5.4.9. The hourly mean, high and low temperatures in fruit cores of Marsh grapefruit replicate 6

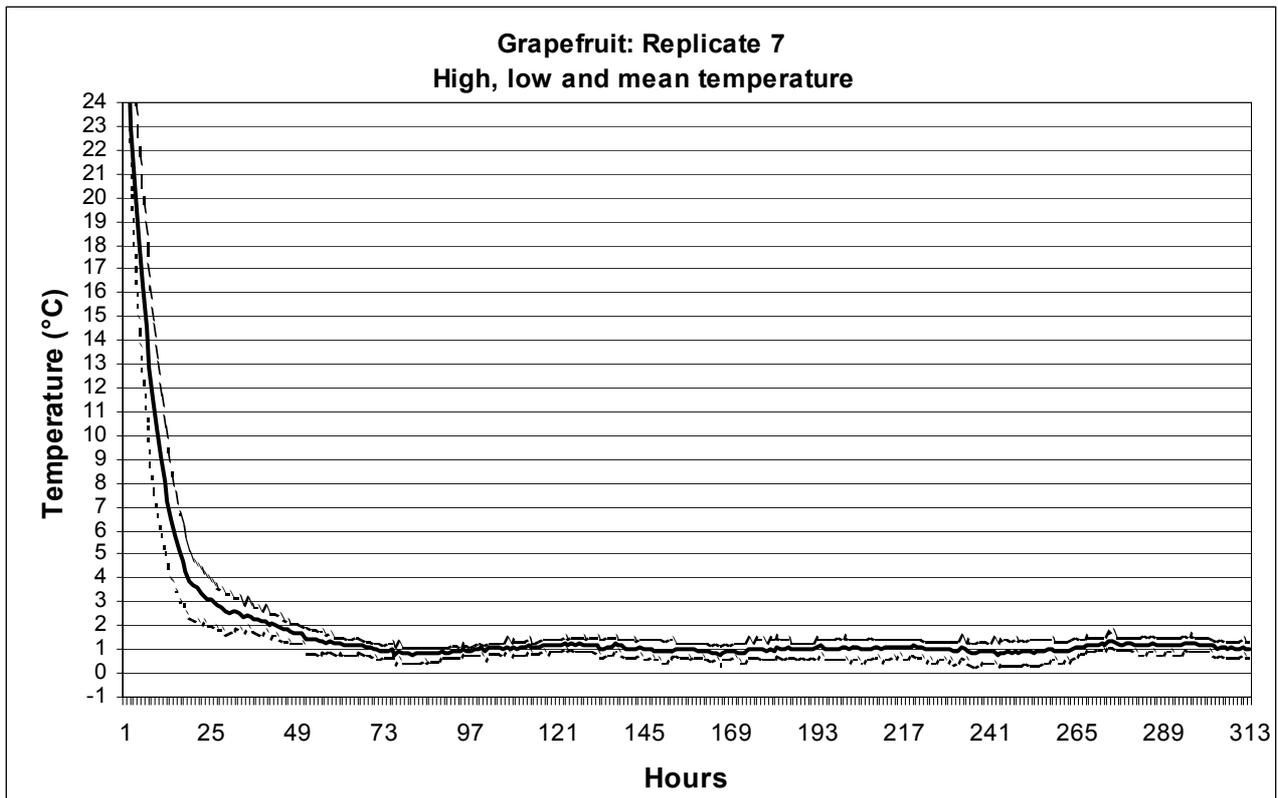


Fig. 3.5.4.10. The hourly mean, high and low temperatures in fruit cores of Marsh grapefruit replicate 7

Valencia oranges. Three experiments were done using Valencia oranges (Table 3.5.4.10). In the first experiment 44.6 eggs were inoculated into each fruit, in the second 43.5 and the third 52.9. The control larval survival rates were 3.0, 4.6 and 10.0/fruit respectively. Fifty-four individuals survived 9 days of treatment in the first orange experiment, 55 in the second and only 20 in the third. However, one individual survived 11 days and five individuals (from two fruit) 13 days treatment in the third replicate.

Table 3.5.4.10. Mortality of larval Mediterranean fruit fly in Valencia oranges after cold treatment of more than 0°C for various periods.

Rep- licate	Exposure (days)	Number of fruit inoculated	Estimated number of eggs inoculated	Estimated number of larvae treated ^a	Actual number of larvae treated	Number of survivors	Estimated mortality (%)	Actual mortality (%) ^b
1	0	100	4460		407	308		24.3
	3	657	29302	2024	2833	1989	1.7	30.0
	5	648	28901	1996	2961	1487	25.5	46.0
	7	654	29168	2014	2388	1184	41.2	50.4
	9	627	27964	1931	2285	54	97.2	97.6
	11	657	29302	2024	2783	0	100	100
2	13	651	29035	2005	3506	0	100	100
	0	105	4568		537	485		9.7
	3	677	29450	3127	2464	1844	41.0	25.2
	5	659	28667	3044	2669	1055	65.3	60.5
	7	681	29624	3145	2912	597	81.0	79.5
	9	686	29841	3168	3220	55	98.3	98.3
3	11	660	28710	3048	3564	0	100	100
	13	672	29232	3104	3719	0	100	100
	0	100	5290		1159	995		14.2
	3	652	34491	6487	10181	8703	-34.7	14.5
	5	670	35443	6667	10983	4311	35.3	60.8
	7	668	35337	6647	9647	427	93.6	95.6
9	663	35073	6597	11266	20	99.7	99.8	

	11	660	34914	6567	11443	1	99.98	99.99
	13	666	35231	6627	10986	5	99.92	99.95

^a This value was calculated by determining the average number of live larvae in each of the control fruit times the total number of fruit inoculated.

^b This value is based on the actual number of dead and live larvae counted.

The Probit analyses of the Valencia orange data are presented in Table 3.5.4.11. Again the two methods of calculating the values (using the estimation of the number of eggs treated and the number of larvae surviving or the actual number of dead and live larvae after treatment) gave similar results in the first replicate. The time to kill 99% of the larvae in the first experiment was calculated to be 9.47 (fiducial limits 8.93 – 10.51) days using the egg data and 9.49 (fiducial limits 8.66 – 12.67) days using the larval data.

The results in the second experiment were somewhat different. Using the data obtained using the number of eggs inoculated into each fruit, it was estimated that it would take 10.75 days to kill 99% of the treated individuals (fiducial limits 9.08 – 20.92). Using the data obtained from the number of dead and live larvae the equivalent results were 10.77 (fiducial limits 9.25 – 15.53) days. These data indicate that any exposure of infested fruit for 16 or 21 days, depending on the calculation technique that was used, will provide phytosanitary security for oranges. Considering that the larval end point is the more direct method, it is suggested that the result from this calculation (16 days) be used in determining the time that will be needed to obtain phytosanitary security for oranges. This was considered to be so different from previous experience that a third experiment was conducted.

In this third experiment one individual survived 11 days treatment but five individuals from 2 fruit survived 13 days exposure to the cold. The analysis could not be done on the full data set as it varied too much from the mathematical model. However, if day 13 data were omitted the analysis could proceed. The result from these data indicates that 8.11 (fiducial limits 8.04 – 8.20) days treatment would result in 99% mortality. Because of the excessive variance of the data, they could not be combined.

Table 3.5.4.11. Probit analyses of Valencia orange data from Table 3.5.4.10. The figures in parentheses indicate the confidence limits of the fiducial values.

Replicate		LT ₅₀	LT ₉₀	LT ₉₉
1 (egg)	Mean	7.35	8.45	9.47
	Lower limit (0.90)	6.88	8.08	8.93
	Higher limit (0.90)	7.69	8.98	10.51
2 (egg)	Mean	5.59	8.01	10.75
	Lower limit (0.90)	2.51	6.57	9.08
	Higher limit (0.90)	6.74	9.76	20.92
3* (egg)	Mean	4.99	6.50	8.05
	Lower limit (0.90)	4.73	6.25	7.60
	Higher limit (0.90)	5.22	6.79	8.68
1 (larva)	Mean	7.62	8.59	9.49
	Lower limit (0.90)	6.55	7.93	8.66
	Higher limit (0.90)	8.26	10.07	12.67
2 (larva)	Mean	5.17	7.75	10.77
	Lower limit (0.90)	3.57	6.86	9.25
	Higher limit (0.90)	6.03	8.93	15.53
3* (larva)	Mean	4.88	6.46	8.11
	Lower limit (0.90)	4.85	6.42	8.04
	Higher limit (0.90)	4.91	6.49	8.20

*Day 13 result aberrant. The data would not fit the model unless removed.

The temperature profiles of the orange experiments are shown in Figures 3.5.4.11 to 3.5.4.13. In the first replicate the temperature initially dropped a little low (0.3°C) but returned to the desired temperature of 1°C within 22 hours of reaching this temperature (Figure 3.5.4.11). A large number of temperature reading spikes were noted in the second replicate (Figure 3.5.4.12) but the reasons for these are unknown. The final orange experiment went smoothly (Figure 3.5.4.13).

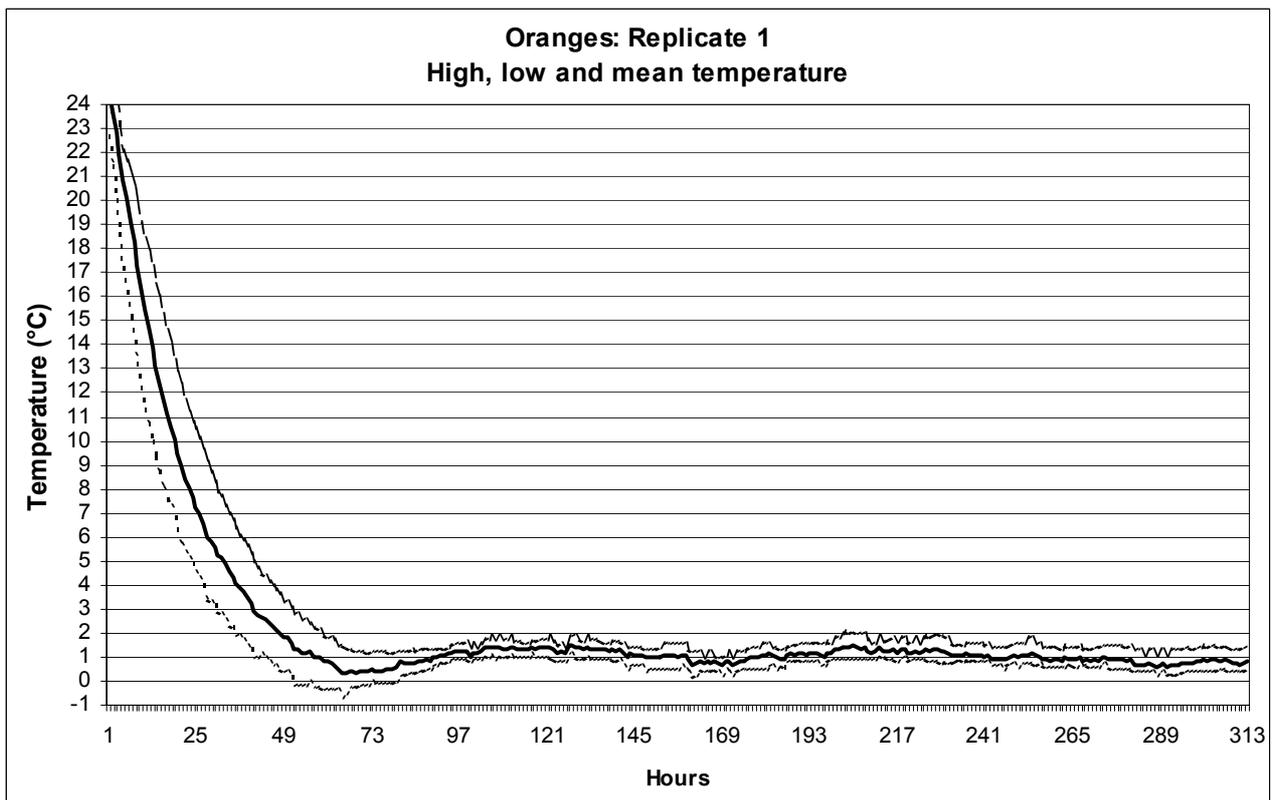


Fig. 3.5.4.11. The hourly mean, high and low temperatures in fruit cores of Valencia oranges replicate 1

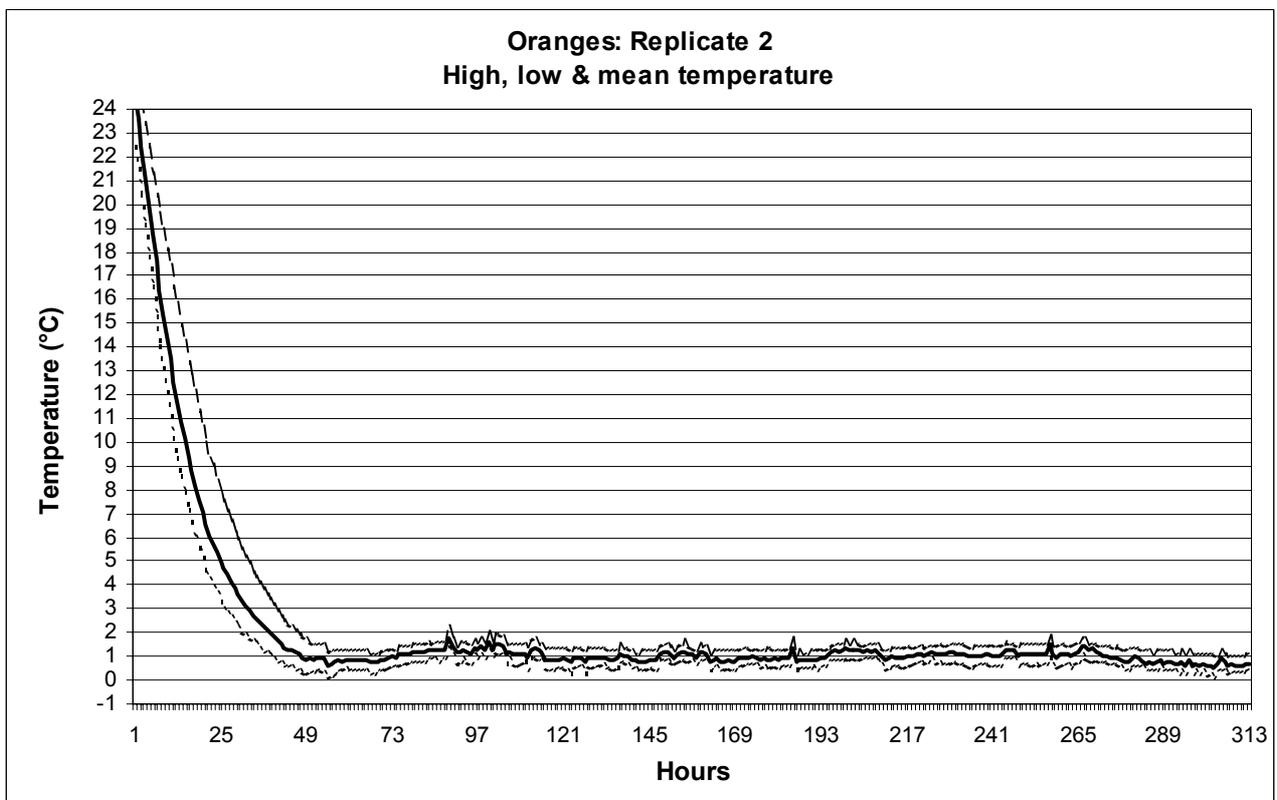


Fig. 3.5.4.12. The hourly mean, high and low temperatures in fruit cores of Valencia oranges replicate 2

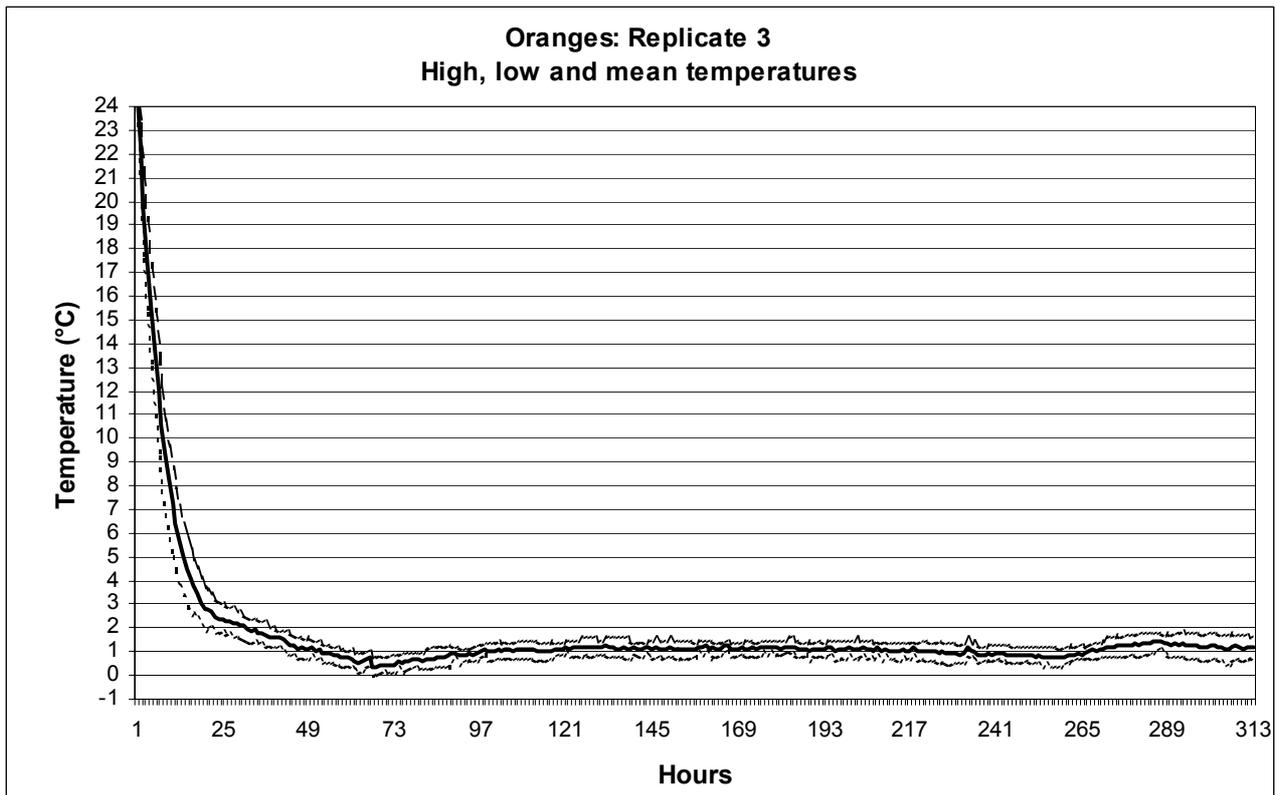


Fig. 3.5.4.13. The hourly mean, high and low temperatures in fruit cores of Valencia oranges replicate 3.

Discussion

The results presented here are considered to be ultra conservative. Firstly the end point of live larvae was used. In research conducted by Heather *et al.* (1996), De Lima *et al.* (2002), Jessup *et al.* (1993), Mason and McBride (1934) and Hill *et al.* (1988) pupae were used as their end point. This was considered appropriate by these researchers as many larvae may be alive after cold treatment but fail to pupate (Mason and McBride, 1934). Furthermore survival of pupae to adult in the absence of cold treatment may only be 80% (Santaballa *et al.*, 1999). The counting of live larvae, as done in this research, would result in an overestimate of the total potential survival. However, the presence of live larvae during inspection by importing authorities, whether or not they would pupate, would be enough for a consignment to be rejected and was therefore considered the more appropriate life stage to use.

Secondly the experiments reported here were assumed to have begun once the fruit was placed in the cold chamber. In practice the treatment is considered to have begun only once the temperature of the fruit core reached the desired temperature. In the above cases this would be some 46 to 91 hours after the fruit was placed in the cold chamber. Probit analyses conducted using these experimental initiating times would result in lower lethal times and fiducial limits e.g. the third orange replicate (without the removal of day 13 data) would have a LT_{99} of 6.17 days (fiducial limits 5.56 – 7.11), nearly 2 days lower than the estimate of 8.20 days (fiducial limits 8.04-8.11) (without day 13 data) that was obtained using the unmodified data.

Thirdly, the counting of the number of live and dead larvae in each fruit was considered to be a better reflection of the number of larvae treated than any extrapolation that was made from the number of eggs inoculated. However, in general there was close agreement of the results using the two methods of analysis demonstrating the robustness of the experimental design and accuracy of the operators.

Because of the low number of control survivors in the third and fourth grapefruit and the first orange replications, the results of these experiments were not considered when deciding which species to use for the definitive large-scale trials. The second orange replicate had both the highest LT_{99} and the highest fiducial limit (Figure 3.5.4.14). In the third orange replicate there were 5 survivors in two fruit after 13 days cold treatment. Based on these results it is recommended that Valencia oranges be considered the host that harbours the most cold tolerant fruit fly larvae and that this species be used for the large-scale experiments and that the results obtained therein be applicable to the lemons, mandarins and grapefruit. These results are in line with the USDA-APHIS-PPQ treatment protocol (Anonymous, 2004) and the extrapolations made by Powell (2003) that fruit should undergo 16 days treatment using temperatures of 1°C.

This research indicates that confirmatory large-scale disinfestation research should be done using 16- day exposure of Mediterranean fruit fly-infested oranges at a temperature of 1°C. Some countries have required that both medium (phase 3) and large-scale (phase 4) research be conducted on each on the citrus species. However, once medium scale trials have demonstrated which citrus species best protects the fruit fly larvae from cold treatment, it is unnecessary to perform large-scale disinfestation research on the other citrus species as the results can be extrapolated to include these species.

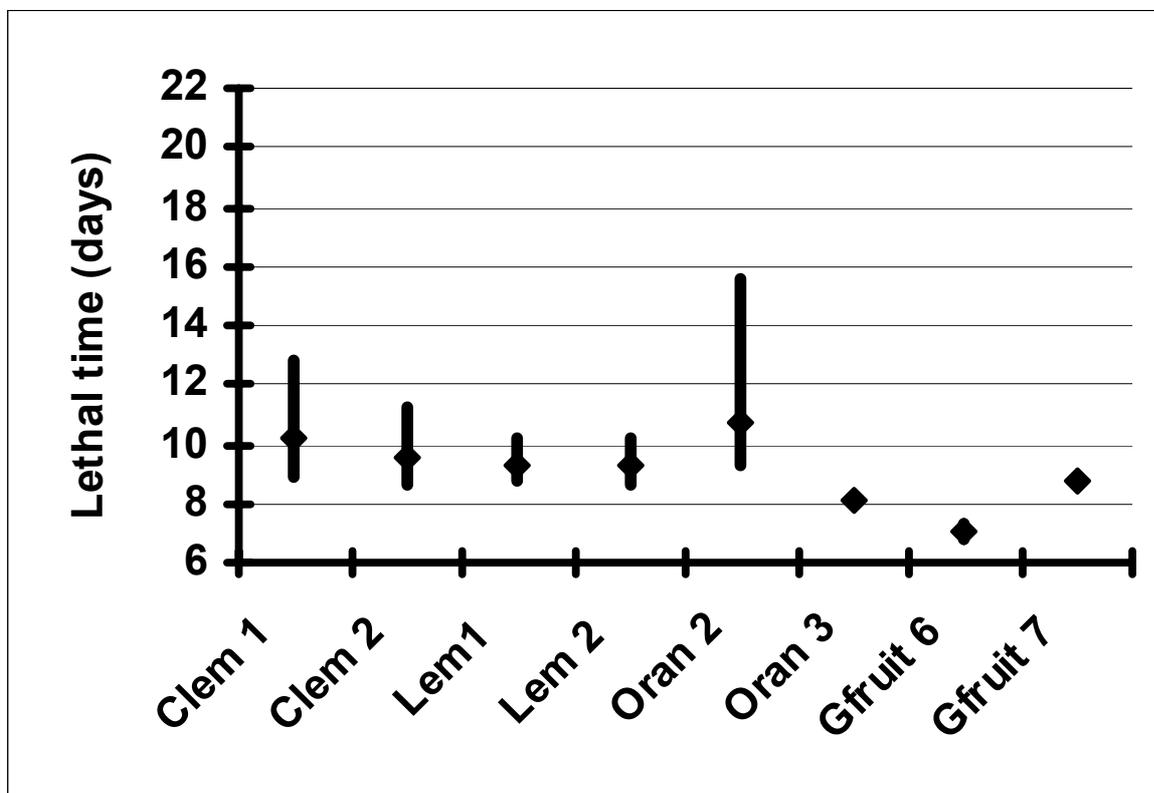


Fig. 3.5.4.14. Summary of Probit analyses using dead and live larval data. The diamond indicates the number of days needed for death to occur in 99% of the population while the lines represent the fiducial limits (some limits too narrow to be reflected on the graph).

Future research

Future research will depend on the outcome of the negotiations with the Japanese authorities

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3.5.5 Cold disinfestation of Mediterranean (*Ceratitidis capitata* [Wiedemann]) and Natal (*Ceratitidis rosa* Karsch) fruit fly-infested litchis (*Litchi chinensis* Sonn.)

Experiment 776 by Tony Ware, Bruce Tate, Peter Stephen, John-Henry Daneel and Rooikie Beck (CRI)

Opsomming

Jong (eerste en tweede instar) en volwasse (derde instar) Mediterreense (*Ceratitidis capitata*) en Natalse (*Ceratitidis rosa*) vrugtevlieglarwes is vir verskeie tye blootgestel aan koue (0.5°C) en die getalle wat oorleef het, is bepaal. Probit analise van die data het getoon dat volwasse Natalse vrugtevlieglarwes die bestandste was. Dit word aanbeveel dat medium- en grootskaalse proewe wat nodig is om die doeltreffendheid van ontsmettingsbehandelings te bepaal, slegs met volwasse Natalse vrugtevlieglarwes uitgevoer word, tensy die land vir wie die navorsing gedoen word daarop aandring dat beide spesies ingesluit word.

Introduction

Fruit flies (Diptera: Tephritidae) attack a wide range of commercial horticultural crops and inhabit all areas in which fruit is grown (White and Elson-Harris, 1992). All countries, even those harbouring specific fruit fly species, are in danger of invasion from an "other" species through the interregional movement of fruit and other produce. In order to alleviate the threat of such accidental importation, many countries place restrictions on the movement of fresh produce unless the commodity has undergone strict quarantine procedures.

Among the quarantine procedures used are fumigation, pesticides, irradiation, hot water immersion, hot air and controlled atmosphere (Sharp and Hallman, 1994). However, cold is the most commonly applied treatment but is only considered effective if it results in a mortality of 99.996832% (or less than 3.2 survivors out of every 100 000 individuals treated). This figure in mathematical terms represents a probit 9 level of security (Baker, 1939). This research was designed to determine the effect of cold treatment on the survival of Mediterranean (*Ceratitidis capitata* [Wiedemann]) and Natal (*Ceratitidis rosa* Karsch) fruit fly in litchis destined for export to those countries deemed to be at risk.

Materials and methods

The complete disinfestation protocol consists of four phases, the first two of which are the subjects of this paper. Phase 1 is designed to determine the duration of the developmental life stages of the flies in litchis. Litchis (Mauritius cultivar) were inoculated with either Mediterranean fruit fly or Natal fruit fly eggs. Eggs of a fruit fly species were placed onto small pieces of blotting paper (±10x10 mm). A flap of litchi peel was partially cut from the fruit and the blotting paper containing the eggs was placed on the flesh. The flap was replaced and the litchis placed into paper bags and then processed as required. Previous trials had shown that few eggs developed if placed directly into the flesh of the fruit.

Both species of fruit fly are maintained in cultures at the Citrus Research International laboratories in Nelspruit in the Mpumalanga Province of South Africa. The Mediterranean fruit flies oviposit through the

gauze of their cages and the eggs were collected from a basin of water positioned under the cage. In the case of Natal fruit fly the flies were allowed to oviposit into apples that are then removed and dissected. The eggs were then washed out of the fruit with water. All eggs used were less than 24 hours old when collected and not more than 30 hours old when placed into the test fruit.

In the phase 1 trial, forty fruit were inoculated with each species (60 eggs/fruit). The fruit was then placed in a constant environment room maintained at 26°C and 60% RH. Five fruit containing each fruit fly species were removed daily and dissected. The fruit fly larvae found were then assigned to their developmental stage (first, second or third instar) using the criteria described by White and Elson-Harris (1992).

The phase 2 trial is designed to determine the most cold-tolerant fruit fly developmental life stage. Fruit was inoculated with eggs that were then allowed to develop at 26°C into the desired developmental stage as described above. Inoculated fruit (350) were placed at 26°C for the length of time the larvae required to become mature or third instar. The second group of 350 fruit was inoculated a few days later and again placed at 26°C until they had developed into young larvae (approximately 50% of the larvae were first instar and 50% second instar). The final group of fruit flies (egg stage) was inoculated and immediately placed into the cold chamber (0.5°C). The trial was designed so that all the developmental stages were placed into the cold chamber at the same time as the eggs. Three days after initial exposure to cold, 50 fruit from each group and each fruit fly species were removed and placed at 26°C. The egg group was held at this temperature for 8 days, the young larvae for 3 days and the mature larvae for 24 hours before the fruit was dissected and the number of survivors determined. The process was repeated every 48 hours. The trial was done twice for each species and Probit analysis (Anonymous, 1987) was done on the data in order to determine the most cold-tolerant developmental stage.

Results and discussion

Phase 1 results are presented in Table 3.5.5.1. The modal distributions of the various developmental stages are essentially the same for both species and are represented in grey in Table 3.5.5.1. Based on these results it was determined that, regardless of species, the fruit fly developed into young larvae (50% first instar and 50% second instar) after approximately 6 days and mature larvae after 8 days. These results were then used in the phase 2 trial.

Table 3.5.5.1. Number of fruit fly larvae over time in each developmental stage in litchi fruit held at 26°C. Numbers were derived from combining the two replicates.

Species	Instar	Developmental period (days)										Total
		1	2	3	4	5	6	7	8	9	10	
Medfly	1	0	3	46	81	127	72	2	0	0	0	331
	2	0	0	1	8	34	68	57	30	0	0	198
	3	0	0	0	0	0	0	64	159	81	33	337
	Total	0	3	47	89	161	140	123	189	81	33	866
Natalfly	1	0	2	21	30	47	12	9	0	0	0	121
	2	0	0	0	0	18	69	61	12	0	0	160
	3	0	0	0	0	0	0	29	126	33	15	153
	Total	0	2	21	30	65	81	99	138	33	15	434

The survival of eggs, young larvae and mature larvae after various periods of exposure to cold treatment of 0.5°C is shown in Tables 3.5.5.2, 3.5.5.3, 3.5.5.4 and 3.5.5.5. The probit analysis of the data indicated that the Mediterranean fruit fly egg stage was the most tolerant to the treatment. However, this result is contrary to those obtained by other researchers (Powell, 2003 and references therein) and ourselves. This fact and the argument that the time delay between the flies laying their eggs, the picking and processing of the fruit before the beginning of the cold treatment would allow most, if not all, eggs to hatch resulting in few, if any, eggs actually undergoing treatment, it was decided not to proceed with the medium and large scale testing with this life stage. Statistically there were no differences between the results from the other life stages (Table 3.5.5.6).

Table 3.5.5.2. Mortality of Mediterranean fruit fly in litchis after various periods of exposure to cold treatment of 0.5°C. (Replicate 1)

Stage	Exposure period (days)	Number of eggs treated*	Number of live larvae recovered	Mortality (%)	Corrected mortality (%)**
Eggs	0	3000	711	76.3	-
	1	3000	223	67.4	68.6
	3	3000	150	95.0	78.9
	5	3000	116	96.1	83.4
	7	3000	15	99.5	97.9
	9	3000	0	100	100
	11	3000	0	100	100
Young larvae	0	3000	711	76.3	-
	1	3000	-	-	-
	3	3000	316	89.5	55.7
	5	3000	31	99.0	95.8
	7	3000	0	100	100
	9	3000	0	100	100
	11	3000	0	100	100
Mature larvae	0	3000	711	76.3	-
	1	3000	232	89.2	54.4
	3	3000	225	92.5	68.4
	5	3000	6	99.8	99.2
	7	3000	0	100	100
	9	3000	0	100	100
	11	3000	0	100	100

* Approximately 60 eggs were placed into each of 50 fruit

** Abbott's formula (1925)

Table 3.5.5.3. Mortality of Mediterranean fruit fly in litchis after various periods of exposure to cold treatment of 0.5°C. (Replicate 2)

Stage	Exposure period (days)	Number of eggs treated*	Number of live larvae recovered	Mortality (%)	Corrected mortality (%)**
Eggs	0	2000	148	92.6	-
	1	2000	-	-	-
	3	2000	97	95.2	35.2
	5	2000	40	98.0	73.0
	7	2000	9	99.6	94.6
	9	2000	0	100	100
	11	2000	0	100	100
Young larvae	0	2000	148	92.6	-
	1	2000	154	92.3	0
	3	2000	116	94.2	21.6
	5	2000	58	97.1	60.8
	7	2000	4	99.8	97.3
	9	2000	0	100	100
	11	2000	0	100	100
Mature larvae	0	2000	148	92.6	-
	1	2000	134	93.3	0.1
	3	2000	93	95.4	37.8
	5	2000	12	99.4	91.9
	7	2000	0	100	100
	9	2000	0	100	100

	11	2000	0	100	100
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* Approximately 40 eggs were placed into each of 50 fruit

** Abbott's formula (1925)

Table 3.5.5.4. Mortality of Natal fruit fly in litchis after various periods of exposure to cold treatment of 0.5°C. (Replicate 1)

Stage	Exposure period (days)	Number of eggs treated*	Number of live larvae recovered	Mortality (%)	Corrected mortality (%)**
Eggs	0	2000	116	94.2	-
	1	2000	81	95.6	24.1
	3	2000	28	98.6	75.9
	5	2000	6	99.7	94.8
	7	2000	0	100	100
	9	2000	0	100	100
	11	2000	0	100	100
Young larvae	0	2000	116	94.2	-
	1	2000	88	95.6	24.1
	3	2000	24	98.8	79.3
	5	2000	4	99.8	96.6
	7	2000	1	99.9	98.3
	9	2000	0	100	100
	11	2000	0	100	100
Mature larvae	0	2000	116	94.2	-
	1	2000	87	95.7	25.9
	3	2000	45	97.8	62.1
	5	2000	0	100	100
	7	2000	0	100	100
	9	2000	0	100	100
	11	2000	0	100	100

* Approximately 40 eggs were placed into each of 50 fruit

** Abbott's formula (1925)

Table 3.5.5.5. Mortality of Natal fruit fly in litchis after various periods of exposure to cold treatment of 0.5°C. (Replicate 2)

Stage	Exposure period (days)	Number of eggs treated*	Number of live larvae recovered	Mortality (%)	Corrected mortality (%)**
Eggs	0	1500	83	94.5	-
	1	1500	39	97.4	52.7
	3	1500	12	99.2	85.5
	5	1500	1	99.9	98.2
	7	1500	0	100	100
	9	1500	0	100	100
	11	1500	0	100	100
Young larvae	0	1500	83	94.5	-
	1	1500	43	97.1	47.3
	3	1500	39	97.4	52.7
	5	1500	13	99.1	83.4
	7	1500	0	100	100
	9	1500	0	100	100
	11	1500	0	100	100
Mature larvae	0	1500	83	94.5	-

	1	1500	68	95.5	48.2
	3	1500	43	97.7	47.3
	5	1500	3	99.8	96.4
	7	1500	0	100	100
	9	1500	0	100	100
	11	1500	0	100	100

* Approximately 30 eggs were placed into each of 50 fruit

** Abbott's formula (1925)

Table 3.5.5.6. Probit analysis (confidence limit of 0.95) of the mortality of the developmental life stages (LT₉₉) of Mediterranean and Natal fruit fly after exposure to 0.5°C for various lengths of time (figures in parentheses the fiducial limits).

Species	Eggs	Young larvae	Mature larvae
Medfly	11.3* (-)	7.1 (5.9-11.0)	5.6 (4.7-8.6)
Natalfly	7.2 (5.7-10.9)	6.9 (5.6-9.4)	6.5 (5.3-11.2)

* Data varied too much from model for fiducial limits to be estimated.

Conclusion

Based on these results it is recommended that the litchi industry address the issue of whether the importing country will accept the proposal that the phase 3 and phase 4 trials be performed on mature Natal fruit fly larvae only and that the results obtained would be applicable to Mediterranean fruit fly. This would speed up the trial and lower the costs.

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3.5.6 Cold Disinfestation of Medfly- and Natalfly-Infested Persimmons (Phases 1 and 2)

Experiment 777 by Tony Ware, Bruce Tate, Peter Stephen and John-Henry Daneel (CRI)

Opsomming

Een van die voorwaardes om toegang tot nuwe markte te verkry, is dat die inkomende produk nie 'n fitosanitêre risiko inhou nie. Verskeie tegnieke is ontwikkel om fitosanitêre sekuriteit te verseker, met koue-behandeling as een van die mees algemene metodes. Die tamatiepruimbedryf het besluit om dié behandeling te ondersoek as voorvereiste vir die sterilisasie van vrugte vir uitvoere na Japan. Die twee spesies van belang is die vrugtevlieë *Ceratitis capitata* (Wiedemann) (Mediterreense vrugtevlieg of Medvlieg) en *Ceratitis rosa* Karsch (Natalse vrugtevlieg of Natalvlieg). Tradisioneel word Probit vlak 9 effektiwiteit beskou as voldoende sekuriteit. 'n Multi-fase benadering word benodig om hierdie waarde te verkry. Die eerste fase is om die duur van die lewensstadiums by 26°C in die teikenvrug te bepaal. Vir beide spesies is die hoogste getal van eerste-instar larwes aangeteken op dag 4, vir tweede-instar op dag 6 en derde-instar op dag 8. Die tweede fase van die navorsing was om die mees koue-verdragsame lewensstadium te bepaal. By 'n temperatuur van 0.5°C was die derde-instar van die Natalse vrugtevlieg die mees-verdraagsame lewensstadium. Dit word aanbeveel dat medium- en grootskaalse ontsmettingsproewe uitgevoer word met hierdie lewensstadium.

Introduction

The persimmon (*Diospyros* spp.) industry is a small but potentially lucrative one situated in the Western Cape Province where a seedless variety (Sharon) is grown. However, in order to achieve their potential the fruit must be exported to those markets that provide the best returns. Before this can occur, the phytosanitary concerns of these countries must be allayed. This project is designed to provide evidence that cold treatment mitigating treatments would protect an importing country from the accidental importation of any pest they deemed to be undesirable.

Traditionally a Probit 9 level of security is required (Baker, 1939) and this quarantine requirement has become the generally accepted norm (Anonymous, 2004). This represents a mortality of 99.99683% or a survival rate of not more than 3 in 100 000 treated individuals. This research examines the response of Mediterranean fruit fly (*Ceratititis capitata* [Wiedemann]) and Natal fruit fly (*Ceratititis rosa* Karsch), both recorded pests of persimmons (White and Elson-Harris, 1992), to cold treatment. The research is divided into four phases. These are, Phase 1: Determination of the larval life stage durations in persimmons for the two species, Phase 2: Determination of the most cold tolerant life stage, Phase 3: Medium-scale disinfestation and Phase 4: Large-scale disinfestation. The first two phases of the research have been completed and are hereby reported.

Materials and methods

Test insects: Mangoes infested with Natafly were collected in 1999 in the Nelspruit area of the Mpumalanga Province of South Africa. The emerging flies were used to initiate the laboratory colony. In order to maintain genetic variability, "wild" flies were introduced on an annual basis. The adult flies were maintained in gauze cages placed in a roofed, gauze-fenced building at Citrus Research International. This resulted in the colony being kept in natural light and at ambient temperature. The adults were allowed to oviposit into apples that had previously been injured to provide the flies with easily accessible oviposition sites (Papaj *et al.*, 1989). The apples were dissected and the eggs washed from the flesh. These were then placed on a food medium where they were allowed to hatch and the resultant larvae develop. The larval development took place in the laboratory normally maintained at 24°C. The larvae were allowed to pupate in sand and the emerging adults returned to the cages where the life cycle was repeated. The following laboratory data were obtained: Ninety-six percent of a sample of 100 eggs placed on wet filter paper and kept at 26°C hatched. From a sample of 500 pupae, 76% adults emerged of which 49.6% were female. These females laid 13 238 eggs (70 eggs per female) over their lifespan. The duration of the life stages were: eggs 2 days, larvae 9 days, pupae 6 days and adults 90 days.

The Mediterranean fruit fly colony was established in 1998 and, like the Natal fruit fly colony, has been supplemented with "wild" flies periodically. The adult colony is maintained in gauze cages in a controlled environment at 26°C ($\pm 2^\circ\text{C}$) and approximately 65% relative humidity and a day/night photoperiod of 14/10 hours. The adults are permitted to oviposit through the gauze and the eggs are collected from a basin of water placed beneath the cage. The eggs are placed onto artificial media where they hatch and develop. The larvae are allowed to pupate in sand. The following data were obtained on the laboratory colony: Ninety-six percent of 600 eggs placed on wet filter paper at 26°C hatched while 92% of 500 pupae successfully emerged. Females made up 50.7% of the population and these laid 113 375 eggs (487 eggs/female) over their lifespan. The observed duration of each life stage was 3 days for eggs, 8 days for larvae, 6 days for pupae and the adults lived for some 30 days.

General inoculation procedure: Eggs were collected within 12 hours of oviposition and placed into water. The water was agitated to ensure even suspension of the eggs. The eggs were drawn into an automatic pipette and inoculated into the fruit. The number of eggs in the sample was determined under a microscope and the amount of water adjusted until approximately 50 eggs were inoculated.

Test fruit: Persimmons were sourced from the Western Cape Province and maintained at 1°C in the Citrus Research International cold rooms until required. The day before the fruit was to be infested they were removed from cold storage and dipped in fungicide (guazatine 210 g/l). A hole was made in the stalk end of the fruit by tearing the calyx from the fruiting body. An aliquot of eggs was then placed in the tear and the calyx replaced. The fruit were individually wrapped in paper towelling and placed stalk end down in wire baskets (allowing excess liquid to drain).

Cold Chamber: The cold chamber was custom built out of Isowall (polystyrene sandwiched between aluminium sheets) on a concrete floor in a warehouse. The room was 4X2.95X2.52 m (lXbXh). The chiller unit was located near the ceiling opposite the sliding door. Heating coils were used for defrosting that occurred every 8 hours for 15 minutes. Fans were switched off and the heating coil switched on during the

defrost cycles. The temperature was controlled using a Carel CR72 universal electronic controller. A Grant 1200 Series (12 bit) Squirrel Meter/logger was used to record and analyse data obtained from the 15 two-wired resistance thermocouples. Temperatures were recorded hourly. The thermoprobes were calibrated by measuring their readings when placed on melting ice. The readings were then adjusted by the difference between these readings and 0°C.

Phase 1: Determination of the larval life stage durations in persimmons for the two species. Medfly eggs were inoculated into 50 fruit as detailed above. The fruit were then placed in a constant environment room that was maintained at 26°C (\pm 2°C) and approximately 65% relative humidity. Five fruit were removed daily from day 2. These fruit were dissected under a microscope and all larvae collected and their larval instar stages determined (White and Elson-Harris, 1992). Natalfly was processed at the same time and in a similar manner as the Medfly. The experiment was done three times.

Phase 2: Determination of the most cold tolerant life stage. Based on the results above, the egg, young larvae (6 day old) and mature larvae (8 day old) were used to determine the most cold tolerant life stage. Fruit (350) were inoculated and placed in a controlled environment room maintained at 26°C (mature larvae). Two days later a further 350 fruit were treated (young larvae) and placed at 26°C and six days later another 350 fruit (eggs) were inoculated. Immediately after the third inoculation all the treated fruit, except for 50 from each group (controls), was placed in the cold chamber. Fruit from each developmental stage were removed after 3, 5, 7, 9 and 11 days of cold treatment. The fruit containing the eggs were placed at 26°C for 8 days and then dissected. All larvae, living and dead, were counted. The young larvae were kept at 26°C for two days after cold treatment before determining the number of survivors. The mature larvae were placed at 26°C for 24 hours before the status of the treated larvae were assessed. All the control fruit was maintained at 26°C for 8 days after which they were dissected as for the treated fruit. The trial was done twice.

Results and discussion

The number of days for the fruit fly to develop through their three life stages is shown in Table 3.5.6.1. Mediterranean fruit fly first instars were recorded from day 2 to day 7 (mode = day 4), second instars from day 3 to 10 (mode = day 6) and third instars from day 7 (mode = day 8). A similar rate of development was recorded for the Natal fruit fly. Here the first instars were recorded from day 2 to day 6 (mode = day 4), second instars from day 4 to day 10 (mode = day 6) and third instars from day 6 onwards (mode = day 8). Based on this information it was determined that the developmental time for young instars (approximately 50% in first instar and 50% in second instar) was on 5 days for Mediterranean fruit fly and between 4 and 5 days for Natal fruit fly. Day 8 was determined to be the time it took for most of the third instars of both species to develop (mature larvae).

Table 3.5.6.1. The number of first, second and third instar larvae recovered from persimmons after various periods of development at 26°C. The three replicates have been summed.

Days	Medfly			Natalfly		
	First instar	Second instar	Third instar	First instar	Second instar	Third instar
2	106	0	0	40	0	0
3	287	1	0	153	0	0
4	385	13	0	339	25	0
5	199	274	0	41	128	0
6	12	326	0	1	226	5
7	1	207	165	0	62	50
8	0	127	309	0	43	121
9	0	9	308	0	0	109
10	0	2	174	0	4	96

The survival of eggs, young and mature larvae after various periods of exposure to 0.5°C is shown in Table 3.5.6.2 for Mediterranean fruit fly and Table 3.5.6.3 for Natal fruit fly. There was no survival of the eggs after 9 days of cold treatment. The young larvae tolerated 7 days of treatment while some mature larvae survived 9 days treatment. Probit analysis using POLO-PC statistical package (LeOra Software, 1987) showed that the mature larvae were most cold tolerant (Table 3.5.6.4).

Table 3.5.6.2. Mortality of Mediterranean fruit fly in persimmons after various periods of exposure to cold treatment of 0.5°C. Fifty fruit were inoculated with 50 eggs for each exposure temperature (total 2500 eggs) in the first replicate and 40 eggs (total 2000 eggs) in the second.

Replicate	Stage	Exposure (days)	Number of live larvae recovered	Mortality (%)	Corrected mortality (%)*
1	Eggs	0	649	74.0	
		3	242	90.3	62.7
		5	3	99.9	99.5
		7	0	100	100
		9	0	100	100
		11	0	100	100
1	Young	0	159	93.6	
		3	29	98.8	81.3
		5	21	99.2	86.8
		7	0	100	100
		9	0	100	100
		11	0	100	100
1	Mature	0	403	83.9	
		3	57	97.7	85.7
		5	20	99.2	95.0
		7	0	100	100
		9	0	100	100
		11	0	100	100
2	Eggs	0	148	92.6	
		3	36	98.2	75.7
		5	52	97.4	64.9
		7	4	99.8	97.3
		9	7	99.7	95.3
		11	0	100	100
2	Young	0	319	84.1	
		3	19	99.1	94.1
		5	4	99.8	98.8
		7	1	99.95	99.7
		9	0	100	100
		11	0	100	100
2	Mature	0	554	72.3	
		3	404	79.8	27.1
		5	70	96.5	87.4
		7	7	99.7	98.8
		9	2	99.9	99.6
		11	0	100	100

* Application of Abbott's (1925) formula

Table 3.5.6.3. Mortality of Natal fruit fly in persimmons after various periods of exposure to cold treatment of 0.5°C. Fifty fruit were inoculated with 50 eggs (total 2500 eggs) for each exposure temperature in the first replicate and 40 eggs (total 2000 eggs) in the second.

Replicate	Stage	Exposure (days)	Number of live larvae recovered	Mortality (%)	Corrected mortality* (%)
1	Eggs	0	328	86.9	
		3	6	99.8	98.2
		5	0	100	100
		7	0	100	100
		9	0	100	100
		11	0	100	100
1	Young	0	176	93	
		3	24	99	86.3
		5	21	99.2	88

		7	7	99.7	96
		9	0	100	100
		11	0	100	100
1	Mature	0	520	79.2	
		3	117	95.3	77.4
		5	44	98.2	91.4
		7	27	98.9	94.8
		9	0	100	100
		11	0	100	100
2	Eggs	0	569	71.6	
		3	30	98.5	94.7
		5	8	99.6	98.6
		7	0	100	100
		9	0	100	100
		11	0	100	100
2	Young	0	284	85.8	
		3	33	98.4	88.
		5	7	99.7	97.5
		7	0	100	100
		9	0	100	100
		11	0	100	100
2	Mature	0	276	86.2	
		3	43	97.9	84.1
		5	12	99.4	95.7
		7	0	100	100
		9	0	100	100
		11	0	100	100

* Application of Abbott's (1925) formula

Table 3.5.6.4. Results of the Probit analysis of the mortality of eggs, young and mature larvae of Medfly and Natalfly in persimmons after various periods of cold treatment of 0.5°C.

Species	Life stage	Lethal time 90% mortality (days)	95% Fiducial limits
Medfly	Egg	6.7	_*
	Young larvae	6.7	5.0-18.7
	Mature larvae	7.7	5.6-14.6
Natalfly	Egg	4.4	3.7-6.6
	Young larvae	7.2	5.4-17.3
	Mature larvae	8.5	5.6-31.2

* Data vary too much from model for fiducial limits to be determined.

The overlapping of fiducial limits implies there is no statistical difference between species and life stages. However, because Natalfly mature larvae required 8.5 days to kill 90% of the treated population, it is nominated most tolerant life stage.

Future research

Phases 3 (medium scale disinfestation trial) and 4 (large scale disinfestation trial) should be carried out on Natal fruit fly mature stage larvae. Cold disinfestation treatment of false codling moth-infested fruit needs to be researched.

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3.5.7 Natal fruit fly monitoring

Experiment 775 by Tony Ware, John-Henry Daneel and Rooikie Beck (CRI)

Opsomming

'n Merk-loslaat-herwinnigstegniek is gebruik met die Mediterreense, Natalse en maroela vrugtevlieg om die effektiwiteit van Capilure, Questlure en Ceratitislure in Sensus lokvalle te bepaal. Capilure-Sensus het groot getalle Mediterreense vrugtevliegmannetjies gelok, terwyl Ceratitislure-Sensus groot getalle maroela vrugtevlieë gelok het. Questlure-Sensus lokvalle het ewe veel Natalse vrugtevliegmannetjies en -wyfies gelok, maar twee keer soveel Mediterreense vrugtevliegwyfies as -mannetjies. Die herwinnig van laboratorium-geteelde vlieë dui daarop dat die vangste relatief ondoeltreffend was aangesien minder as 3% van enige geslag herwin is in enige van die lokaas-lokval kombinasies.

Introduction

Since the alleged intervention of South African Natal fruit fly-infested citrus in Spain the species has assumed phytosanitary significance. This has resulted in the industry reassessing the treatment and monitoring of fruit fly in general and Natal fruit fly in particular. The research was undertaken to assess the effectiveness of Questlure, Capilure and Ceratitislure in Sensus traps for monitoring fruit fly species.

Materials and methods

Four Capilure-Sensus, four Questlure-Sensus and four Ceratitislure-Sensus traps were placed concentrically 10 metres from a central release point. Further sets of traps were placed at 20 and 30 metres from this point. Six thousand Dayglo-marked laboratory-reared fruit flies (2000 Natal fruit fly, 2000 Mediterranean fruit fly and 2000 marula fruit fly, with both sexes of each species) were released on 31 February 2004 at Bakgat Farm near Nelspruit. The trials were repeated on 17 February 2004 at Larten, also near Nelspruit. The traps were emptied daily for 9 days and all flies caught were separated into species and then separated into wild and laboratory-reared groups. They were further separated according to sex.

Results and discussion

The numbers of flies caught in the Sensus traps with the three different attractants are shown in Tables 3.5.7.1-6. The Questlure/Sensus combination trapped 1.3% of the laboratory-reared female Mediterranean fruit fly (13 out of the 1000 females released) at the Larten trial site (Table 3.5.7.1). If one assumes that this is a measurement of the attractiveness of this lure then the lure will also have trapped 1.3% of the wild population's females. This translates to there being approximately 4769 female Mediterranean fruit fly in the area (62 wild female flies trapped against 13 released females). Seven wild and one laboratory-reared male Natal fruit flies were trapped (Table 3.5.7.1) and from this result it was estimated that the naturally occurring population of Natal fruit fly males was approximately 7000. No laboratory-reared female Natal fruit flies were trapped so that the population estimate of this sex in excess of 8000. The number of wild marula fruit flies in the orchard was calculated to be 7000 males (14 wild and 2 laboratory-reared males) and 28 000 females (28 wild and no laboratory-reared females). While there may be a correlation between the male and female population estimates for Natal fruit fly, there is clearly no relationship between the numbers of male and female marula fruit fly trapped.

In a similar exercise, the computation of the naturally occurring population was done using the number of Mediterranean fruit fly males caught with the Capilure/Sensus combination (59 released males and 603 wild males) (Table 3.5.7.2). The 59 laboratory-reared males represent 5.9% of the 1000 males released and the 603 wild males caught translates into 10 220 males in the vicinity. Four laboratory-reared male Natal fruit flies (1000 were released) were recaptured in the Capilure/Sensus traps. The 25 wild flies caught therefore translates into there being some 6250 wild males in the vicinity of the traps or 12 500 of both sexes if one assumes an equal sex ratio.

Similar calculations were done on the fruit fly trap catches from the Ceratitislure/Sensus combination (Table 3.5.7.3). These were: 32 laboratory-reared male Mediterranean fruit flies (3.2% of the males released) and 82 wild flies and this represents a natural population of some 2563 males in the area. Using the female counts: 14 laboratory-reared flies were trapped against the 22 wild flies and this translates to there being

approximately 1571 wild females in the area. If one assumes an equal sex ratio in the field then there is no relationship between the male and female natural population estimations. The small number of Natal fruit fly trapped in the Ceratitis/Sensus trap combination indicated that there was a small population of some 500 females of this species in the area.

Based on the above results it was estimated that there were 9538 Mediterranean fruit fly (both sexes) using the Questlure/Sensus combination, 20 440 using the Capilure/Sensus trap counts and only 5126 with the Ceratitis/Sensus results. Clearly there is no correlation between the marked-release-recapture results and the extrapolated number of wild Mediterranean fruit flies in the field. Using the Questlure/Sensus trap results it was estimated that there were 14 000 Natal fruit fly in the orchard, using the Capilure trap results there were 12 500 and using the Ceratitis trap results only 1000. The reasons for these differences are not known but they do indicate that any extrapolation of the trap counts to estimate population levels should be treated with circumspection.

The trial was repeated at Bakgat (Tables 3.5.7.4-6). In this case there were four released Mediterranean fruit fly females recaptured (out of 1000 females released) and 11 wild females trapped in the Questlure/Sensus lure/trap combination (Table 3.5.7.4). This translates to there being some 2750 Mediterranean fruit fly females in the vicinity of the traps. As in the Larten site, no Natal fruit fly females were recaptured with the Questlure/Sensus traps. The confirmation of this result perhaps indicates that Questlure is not particularly good at luring this species. No wild marula fruit flies were trapped although 4 females were recaptured. This result implied that the natural population of this species was low.

Seven of the 1000 laboratory-reared Mediterranean males and 13 wild males were sampled from the Capilure/Sensus traps (Table 3.5.7.5). This result suggests that there were 1857 males in the vicinity of the traps. Two laboratory-reared Natal fruit fly females were sampled against the 21 wild flies resulting in an estimation of 10 500 females in the orchard.

There were seven laboratory-reared and 12 wild male and six laboratory-reared and 14 wild female Mediterranean fruit flies in the Ceratitis/Sensus traps (Table 3.5.7.6). The naturally occurring male population for the species was calculated to be 1714 and the female population 2333. There were 15 laboratory-reared and 71 wild male Natal fruit fly captured with this attractant/lure combination from which a male population estimate of 4733 was made. No laboratory-reared female Natal fruit flies were caught indicating that this attractant/lure combination is an effective trapping tool for males but not females. When comparing the Questlure/Sensus with the Ceratitis/Sensus Natal fruit fly trap data, it is evident that any conclusion drawn from the data generated by the Questlure trap catches is likely to be erroneous. This is particularly serious in view of the phytosanitary significance of Natal fruit fly. Ceratitis/Sensus trap combination was efficient at recapturing the released marula fruit fly males (7.4% recaptured). Based on the number of wild fruit flies trapped the naturally occurring population appear to be low (estimated to be 730 males in the vicinity).

In general the three attractant/trap lure combinations gave similar population estimates for Mediterranean fruit fly (Questlure 5 500; Capilure 3714; Ceratitis 3428 - based on female captures, and 4666 - based on male captures) at the Bakgat site. The results obtained using the Natal fruit fly data were more variable (Questlure not calculated but would be in excess of 18 000; Capilure 21 000 and Ceratitis 9466). Both Questlure and Ceratitis trap results indicated that there were low populations of marula fruit fly in the area (Capilure does not attract this species).

Table 3.5.7.1. Trap catches of fruit fly in Sensus-Questlure traps placed concentrically about a central release point at Larten near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	6	22	8	36
		Laboratory	3	6	0	9
	Female	Wild	25	30	7	62
		Laboratory	7	6	0	13
<i>C. rosa</i>	Male	Wild	2	2	3	7
		Laboratory	1	0	0	1
	Female	Wild	1	3	4	8
		Laboratory	0	0	0	0

<i>C. cosyra</i>	Male	Wild	4	5	5	14
		Laboratory	2	0	0	2
	Female	Wild	6	14	8	28
		Laboratory	0	0	0	0

Table 3.5.7.2. Trap catches of fruit fly in Sensus-Capilure traps placed concentrically about a central release point at Larten near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	162	205	236	603
		Laboratory	25	18	16	59
	Female	Wild	11	1	1	12
		Laboratory	5	0	0	5
<i>C. rosa</i>	Male	Wild	8	5	12	25
		Laboratory	3	0	1	4
	Female	Wild	0	0	0	0
		Laboratory	0	0	0	0
<i>C. cosyra</i>	Male	Wild	0	0	0	0
		Laboratory	0	0	0	0
	Female	Wild	0	0	0	0
		Laboratory	0	0	0	0

Table 3.5.7.3. Trap catches of fruit fly in Sensus-Ceratitris traps placed concentrically about a central release point at Larten near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	43	20	19	82
		Laboratory	17	9	6	32
	Female	Wild	16	5	1	22
		Laboratory	13	0	1	14
<i>C. rosa</i>	Male	Wild	5	1	5	11
		Laboratory	2	0	1	3
	Female	Wild	1	0	0	1
		Laboratory	0	2	0	2
<i>C. cosyra</i>	Male	Wild	83	66	28	177
		Laboratory	3	5	3	11
	Female	Wild	3	1	1	5
		Laboratory	1	0	0	1

Table 3.5.7.4. Trap catches of fruit fly in Sensus-Questlure traps placed concentrically about a central release point at Bakgat near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	0	0	0	0
		Laboratory	0	0	0	0
	Female	Wild	6	1	4	11
		Laboratory	2	0	2	4
<i>C. rosa</i>	Male	Wild	3	7	9	19
		Laboratory	0	0	0	0
	Female	Wild	0	3	15	18
		Laboratory	0	0	0	0
<i>C. cosyra</i>	Male	Wild	0	0	0	0
		Laboratory	0	0	1	0
	Female	Wild	0	0	0	0
		Laboratory	0	1	3	4

Table 3.5.7.5. Trap catches of fruit fry in Sensus-Capilure traps placed concentrically about a central release point at Bakgat near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	5	6	2	13
		Laboratory	5	1	1	7
	Female	Wild	0	0	0	0
		Laboratory	0	3	0	3
<i>C. rosa</i>	Male	Wild	3	7	11	21
		Laboratory	1	1	0	2
	Female	Wild	0	0	2	2
		Laboratory	0	0	0	0
<i>C. cosyra</i>	Male	Wild	0	0	1	1
		Laboratory	0	0	0	0
	Female	Wild	0	0	0	0
		Laboratory	0	0	0	0

Table 3.5.7.6. Trap catches of fruit fly in Sensus-Ceratitis traps placed concentrically about a central release point at Bakgat near Nelspruit.

Species	Sex	Group	Distance from release point			Total
			10 m	20 m	30 m	
<i>C. capitata</i>	Male	Wild	2	8	2	12
		Laboratory	5	1	1	7
	Female	Wild	3	8	3	14
		Laboratory	1	4	1	6
<i>C. rosa</i>	Male	Wild	5	29	37	71
		Laboratory	10	4	1	15
	Female	Wild	1	2	2	5
		Laboratory	0	0	0	0
<i>C. cosyra</i>	Male	Wild	15	27	12	54
		Laboratory	17	35	22	74
	Female	Wild	0	0	0	0
		Laboratory	0	0	0	0

In general the recapture of the marked flies was greatest nearest the release point. However, Table 3.5.7.6 indicated that male marula fruit fly recovery was lowest nearest the point of release. The reason for this is not known but may be a result of species-specific dispersal behaviour of marula fruit fly.

Table 3.5.7.7. Wild flies caught at Larten and Bakgat farms using Capilure-Sensus, Questlure-Sensus and Ceratitislure-Sensus traps (12 traps of each on each farm) over a nine-day trapping period.

Attractant	Farm	Medfly		Natafly		Marula fruit fly	
		Male	Female	Male	Female	Male	Female
Questlure	Larten	36	62	7	8	14	28
	Bakgat	0	11	19	18	0	0
Total		36	73	26	26	14	28
Capilure	Larten	603	12	25	0	0	0
	Bakgat	13	0	21	2	1	0
Total		616	12	46	2	1	0
Ceratitislure	Larten	82	22	11	1	177	5
	Bakgat	12	14	71	5	54	0
Total		94	36	82	6	231	5

Twice as many Mediterranean and marula fruit fly females as males were caught in the Questlure-Sensus traps (Table 3.5.7.7). This contrasted with the Natal fruit flies where equal numbers of both sexes were caught. Capilure was the superior attractant for Mediterranean fruit fly males while Ceratitislure-Sensus traps caught the most marula fruit fly.

Future research

Other attractants such as terpinyl acetate will be tested as a Natal fruit fly attractant. Research will also be conducted into modifying the Sensus trap to make it more accessible to Natal fruit fly.

3.5.8 Maputo Corridor Fruit Fly Survey

Experiment 727 by John-Henry Daneel and Tony Ware (CRI)

Opsomming

Metiel eugenol, Cuelure en Questlure is gebruik tussen Maputo en Nelspruit om 'n opname van vrugtevlieë te doen. Veertien vrugtevliespesies is gevang, maar nie een hiervan was 'n uitheemse spesie nie. Dit dui daarop dat geen indringing van die gebied plaasgevind het nie.

Introduction

One of the biggest threats to the citrus industry is the accidental introduction of pests and diseases from other parts of the world. One such group of pests is fruit flies, in particular the *Bactrocera* species from Asia and Africa. The fruit fly survey was undertaken in order to determine whether any of these species had invaded the eastern part of our country without our knowledge.

Materials and methods

Three attractant lures (Questlure, Cuelure and methyl eugenol) were used in combination with the Sensus trap (with DDVP) to survey the flies. A set of three traps was placed at five sites (Maputo, Komatipoort, Malelane, Nelspruit and IYSIS) at suitable secure sites (home gardens, hotels etc). The trees were not sprayed during the survey period. The traps were emptied every seven days and the flies brought back to the laboratory where they were identified to species. The lures and DDVP were replaced every 6 weeks.

Results and discussion

Table 3.5.8.1 shows the 14 fruit fly species trapped with the three lures. No exotic fruit flies were detected over the sampling period.

Table 3.5.8.1. Fruit fly species caught in Sensus traps primed with Questlure, methyl eugenol or Cuelure and placed between Maputo and Nelspruit. (+) = small numbers caught, (++) = moderate numbers caught and (+++) = large numbers caught.

Questlure	Cuelure	Methyl eugenol
<i>Ceratitis capitata</i> (+)	<i>Dacus</i> sp. 1 (+)	<i>Ocnerioxa pennata</i> (+)
<i>Ceratitis cosyra</i> (+)	<i>Dacus</i> sp. 2 (+)	<i>Perilampus diademata</i> (+)
<i>Ceratitis rosa</i> (+)	<i>Dacus bivittatus</i> (+++)	
<i>Coelotrypes</i> sp. 1 (+)	<i>Dacus ciliatus</i> (+)	
<i>Coelotrypes vittatus</i> (+)	<i>Dacus punctatifrons</i> (++)	
<i>Dacus vertebrates</i> (++)		
<i>Dacus ciliatus</i> (+)		

Conclusion

No exotic flies were recorded indicating that there was probably no establishment of these species in the area.

Future research

None planned. The programme should become a government function.

3.5.9 Modifications to the M3 bait station

3.5.9.1 The use of absorbent paper

Experiment by Tony Ware, Hennie le Roux, John-Henry Daneel and Henry Skinner (CRI)

Opsomming

Veranderde omstandighede vereis dikwels dat 'n produk verdere ontwikkeling moet ondergaan. Die M3-lokaasstasie is oorspronklik ontwikkel vir plaaslike gebruik. Met die besluit om die lokaasstasie aan Spanje te verskaf, was die lywige sponse te duur vir vervoer. Dit het die soektog na 'n minder lywige materiaal genoodsaak die absorberende papier is gevolglik as plaasvervanger getoets. Die papier was net so doeltreffend as die spons maar het "gelek". Die swamdoder wat in die lokaasstasie gebruik is, is nie meer beskikbaar nie en 'n alternatief word dringend benodig. Biede Kaptan en Euparen-M was effektief en het nie die vrugtevlieë verdryf nie.

Introduction

The M3 bait station has been developed as an IPM-compatible alternative to conventional fruit fly control. It has many advantages over the traditional treatments in that it uses attract-and-kill technology thereby avoiding the application of insecticides directly to the tree and the environment. It has the further advantage that it is rain-fast. However, it is viewed as being expensive. One of the major costs of the M3 bait station is that of transport. The sponges currently used in the commercial M3 are bulky and are extremely messy when assembling or recharging the bait station. The aim of the trial was to investigate alternatives to sponges while maintaining effectiveness.

Materials and methods

Quest Developments CC chose a carbon paper base impregnated with cotton as the viable alternative to the sponge that is currently used in the M3 bait station. Bait station substrates made up with this paper were designated the M4. The volume of lure (Questlure) applied to the paper was the same as applied to the conventional sponges. Quest Developments assembled the lures prior to their delivery to CRI.

The lures were placed in Sensus traps together with dichlorvos and were hung in three orchards on the farm Bakgat in the Schoemanskloof Valley near Nelspruit on 1 May 2004. One orchard was a variety block and was monitored with ten M3 and ten M4 Sensus traps on 11 May, 22 May, 12 June, 16 June, 25 July and 22 September. This orchard was never treated for fruit fly. The second was a Nova variety orchard treated with conventional M3 bait stations with the exception of those trees used for this experiment. Twenty Sensus traps containing each lure type were used and the Sensus traps were monitored on 11 May, 16 June, and 25 July. The M3 bait stations were removed a week prior to the last observations being made. The final orchard contained trees of the Cara Cara variety and were monitored using 13 M4 Sensus traps and 13 M3 Sensus traps that were monitored on 16 May, 22 May, 16 June and 25 July. This orchard was treated for fruit fly although the adjacent one was not. All fruit flies caught in the traps were taken to the laboratory where they were counted, sexed and identified to species.

Results and discussion

A total of 2150 flies were trapped of which the conventional M3 bait substrate attracted 54% (36% of the flies trapped were males) (Table 3.5.9.1.1). 59% of the flies caught were Medfly, 16% Natalfly and 25% marula fruit fly, Of the Medfly trapped, 49% were attracted to the M3 Sensus traps (30% males) and 51% to the M4 Sensus trap (26% males). Based on these results it appears that the two lures are similar in their overall attractiveness to flies and both types attract Medfly in a ratio of approximately 7 females: 3 males.

Of the 339 Natalfly caught, 59% were males (Table 3.5.9.1.1). 58% of this species were trapped in the M3 Sensus traps (60% males). In the M4 Sensus traps, 58% of the flies trapped were males. Both lures attracted similar numbers of males to females. One noted that the ratio of 3 males: 2 females for Natalfly is different from that of Medfly in that more males than females were trapped. This finding confirms that obtained earlier (Ware and Daneel, 2003a and 2003b).

553 marula fruit fly were trapped of which 226 (41%) were male (Table 3.5.9.1.1). More Marula fruit flies (64%) - 41% of these were males - were trapped in the M3 Sensus traps as against 46% (39% males) in the M4 traps. Although there was a large difference in the number of marula fruit fly caught in the M3 and M4 Sensus traps, the sex ratio was consistent.

Table 3.5.9.1.1. A comparison of fruit fly catches between the conventional M3 lure in a Sensus trap and the experimental M4 lure in a Sensus trap at Bakgat Farm

Cultivar	Lure	Sex	Medfly	Natalfly	Marula	Total	
Mixed	M3	Male	152	74	120	346	
		Female	334	38	186	558	
		Total	486	112	306	904	
	M4	Male	128	28	59	215	
		Female	373	31	95	499	
		Total	501	59	154	714	
	M3 and M4	Total	987	171	460	1618	
	Nova	M3	Male	7	14	5	26
			Female	37	10	5	52
Total			44	24	10	78	
M4		Male	7	17	5	29	
		Female	50	15	11	76	
		Total	57	32	16	105	
M3 and M4		Total	101	56	26	183	
Cara Cara		M3	Male	25	28	18	71
			Female	57	31	18	106
	Total		82	59	36	177	
	M4	Male	34	38	19	91	
		Female	54	15	12	81	
		Total	88	53	31	172	
	M3 and M4	Total	170	112	67	349	

The majority of the fruit flies were trapped in the second and the third sampling of the orchard (Figure 3.5.9.1.1). There was little difference between the total numbers of fruit flies trapped with the two different lure types. It was noted that the number of flies trapped tapered off over time. It is uncertain whether this result reflects the lower number of fruit flies present or was a result of the diminished attractiveness of the bait. In either case the two systems reacted similarly.

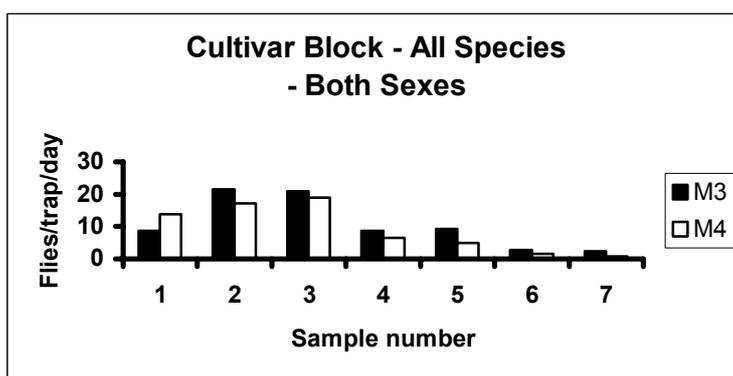


Fig. 3.5.9.1.1. Fruit flies caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

Figures 3.5.9.1.2 – 3.5.9.1.7 show the sex-biased trap catches of the three species indicating that on a species and sex level the paper and the sponge were comparable.

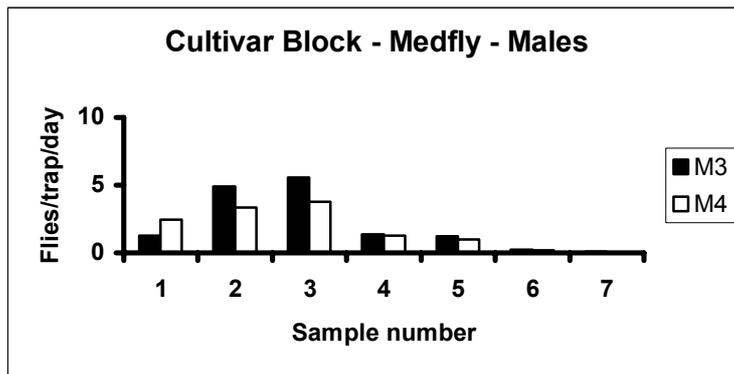


Fig. 3.5.9.1.2. Male Medfly caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

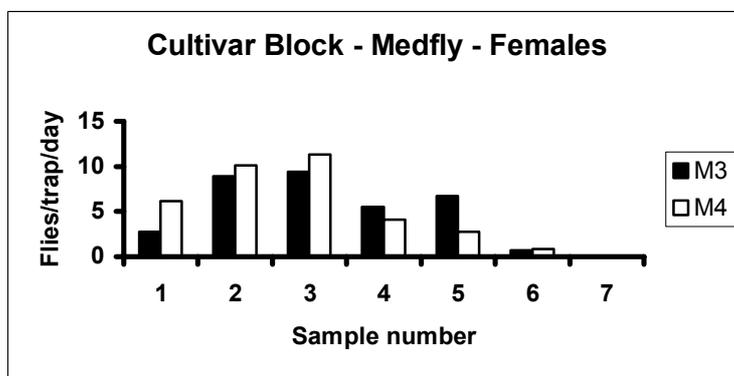


Fig. 3.5.9.1.3. Female Medfly caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

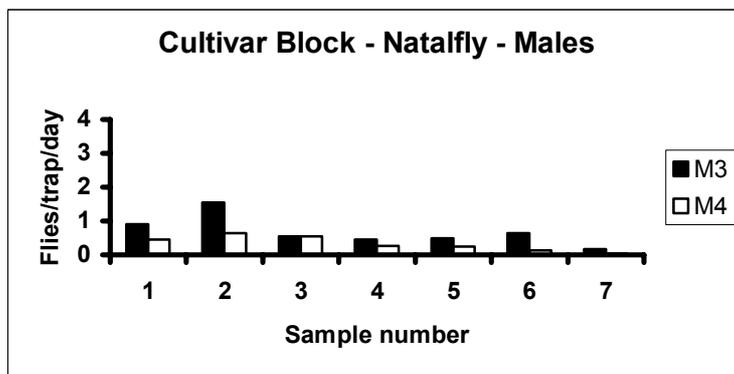


Fig. 3.5.9.1.4. Male Natalfly caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

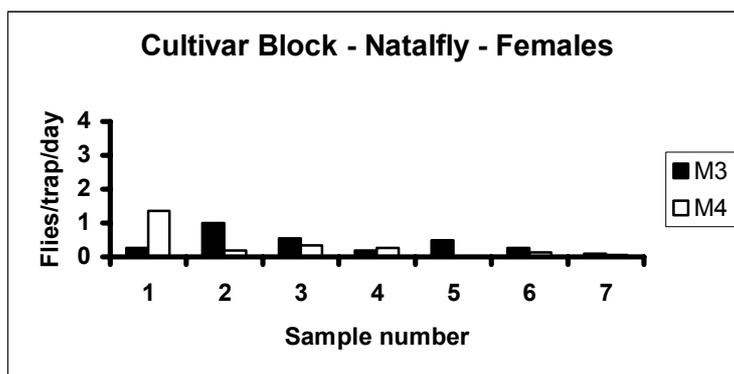


Fig. 3.5.9.1.5. Female Natalfly caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

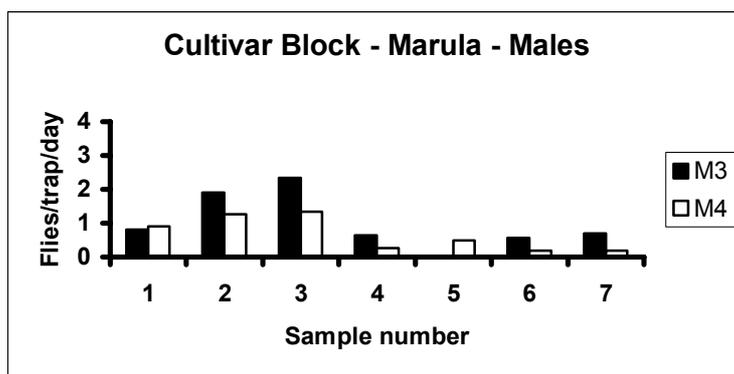


Fig. 3.5.9.1.6. Male marula fruit flies caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

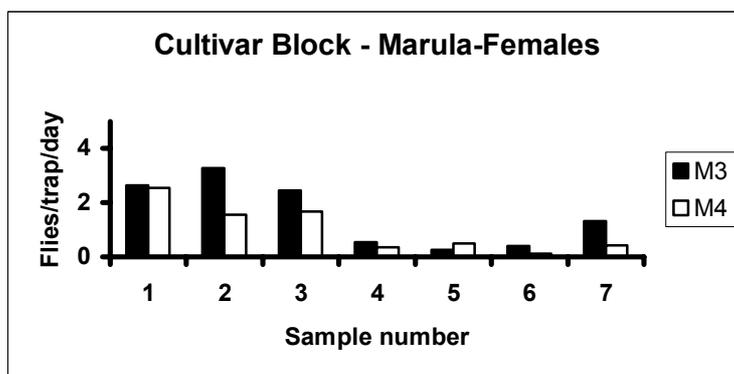


Fig. 3.5.9.1.7. Female marula fruit flies caught in Sensus traps containing Questlure impregnated sponge (M3) or Questlure impregnated paper (M4) placed in a mixed cultivar block on the farm Bakgat.

Based on these results it appears that there is little difference between the paper and the sponge. Although it was observed that the paper “leaked” more than the sponges.

Conclusions

The absorbent paper can be substituted for the sponge in the bait station without any appreciable loss of effectiveness.

References cited

Ware, A.B. and Daneel, J.-H. 2003a. Area-wide control of fruit fly: Laughing Waters – Litchis - Onderberg. CRI Group Annual Research Report: 166-167.

3.5.9.2 Replacement fungicide for the M3 bait station

Experiment by Tony Ware, John-Henry Daneel, Hennie le Roux and Laura Huisman (CRI)

Opsomming

Kaptafol (difolatan), die swamdoder wat tans in die M3-lokaasstasies gebruik word, word nie meer vervaardig nie en 'n plaasvervanger word dringend benodig. Benlate (benomyl), Kaptan (captan), Bravo (chlorothalonil), Sporekill (dimethyldidecylammonium chloride) en Euparen M (tolyfluaniid) is getoets met en sonder Benlate teen die swam, *Cladosporium cladosporioides*. Kaptan en Euparen M was net so doeltreffend as Kaptafol en veldtoetse het getoon dat hierdie middels nie afwerend is nie.

Introduction

Captafol (difolatan), the fungicide currently used in the M3 bait station, is no longer manufactured. It was therefore imperative that an alternative be found. This research reports on a laboratory investigation in determining suitable alternatives that then underwent field-testing.

Materials and methods

Various fungicides, or combinations thereof (concentrations shown in Table 3.5.9.2.1), were mixed with 50 ml Questlure and each placed into a single M3 bait station sponge. In addition to the single dosage another set of concentrations at five times this dosage was used. The sponges were stored for 7, 10 and 14 weeks at 25°C and 35°C in a laboratory oven. A water suspension of *Cladosporium cladosporioides* was made and then plated out on agar. A 1 cm² square was cut from each sponge and placed in the centre of a plate. The fungus was allowed to grow and the zone of inhibition was measured five days later. Difolatan, the fungicide currently used in the bait stations, served as the standard while untreated plates served as the untreated control.

Kaptan (3 g/l) and Euparen-M (10 g/l) were mixed with Questlure and placed in the Sensus traps with dichlorvos strips on 20 October 2004. The traps were emptied weekly and the flies caught separated into species and sexed.

Table 3.5.9.2.1. Concentrations of fungicides used in M3 bait stations

Treatment number	Product	Active	Dose /50 ml Questlure
1	Kaptan	Captab	0.5 g
2	Bravo	Chlorothalonil	0.125 ml
3	Benlate	Benomil	0.025 g
4	Euparen M	Tolyolfluaniid	0.15 g
5	Sporekill	Didecyl dimethyl-ammonium-chloride	0.05 g
6	1 and 3		0.3 g plus 0.015 g
7	2 and 3		0.08 g plus 0.015 g
8	4 and 3		0.08 g plus 0.015 g
9	5 and 3		0.03 ml plus 0.015 g
10	Captafol	Difolatan	0.5 ml
11	Control		0

Results and discussion

The results of the experiment are shown in Table 3.5.9.2.2. Benlate showed poor activity at the lower dosage and temperature apparently also affected its effectiveness. The addition of Benlate to the other products did not produce any synergistic effect and it was dropped from the assessment. The effectiveness of Bravo decreased at 10 weeks and no further assessments were made. Kaptan and Euparen M showed decreases in activity over time but there was no marked difference in the zone size based on dose or temperature. Both these fungicides were considered to be as effective as difolatan and could be used as replacements for this compound. However, before this decision is taken field trials need to be conducted to ensure that these compounds are not repellent.

Table 3.5.9.2.2. *Cladosporium cladosporoides* growth inhibition zones (diameter cm) of various fungicides after various periods of storage at 25 and 35°C.

Temperature	Number of weeks at 25°C or 35°C	Treatment number	25°C		35°C	
			Single dosage	5 times dosage	Single dosage	5 times dosage
7	1		4.5	4.5	5.5	5.5
	2		2.0	4.5	2.5	3.0
	3		0	3.5	0	1.2
	4		3.0	3.5	4.0	4.0
	5		2.2	2.0	2.0	2.5
	6		4.5	5.0	5.2	5.0
	7		2.5	2.8	3.0	3.0
	8		3.0	3.5	3.2	4.0
	9		3.0	2.0	0	1.6
	10		3.0	4.5	3.6	6.5
	11		0	0	0	0
10	1		3.8	3.2	3.8	4.4
	2		1.0	1.0	1.5	1.2
	4		3.6	3.6	2.5	2.8
	10		2.6	2.8	2.5	3.0
	11		0	0	0	0
14	1		2.5	2.6	2.8	2.7
	4		1.6	2.2	1.7	2.0
	10		2.6	3.0	3.0	2.8
	11		0	0	0	0

The weekly number of flies trapped are combined as only repellent effects were being investigated. These results are shown in Table 3.5.9.2.3 and indicate that the addition of the fungicides did not result in flies being repelled from the Sensus traps.

Table 3.5.9.2.3. Fruit fly trap catches in Sensus traps using Questlure, Euparen-M/Questlure (3 g/l) and Kaptan/Questlure (10 g/l) at the Lowveld Agricultural College.

	Medfly		Natalfly		Marula fruit fly		Total
	♂	♀	♂	♀	♂	♀	
Questlure	0	1	9	4	7	23	44
Euparen-M	1	3	8	6	13	19	50
Kaptan	0	2	12	10	16	21	61

Conclusion

Kaptan and Euparen M appear to be suitable as replacements for Captafol.

Future research

None.

3.6 PROJECT: MEALYBUG AND OTHER PHYTOSANITARY PESTS

Project manager: Sean Moore (CRI)

3.6.1 Project summary

Four experiments were registered under this project. In the first, a survey was conducted for the parasitoids of oleander mealybug, *Paracoccus burnerae* (3.6.2). No parasitoids were captured. This was ascribed to shortcomings in the trial protocol. In the second trial Ultracide, Parathion and Tokuthion, applied preventively, all gave 100% control of mealybug (3.6.3). In a corrective control trial, Applaud was the most effective treatment. Research work on the third trial, regarding descriptions of mealybugs found on citrus, was completed during 2003 but no samples of *Delotococcus elisabethae* could be found. Recently, a manuscript on the work was submitted to a scientific journal for publication and is not included here. The fourth trial was scheduled to conduct a survey of grain chinch bug in fruit orchards. Work on this trial will only be initiated in March 2005.

Projekopsomming

Vier eksperimente is onder hierdie projek geregistreer. In die eerste eksperiment is 'n opname van die parasiete van oleander wltuis, *Paracoccus burnerae*, uitgevoer (3.6.2). Geen parasiete is gekry nie. Hierdie word aan tekortkominge in die proef protokol toegeskryf. In die tweede proef het Ultracide, Parathion en Tokuthion, wat voorkomend gespuit is, almal 100% wltuis beheer veroorsaak (3.6.3). In 'n korrektiewe beheer proef was Applaud die mees effektiewe behandeling. Navorsing op die derde proef, wat wltuis spesies wat op sitrus voorkom beskryf het, is gedurende 2003 klaargemaak maar geen *Delotococcus elisabethae* monsters is gekry nie. Onlangs is 'n manuskrip van die werk by 'n wetenskaplike joernaal voorgelê vir publikasie en is nie hier ingesluit nie. Die vierde proef is geskeduleer om 'n opname van die graanstinkbesie in vrugte boorde uit te voer. Werk op hierdie proef sal eers in Maart 2005 begin word.

3.6.2 Investigating biocontrol agents of mealybug species other than citrus mealybug

Experiment 692 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Die plaagstatus en derhalwe die belangrikheid van *Paracoccus burnerae* op sitrus, het in Suid-Afrika verhoog, veral in die Oos-Kaap. Belangrike en doeltreffende parasitoïede van 'n paar wltuisspesies wat op sitrus voorkom, is geïdentifiseer. Soortgelyke inligting word egter nog vir *P. burnerae* benodig. 'n Proef is uitgevoer om sulke inligting te versamel. Beide *Leptomastix* sp. en *Coccidoxenoides perminutus* is in 'n opname gedurende November 2003 as parasitoïede van *P. burnerae* geïdentifiseer, maar geen parasitoïede van dié wltuisspesie is in die laaste twee opnames in dieselfde seisoen gekry nie. Die verskynsel word aan die vinnige uitdroging van wltuisbesmette saailinge toegeskryf. As gevolg daarvan is groter saailinge wat in groter potte geplant is, gedurende die 2004/05-seisoen gebruik om die lewensduur van die plante te verleng. Ten spyte hiervan is weereens geen parasitisme van *P. burnerae* gedurende die eerste twee opnames van die seisoen gekry nie. In die toekoms sal die probleme met die proefprotokol eers uitgestryk moet word. Nadat die belangrikste parasitoïeds spesies van *P. burnerae* geïdentifiseer is, sal die moontlik gebruik van hierdie spesies vir aanvullende loslatings nagevors word. Opnames van wltuisspesies sal ook in die hele Suid-Afrika uitgevoer word.

Introduction

Citrus mealybug, *Planococcus citri*, is known to be effectively controlled by natural enemies. This control has been substantially enhanced with the development of the augmentation technique for the parasitoid *Coccidoxenoides perminutus* (*peregrinus*). However, it has been shown that the oleander mealybug, *Paracoccus burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997). What makes this a serious situation justifying further investigation is that *P. burnerae* is regarded by certain important markets e.g. USA and Korea (and potentially many others) as being a phytosanitary pest; and that *P. burnerae* has increased in dominance in citrus orchards, at least in the Eastern Cape. Important and effective natural enemies of *P. burnerae*, and other important mealybug species, should be identified. Ultimately, the objective should be to establish an augmentation technique with natural enemies effective against these "other" species of mealybug. Recently, Wakgari & Gilliomee (2002) qualified and quantified the species of parasitoids in the natural enemy complexes attacking citrophilous mealybug, *Pseudococcus calceolariae*, longtailed mealybug, *Pseudococcus longispinus*, and *P. citri*. However, this has not been done for *P. burnerae*.

Materials and methods

2003/04 season

A starter culture of *P. burnerae* was obtained from Bruce Tate (CRI, Nelspruit). Their identification was confirmed by Dr. Ian Millar of the Biosystematics Unit of the Plant Protection Research Institute (PPRI). The mealybugs were transferred onto Clementine mandarin seedlings, which were kept under Growlux (UV-B) lights in the laboratory. Trees were watered and fertilised regularly. Small trifoliolate seedlings (approximately 20 cm from soil to tip) were obtained from the Citrus Foundatin Block (CFB). Mealybug infested leaves from the Clementines were removed and placed onto the trifoliolate seedlings to facilitate movement of the mealybug onto the trifoliolate seedlings.

Two orange orchards, in which there were conspicuous levels of mealybug infestation, were selected. One of these was in the Gamtoos River Valley (GRV) (Tierhok Farm, orchard 4, Delta Valencias) and the other in the Sundays River Valley (SRV) (Rosedale Farm, orchard 3, Washington navels). Two gauze cages were assembled and each was fitted onto a platform on the top of a pole. These were inserted into the ground in each orchard, so that the cage was about 1.5 m above the ground, and the cage was not in contact with any of the trees in the orchard. Twenty mealybug-infested trifoliolate seedlings were placed into each cage (approximately 1 week after mealybug infested Clementine leaves had been placed onto the seedlings). Tangletrap glue was smeared around each pole so that ants and other wingless predators could not access the cages. Seedlings were retrieved from each orchard after 1 week. Numbers and life-stages of mealybug were estimated and seedlings were placed into emergence boxes. Emerging parasitoids were collected, placed into 70% ethanol, identified and counted. This survey was conducted monthly in each orchard. Results from the earlier part of the 2003/04 season are recorded in a previous report (Moore & Kirkman, 2003). Only the results of the trial conducted during December 2003 and February 2004 are presented here.

2004/05 season

Due to the collapse of the *P. burnerae* laboratory culture, a second starter culture was obtained from Welma Pieterse (Department of Agriculture, Stellenbosch). Due to flaws in the trial protocol, certain changes were made in an attempt to remedy these. Instead of the small seedlings used previously for exposing mealybug in orchards, larger Carrizo seedlings (approximately 40 cm from soil to tip) were used. Seedlings were again obtained from the CFB. Mealybug-infested leaves from the Clementines in the laboratory culture were removed and placed onto 12 Carrizo seedlings to facilitate movement of the mealybug onto them.

Two orange orchards, in which there were conspicuous levels of mealybug infestation, were selected. Both of these were in the Sundays River Valley (SRV). One of them was the same organic orchard of Washington navel orange trees on Rosedale Farm that had previously been used. The other was an orchard of mature Palmer navel orange trees on Sun Orange Farm. Gauze cages were used, as previously described, except that cages were now slightly larger in order to facilitate the larger seedlings – six per cage. This survey was conducted twice in each orchard, from September to November 2004.

Results and discussion

2003/04 season

In the first survey, conducted during November 2003, both *Leptomastix* sp. and *Coccidoxenoides perminutus* were found attacking *P. burnerae*. *Leptomastix* sp. was the dominant species, parasitising 7.6% and 8.2% of the mealybug recovered from the two trial sites (Moore & Kirkman, 2003). Despite this, no parasitism of *P. burnerae* was recorded during the two later surveys conducted during the same season (Table 3.6.2.1).

Table 3.6.2.1. Mealybug placed into emergence boxes and parasitoids collected from emergence boxes in 2003/04 season survey.

		Vergenoeg Farm (GRV)		Rosedale Farm (SRV)	
		23-30 Dec	3-10 Feb	23-30 Dec	3-10 Feb
Mealybug life-stages counted before placement in emergence box	Exposure period of mealybug in orchard				
	Egg sacs	17	5	9	6
	Crawlers (1st & 2nd instars)	7	0	26	3
	Sub-adults (3rd instar)	19	28	44	2
	Adults	37	17	28	6
Parasitoids collected from emergence box	<i>Leptomastix</i> sp.	0	0	0	0
	<i>Coccidoxenoides perminutus</i>	0	0	0	0

2004/05 season

In the surveys conducted during the 2003/04 season, the seedlings placed into the orchards did not always survive very well. Due to the small size of the seedlings and the small size of the bags into which they were potted, trees desiccated too rapidly. It was therefore not uncommon to retrieve seedlings from orchards after the one-week exposure period, which were dead or dying. This was thought to possibly be effecting the survival of the mealybug on the seedlings and the attractiveness of the mealybug and their host plants to parasitoids. Consequently, the trial protocol was changed for the 2004/05 season i.e. larger seedlings and pots were used. Consequently, there was far better survival of seedlings. Despite this, no parasitism of *P. burnerae* was recorded (Table 3.6.2.2). It is not clear where the fault in the protocol lies. The natural enemy complex of mealybug is known to be very effective and is known to peak during January and February in the Eastern Cape (Hattingh & Moore, 2003) (although this does pertain chiefly to *P. citri*). It is therefore highly improbable that there was a dearth of parasitoids in the trial orchards, both of which were subjected to natural enemy friendly management (i.e. IPM or organic). From the results of the very first survey conducted (Moore & Kirkman, 2003) it was seen that a meaningful level of parasitism could be obtained with this general protocol. However, it is possible that if the infested seedlings are left in an orchard for longer than one week, the probability of capturing parasitoids could be significantly improved.

Table 3.6.2.2. Mealybug placed into emergence boxes and parasitoids collected from emergence boxes in 2004/05 season survey.

		Sunorange Farm		Rosedale Farm	
		22 Sep-1 Oct	4-11 Nov	1-8 Oct	4-11 Nov
Mealybug life-stages counted before placement in emergence box	Exposure period of mealybug in orchard				
	Egg sacs	24	10	13	13
	Crawlers (1st & 2nd instars)	25	21	6	45
	Sub-adults (3rd instar)	173	20	23	34
	Adults	38	9	19	34
Parasitoids collected from emergence box	<i>Leptomastix</i> sp.	0	0	0	0
	<i>Coccidoxenoides perminutus</i>	0	0	0	0

Conclusion

No parasitism of *P. burnerae* was recorded during the final two surveys conducted in the 2003/04 season. Consequently, larger seedlings and pots were used in the surveys conducted in the 2004/05 season. This was to reduce the desiccation of the seedlings. Despite this, no parasitism was recorded during the two early season surveys conducted during the 2004/05 season. The problem with the trial protocol must be identified and remedied.

Future research

This experiment is ongoing. Firstly, the problems with the trial protocol must be remedied. Once the dominant parasitoid species, attacking *P. burnerae*, are identified, the possible use of these species for augmentative biocontrol will be investigated. Surveys of mealybug will be conducted in citrus producing regions in South Africa, outside of the Eastern Cape. If other species of mealybug, such as *P. longispinus*, are found to be sufficiently important, biocontrol of these species will also be investigated.

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3.6.3 Preventive and corrective chemical treatments for control of mealybug on citrus Experiment 755 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

In 'n proef wat uitgevoer is om die werking van verskeie produkte vir die voorkomende bestryding van witluis te vergelyk, het Ultracide, Parathion en Tokuthion die plaag volkome bestry. Ongelukkig was die vlak van besmetting, selfs in die onbehandelde kontrole, baie laag. Die resultate van hierdie proef word daarom nie as 'n akkurate beeld van die werking van die onderskeie produkte beskou nie. In 'n korrektiewe bestrydingsproef was Applaud die doeltreffendste behandeling en die produk se uitklopaksie was duidelik baie langer as dié van enige van die organofosfaatbehandelings.

Daar is in 'n proef bevind dat, alhoewel 90% van die vrugte in Februarie ten tye van bespuiting, met witluis besmet was, het dit teen einde-Maart tot 34% in die onbehandelde kontrole afgeneem. Dit is bewys van die uitwerking van die biologiese beheerkompleks op witluis. Dit plaas 'n vraagteken oor die waarde van korrektiewe bespuitings vir witluisbestryding, selfs in swaarbesmette boorde. Gedurende die volgende navorsingsiklus sal 'n tweede voorkomende bestrydingsproef geëvalueer word. Dié navorsing sal daarna afgesluit word.

Introduction

The pest status of mealybugs on citrus has increased during the last few years. This is partly due to the increased levels of oleander mealybug, *Paracoccus burnerae* (Moore & Kirkman, 2003). Consequently, chemical treatment of mealybug is becoming more necessary than has been the case for several years. There is a lack of clarity as to how the efficacy of the various registered and available pesticides compare with one another. This coupled with rumours and misinformation about the various products makes decision making difficult for growers. The last trial to compare the various products registered for mealybug control was conducted approximately nine years ago (Hattingh *et al.*, 1995). Consequently, it is considered important and urgent to determine the relative efficacy of all products, both as preventative and corrective treatments.

Materials and methods

An orchard of nine-year-old Robyn navel oranges on Vergenoeg Farm in the Gamtoos River Valley, which had experienced severe mealybug infestation for the past two seasons, was selected for the first trial. The trial was laid out in a random block design with 12 single-tree replicates per treatment. Eight treatments were sprayed and untreated trees were retained for comparison (Table 3.6.3.1). An average of 17.5 ℓ of spray mix was applied per tree using hand-held spray guns. The trial was evaluated on 5 February 2004 by inspecting 10 randomly selected fruit on each tree and recording any mealybug infestation.

Table 3.6.3.1. Treatments applied for the preventative control of mealybug in a Robyn navel orange orchard on Vergenoeg Farm.

Number	Treatment (concentrations per 100 L water)	Timing of application	Date of application
1	Untreated control	-	
2	Ultracide (150 ml) + Agral 90 (18 ml)	100% petal drop +	21 October 2003
3	Parathion (150 ml) + Agral 90 (18 ml)	50-90% petal drop	9 October 2003
4	Selecron (100 ml) + Agral 90 (18 ml)	50-90% petal drop	9 October 2003
5	Tokuthion (50 ml) + Agral 90 (18 ml)	50-90% petal drop	9 October 2003
6	Folimat (50 ml) + Agral 90 (18 ml)	100% petal drop +	22 October 2003
7	Chlorpyrifos WG (64 g) + Agral 90 (18 ml)	100% petal drop +	22 October 2003
8	Applaud (30 g) + Agral 90 (18 ml)	100% petal drop +	22 October 2003
9	Mevinphos EC (165 ml) + Agral 90 (18 ml)	100% petal drop +	21 October 2003

A second trial was conducted to compare various treatments for the corrective control of mealybug. An orchard of five-year-old Lane Late navel oranges on Atmar Farm in Sundays River Valley was selected for the trial. A pre-spray assessment of mealybug infestation was conducted on 3 February 2004. The trial was laid out in a random block design with 12 single-tree replicates per treatment. Five treatments were sprayed on 6 February and untreated trees were retained for comparison (Table 3.6.3.2). An average of 9.8 ℓ of spray mix was applied per tree using hand-held spray guns. The trial was evaluated by inspecting 10 randomly selected fruit on each tree and recording any mealybug infestation. This was conducted twice: on 24 February and again on 30 March 2004.

Table 3.6.3.2. Treatments applied for the corrective control of mealybug in a Lane Late navel orange orchard on Atmar Farm on 6 February 2004.

Number	Treatment (concentrations per 100 ℓ water)
1	Untreated control
2	Mevinphos (165 ml) + Agral 90 (18 ml)
3	Mevinphos (165 ml) + oil (500 ml)
6	Ultracide (150 ml) + Agral 90 (18 ml)
7	Chlorpyrifos WG (64 g) + Agral 90 (18 ml)
8	Applaud (30 g) + Agral 90 (18 ml)

A second preventive treatment trial was applied in the spring of the 2004/05 season. An orchard of six-year-old Lane Late navel oranges on Atmar Farm in Sundays River Valley, which had a history of high mealybug infestation, was selected for the trial. The trial was laid out in a random block design with 12 single tree replicates per treatment. The same nine treatments that were used in the preventive treatment trial at Vergenoeg Farm during the previous season were again applied (Table 3.6.2.3). An untreated control was retained for comparison. An average of 17.5 ℓ of spray mix was applied per tree using hand-held spray guns. Evaluations of treatments will be conducted in January 2005.

Table 3.6.3.3. Treatments applied for the preventative control of mealybug in a Lane Late navel orange orchard on AtmarVergenoeg Farm.

Number	Treatment (concentrations per 100 ℓ water)	Timing of application	Date of application
1	Untreated control	-	
2	Ultracide (150 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
3	Parathion (150 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
4	Selecron (100 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
5	Tokuthion (50 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
6	Folimat (50 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
7	Chlorpyrifos WG (64 g) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
8	Applaud (30 g) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
9	Mevinphos EC (165 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004

Results and discussion

At the time of spraying the first trial at Vergenoeg Farm, very little mealybug was evident. However, this was expected at this early stage of the season, even in an orchard in which infestation would reach a high level later in the season. Unfortunately, this did not happen. Even in the untreated control, mealybug infestation did not exceed 7% fruit infested. Ultracide, Parathion and Tokuthion all gave 100% control of mealybug (Table 3.6.3.4). Although this was not significantly better than any of the other treatments. Mealybug infestation in all of the treatments was significantly reduced from that in the untreated control (Table 3.6.3.4). Due to the very low level of infestation throughout, the results of this trial are not considered as an accurate comparative measure of the efficacy of the various treatments.

Table 3.6.3.4. Mealybug infestation of fruit (evaluated on 5 February 2004) for various treatments applied preventatively in a Robyn navel orange orchard on Vergenoeg Farm in October 2004.

Number	Treatment	% Fruit infested
1	Untreated control	6.67a
2	Ultracide	0b
3	Parathion	0b
4	Selecron	0.83b
5	Tokuthion	0b
6	Folimat	0.83b
7	Chlorpyrifos	2.50ab
8	Applaud	0.83b
9	Phosdrin	2.50ab

The pre-harvest evaluation of fruit from the corrective trial site on Atmar Farm, conducted on 3 February, revealed that 100% of fruit inspected were infested with mealybug. Most of the mealybug, which could be identified to species was recognised to be oleander mealybug, *P. burnerae*. Eighteen days after spraying, all treatments had significantly reduced mealybug infestation (Table 3.6.3.5). Applaud showed the lowest level of reduction in infestation and Ultracide appeared to be the most effective treatment, being the only treatment which was significantly more effective than Applaud. However, the second evaluation, conducted 53 days after spraying, revealed a different picture. As infestation in the untreated control had declined dramatically (from 90% to 34% fruit infested), infestation was not significantly lower for any of the treatments. Although, Applaud now showed up as the most effective treatment and infestation on Ultracide treated trees had increased substantially. This indicated that Applaud takes a long time to show its full effect on mealybug. This stands to reason, as Applaud is an insect growth regulator (IGR) and will therefore not have a knockdown action like the organophosphates do. This change in infestation also indicated that despite the good impact of Ultracide initially, its detrimental impact on mealybug parasitoids (Ware *et al.*, 1999) probably prohibited any biocontrol maintenance of its knockdown. Both Mevinphos and Chlorpyrifos were disappointing as corrective treatments for mealybug (Table 3.6.3.5). Mevinphos with oil, instead of a wetter, performed slightly better, although not significantly so. This combination was included, as field observations indicated that the addition of oil instead of a wetter might improve the efficacy of Mevinphos. The dramatic decline in mealybug infestation in the control was encouraging. This was testament to the incredible effectiveness of the mealybug biocontrol complex, even late in the season. It raises the question of the value of corrective spraying for mealybug, even in heavily infested orchards.

Table 3.6.3.5. Mealybug infestation of fruit for various treatments applied correctively in a Lane Late navel orange orchard on Atmar Farm on 6 February 2004.

Number	Treatment	% Fruit infested	
		24 February	30 March
1	Untreated control	90.0a	34.0ab
2	Mevinphos + wetter	34.0cd	40.0a
3	Mevinphos + oil	33.0cd	26.0ab
4	Ultracide	19.0d	33.0ab
5	Chlorpyrifos	44.0bc	35.0a
6	Applaud	54.0b	17.0b

In trials conducted in spring by Hattingh *et al.* (1995), Applaud was the most effective treatment and Phosdrin (mevinphos) was the least effective treatment. These findings are not in disagreement with the results of the two trials conducted here in the 2003/04 season.

Conclusion

In a trial conducted to compare the efficacy of various products as preventative control measure for mealbug, Ultracide, Parathion and Tokuthion all gave 100% control. However, due to the very low level of infestation, even in the untreated control, the results of this trial are not considered as an accurate comparative measure of the efficacy of the various treatments. In a corrective control trial, Applaud was the most effective treatment. However, knockdown time was clearly far longer than that of the organophosphate treatments. Despite 90% of fruit being infested with mealybug in February at the time of spraying, by the end of March only 34% of the fruit in the untreated control were infested, bearing testimony to the effectiveness of the mealybug biocontrol complex.

Future research

During the next research cycle, the second preventative control trial will be evaluated. Thereafter, no further work will be conducted on this experiment.

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3.7 PROJECT: PRODUCTION PESTS

Project coordinator: Tony Ware (CRI)

3.7.1 Project summary

An unscheduled trial using mayonnaise type horticultural mineral oil was undertaken (3.7.2). The oil was found to be phytotoxic and no further research is planned. On the psylla front (3.7.3) suitable populations of citrus psylla were only found at one site in January 2005 but the results are included here for convenience. Tracer and Envidor were ineffective, oil had some effect but only at 1%, and both abamectin plus oil and kaolin showed some promise.

Projekopsomming

'n Ongeskeduleerde proef is uitgevoer met mayonnaise-tipe tuinboukundige mineraalolie (3.7.2). Die olie was fitotoksies en geen verdere proewe word beplan nie. Geskikte populasies van sitrussilla is by slegs een plek gevind in Januarie 2005, maar die resultate word gerieflikheidshalwe hier ingesluit (3.7.3). Tracer en Envidor was ondoeltreffend, olie het 'n effek gehad maar slegs teen 1%, en beide abamectin plus olie en kaolien het belofte getoon.

3.7.2 Control of red scale with mayonnaise oil

Experiment by Tony Ware and John-Henry Daneel (CRI)

Opsomming

'n Tuinboukundige mayonnaise-tipe mineraalolie wat gebruik word in die sagtevrugbedryf om dormansie op te hef is getoets as 'n rooidopluismiddel op sitrus, aangesien dit by verre goedkoper is as die mineraalolies met 'n nou distillasiereeks wat tans gebruik word. Dit was effektief as 'n dopluisdoder maar het kleurontwikkeling vertraag. Meer belangrik is dat die gebruik daarvan blomvorming die volgende seisoen verhoed het.

Introduction

The use of horticultural mineral spray oils is considered an IPM compatible option for the control of red scale on citrus. However, the recent increase in global oil prices has resulted in the narrow distillation range mineral oils becoming expensive. Mr Theuns Maritz (Cropchem) approached CRI with the idea of

substituting these oils with a cheaper mayonnaise type oil. This report details the work done in determining the effectiveness and phytotoxicity of the product.

Materials and methods

The oil was supplied by Cropchem with a label proclaiming it to be 86% Winter Oil for use on deciduous trees for dormancy breaking. Five year-old red scale-infested navel trees on the farm Oliphanthoek in the Burgersfort district were sprayed with 1.75% (6 trees), 2.0% (6 trees), 3.5% (4 trees) or 4.0% (4 trees) oil. The oil was applied as a full cover spray. Untreated trees and farmer-treated trees (Applaud plus 0.5% BP medium oil) acted as the control groups. The farm was visited at irregular intervals in order to assess any phytotoxic reaction. The red scale population was estimated by counting 20 randomly selected fruit on each tree that had more than 10 scale, 1-10 scale or were clean. At harvest the size and colour of the fruit was determined using standard laboratory methods. The number of flowers set the following season was estimated on a scale 0-5 with 0 indicating the trees had no flowers and 5 indicating those trees that were flowering copiously.

Results and discussion

For many weeks after application of the oil the trees were visually much shinier than the untreated trees and the orchard smelt as if someone had spilt old gun oil in the vicinity. The oil treatment effectively controlled scale (Table 3.7.2.1). There was an increase in red scale in the farmer-treated trees indicated that there was some repercussions due to his treatment. Natural enemies helped to control the red scale in the untreated block.

Table 3.7.2.1. Number of fruit (%) with no scale, between 1 and 10 scale and more than 10 scale after treatment with various concentrations of mayonnaise type horticultural mineral oil

Number of scale	Untreated	Farmer treated	1.75%	2.0%	3.5%	4.0%
0	71	29	87	81	98	94
1-10	24	33	12	17	2	6
11+	5	38	1	3	0	0

There was approximately one colour plate shift of fruit colour between the trees receiving the oil and the untreated controls (Table 3.7.2.2). There was some variation in fruit size but this could not be attributed to the treatments (Table 3.7.2.2). Oil-treated trees had far fewer flowers than the untreated trees (Table 3.7.2.2) with the result that they set a small crop.

Table 3.7.2.2. Colour, size and flowering of navel trees treated with various concentrations of mayonnaise type horticultural mineral oil.

Parameter (n=12)	Untreated	Farmer treated	1.75%	2.0%	3.5%	4.0%
Colour	5.1	5.2	6.0	6.1	6.1	6.3
Size (mm)	78.3	82.3	76.9	76.3	75.4	78.9
Flowering	3.5	2.0	0.7	0.6	0	0

Conclusion

Mayonnaise oil was effective in controlling red scale but was phytotoxic in that the trees did not set flowers the following season.

Future research

No further research is to be conducted.

3.7.3 IPM-compatible treatment options for citrus psylla *Trioza erytreae* Experiment 586 by Tim Grout and Peter Stephen (CRI)

Opsomming

Pogings om veldproewe uit te voer vir die evaluering van nuwe behandelings teen sitrusbladvlou het in 2002 en 2003 misluk as gevolg van ongunstige lenteweer en populasies wat onreëlmatig in die boord versprei was. In 2004 is besluit om sitrusplante in potte te behandel en in boorde met sitrusbladvlou-besmetting te plaas. Dit was egter nie prakties nie aangesien nuwe groei op die gepotte plante nie saamgeval het met die aanwesigheid van silla in die boord nie. 'n Boordproef is uiteindelik in Januarie 2005 gedoen. Geeneen van die blaarbespuitings was so effektief as endosulfan nie. Tracer (spinosad) teen die geregistreerde dosis vir sitrusblaaspootjie was geheel en al ondoeltreffend, asook Envidor (spirodiclofen) teen die dosis geregistreer vir die beheer van sekere myte. Orchem tuinboukundige olie het nie veel impak gehad teen 0.3% nie, maar teen 1.0% het dit volwassenes en eierlegging tot 'n mate afgeweer en was dit meer effektief om nimfe te dood as die meeste ander behandelings. Kaolien was nie so doeltreffend as 1% olie om nimfe te dood nie, maar was meer effektief om volwassenes af te weer en eierlegging te voorkom. Abamectin plus 0.3% olie was net so doeltreffend as kaolien om nimfe te dood, maar minder effektief om volwassenes af te weer en eierlegging te verhoed. Verdere navorsing met olie, abamectin en kaolien is geregverdig om afhanklikheid te verminder op ou organofosfate wat in die toekoms nie meer beskikbaar mag wees nie.

Introduction

In 2001, citrus psylla populations went out of control and the need for new IPM-compatible control options was highlighted because the greening disease (*Liberobacter africanum*) that is transmitted by this vector was spreading rapidly. In the two subsequent seasons, citrus psylla populations were low due to unusual weather so research on additional control measures could not be conducted. Monocrotophos is no longer available and the use of dimethoate has been reduced to one preblossom treatment on bearing trees due to the MRL in EU being lowered markedly. Registered insecticide options are therefore dwindling and new alternative treatments need to be found. The currently registered products can be found in Nel et al. (2002) but not many of these can be used late in the season due to residue problems. No recent work has been conducted on the chemical control of citrus psylla. An evaluation of various treatments was therefore planned for spring and summer 2004.

Materials and methods

Due to difficulties in finding orchards with sufficient infestations of citrus psylla for trials, an attempt was made to use potted citrus plants that could be sprayed with various treatments, then placed in zones in an orchard where citrus psylla had been noticed. Potted plants were therefore prepared for this purpose but when they had suitable growth flush for citrus psylla in spring there were no orchards with citrus psylla. One farm was later found with low numbers of citrus psylla but by this time the leaves on the potted plants had hardened off and were no longer attractive to citrus psylla. This potted-plant approach was therefore abandoned.

No suitably infested citrus orchards were found until January 2005 when an orchard of Empress mandarins was found on Hilltop farm south of Nelspruit where citrus psylla was extremely abundant and the grower allowed us to do a quick trial before he sprayed out the orchard. A nested block design was used with the orchard being split in half and each half being subdivided into eight treatment blocks. Each block comprised three rows and was at least six trees long. Sprays were applied by hand using a high-pressure (30 bar) spray machine and applying approximately 6.8 l spray mixture per tree on 21 January 2005. The treatments used are shown in Table 3.7.3.1. They were evaluated 11 days after treatment on 2 February 2005. Only a single evaluation was conducted due to the urgent need to spray out the orchard. Data trees were primarily selected from the central row in each block and if there were insufficient trees in the centre row, the sides of trees closest to the central row were used as well. Counts were based on 10 branch terminals per data tree having new leaves suitable for citrus psylla. Each terminal was rated for the presence or absence of fresh eggs, live nymphs or adults. Data were transformed to the square root of the arc sine, then analysed by two-way ANOVA and means further compared using Student-Newman-Keul's test at $P=0.05$.

Results and discussion

Although only one evaluation could be conducted due to time constraints, the results were good due to the extremely high population density of citrus psylla. It was obvious that neither Envidor nor Tracer plus oil had any effect on citrus psylla (Table 3.7.3.1). This is disappointing as both products are relatively IPM-

compatible. The standard treatment of endosulfan was the most efficacious treatment against all three life stages. Orchex horticultural oil at 1% was the second best treatment against the nymphs and this confirmed research by Rae et al. (1997) on the Asian citrus psyllid *Diaphorina citri*. However, oil at this rate was only the third best treatment in preventing oviposition or reducing adult infestation. This contrasted with work by Erler (2004) who found that 1% oil prevented all oviposition by the pear psylla *Cacopsylla pyricola*. Oil at 0.3% had no significant effect on nymphs and only a slight effect on the other life stages. Abamectin plus 0.3% oil was only significantly different from the 0.3% oil alone when the egg infestation was considered. The kaolin (Fruitcote) treatment was less effective than 1% oil in reducing nymphal infestation but the second most effective treatment in preventing oviposition and adult infestation. This must be due to the white coating of the kaolin being a visual repellent as has been found with the pear psylla (Glenn & Puterka 2005). Perhaps if this product was applied at bud burst it would prevent citrus psylla from locating the new foliage. A single spray at this time of the year may be less likely to result in pest repercussions that have been observed with multiple sprays later in the season (Ware 2003).

Table 3.7.3.1. The effect of various treatments against different life stages of citrus psylla at Hilltop farm near Nelspruit.

Foliar treatments	Infestation of shoot terminals 11 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	94.4 a	93.8 a	88.9 a
Endosulfan (475 WP) 250 g/hl	33.1 e	6.9 d	11.9 c
Fruitcote (kaolin) 3 kg/hl	41.9 de	64.4 b	25.6 c
Abamectin (18 EC) 20 ml plus Orchex horticultural oil 300 ml/hl	66.3 c	70.0 b	58.8 b
Tracer (spinosad 480 SC) 15 ml plus Orchex 300 ml/hl	92.5 a	90.0 a	76.3 a
Orchex 300 ml/hl	78.1 b	80.0 ab	48.8 b
Orchex 1 l/hl	52.5 d	38.1 c	26.3 c
Envidor (spirodiclofen 240 SC) 15 ml/hl	88.8 ab	92.5 a	81.3 a

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

Conclusion

Suitable trial sites were difficult to find but one trial was successfully conducted that showed Tracer and Envidor to be ineffective against citrus psylla, oil to have some effect but only at 1% and both abamectin plus oil and kaolin to show some promise.

Future research

Further research should be conducted with kaolin and possible kaolin combinations but this will be difficult as farmers do not tolerate populations of citrus psylla in their orchards.

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4 PROGRAMME: DISEASE MANAGEMENT

4.1 PROGRAMME SUMMARY

By Tim G. Grout (Research & Technical Manager: CRI)

The Disease Management programme received the most funding of the four research programmes during 2004 and this was primarily used for research against the continued threat to production of graft transmissible diseases and reducing export losses due to citrus black spot. Star Ruby trees preimmunised with the Citrus Tristeza Virus isolates GFMS 35 and GFMS 78 had the best production over a five-year period and the presently-used isolate LMS 6 for sweet orange and mandarin still appears to be the best. Research on citrus blight indicates that trees on rootstocks such as C35 citrange, Empress mandarin and Carrizo citrange were the most affected by blight whereas trees on X639, Sun Chu Sha and Sunki rootstocks were the most tolerant. Greening disease requires more attention and if recent climatic changes are sustained it may spread to new areas. The appointment of Prof. Gerhard Pietersen to CRI at the University of Pretoria led to the development of PCR methods to detect South African greening in addition to the Asian and American greening diseases. Screening of many new and old mandarin cultivars showed that only Primasol, Cami, M26, Fremont and Bay Gold were resistant to *Alternaria* brown spot. The mystery disease affecting Clementines in the Knysna area was successfully demonstrated to be *Phytophthora citrophthora*. Final results of screening rootstocks for resistance to citrus nematode have shown that C35, Swingle and C32 citrange could be recommended as replant rootstocks. Scoparone in roots was not correlated with tolerance to *P. nicotianae* but increases in soluble phenolic concentrations and the accumulation of specific phenolic compounds such as U82 in tolerant rootstocks suggest a biochemical mechanism of resistance.

The PCR (DNA) method of detecting citrus black spot (CBS) on dead leaves and in the soil has been refined and is being used successfully. A new inoculum monitor has also been developed and is being used to detect potential ascospore outbreaks. Although CBS incidence in the field was low in 2004, good progress was achieved in breaking the life cycle and improving management strategies. Ways have been found to overcome the stippling associated with copper sprays and the combination of Sporekill with mancozeb and copper seemed to improve control. Post-harvest research on new formulations of imazalil and thiabendazole may result in new registrations. The quaternary ammonium compounds and organics tested gave poor efficacy. Screening of samples of *Penicillium* fungal spores from the Eastern Cape showed some to be resistant to imazalil and further research on imazalil resistance will be conducted as a result.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

Die Siektebestuurprogram het die meeste befondsing van die vier navorsingsprogramme ontvang in 2004 en dit is hoofsaaklik aangewend vir navorsing gerig teen die voortdurende gevaar vir produksie van entoordraagbare siektes en die vermindering van uitvoerverliese as gevolg van sitruswartvlek. Star Ruby bome wat preïmmuniseer was met die Sitrus Tristeza-virus isolate GFMS 35 en GFMS 78 het die beste produksie oor 'n tydperk van vyf jaar gelewer en isolaat LMS 6 wat tans gebruik word vir soetlemoen en mandarin, lyk nog steeds die beste. Navorsing oor sitruskroei het getoon dat bome op onderstamme soos C35 citrange, Empress mandaryn, en Carrizo citrange meeste deur die siekte aangetas is, terwyl bome op X639, Sun Chu Sha en Sunki onderstamme wortelstokke die verdraagsaamste was. Vergroening verg meer aandag en indien onlangse klimaatsveranderinge blywend is, kan dit versprei na nuwe gebiede. Die aanstelling van Prof Gerhard Pietersen in CRI by die Universiteit van Pretoria het tot die ontwikkeling van PCR-metodes gelei vir die opsporing van Suid-Afrikaanse vergroening sowel as Oosterse en Amerikaanse vergroening. Toetsing van talle nuwe en ou mandarinkultivars het getoon dat slegs Primasol, Cami, M26, Fremont en Bay Gold weerstandbiedendheid het teen *Alternaria*-bruinvlek. Die vreemde siekte wat Clementines in die Knysna-gebiede aangetas het, is suksesvol identifiseer as *Phytophthora citrophthora*. Finale resultate met die toetsing van onderstamme vir weerstand teen die sitrusaalwurm, het getoon dat C35, Swingle en C32 citrange aanbeveel kan word as herplantonderstamme. Skoparoon in wortels was nie gekorrelleer met verdraagsaamheid teenoor *P. nicotianae* nie, maar verhoging in oplosbare fenoliese konsentrasies en die ophoping van spesifieke fenoliese verbindings soos U82 in verdraagsame onderstamme dui op 'n biochemiese meganismes van weerstand.

Die PKR (DNS) metode om sitruswartvlek (SSV) op dooie blare en in die grond aan te dui, is verfyn en word suksesvol gebruik. 'n Nuwe inokulummonitor is ontwikkel en word gebruik om potensiële askosporuitbrake aan te dui. Alhoewel die voorkoms van SSV in die veld laag was in 2004, is goeie vordering gemaak met die breking van die lewensiklus en verbetering van beheerstrategieë. Maniere is gevind om die stippeling wat geassosieer word met koperbespuitings te voorkom en die kombinasie van Sporekill met mankoseb en koper lyk of dit beheer verbeter. Na-oes navorsing op nuwe formulasies van imazalil en tiabendazool mag lei tot nuwe registrasies. Die kwaternêre ammonium en organiese verbindings

wat getoets is, het swak resultate gelewer. Toetsing van monsters van *Penicillium*-spore van die Oos-Kaap het 'n mate van bestandheid teenoor imazalil aangedui en verdere navorsing oor imazalil-bestandheid sal gevolglik gedoen word.

4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project Co-ordinator: S.P. van Vuuren (CRI)

4.2.1 Projekopsomming

Vordering in navorsing help om siektes beter te verstaan en daardeur word die toepassing van beheermaatreëls bevorder. Vinnige, sensitiewe, akkurate diagnose en identifikasie van patogene is onderliggend tot hul suksesvolle bestuur, beheer en navorsing op die patogene. Aangesien *Sitrus tristeza virus* (STV) endemies is in suider Afrika as gevolg van die oorfloed van plantluis vektore, is ligte ras kruisbeskerming die enigste beheermaatreël. Die langtermyn waardebeplanning van nuwe verbeterde kruisbeskermings-isolate is 'n beperking wat optimale beheermaatreëls vertraag om maksimum produksie te verkry. Pomelos vorm 'n belangrike deel van die suider Afrikaanse sitrus bedryf, maar die produksie en vruggrootte word grootliks beïnvloed deur STV. Daar is ook bewys dat soetlemoene en mandaryne baat vind by kruisbeskerming waar produksie en vruggrootte verbeter word. Die soeke na beter kruisbeskermings-isolate is daarom 'n voortdurende aksie. Die her-indeksering van moederbronne by die Sitrus Grondvesblok (SGB) te Uitenhage om die virulensie van kruisbeskermings-isolate te monitor is tydrowend, gegrond op biologiese reaksie van indikatore en raak jaarliks meer omvangryk. Voorvoeders wat in getru-transkribeerde "nested" polimerase kettingreaksie gebruik kan word om tussen virulente en avirulente rasse te onderskei is plaaslik ontwikkel en kan met sukses toegepas word. Met die nodige aanpassings kan die tegniek gebruik word om moederbome by die SGB wat virulente rasse huisves, te identifiseer sodat hulle vermy kan word as enthout bronne (afdeling 4.2.2). 'n Virusvrye genebron is gevestig waar 213 kultivars en seleksies gevestig is. Nuwe plantmateriaal wat gevrywaar is van entoordraagbare siektes d.m.v. groeipunt enting sal jaarliks by die versameling gevoeg word (afdeling 4.2.3).

Marsh en Star Ruby pomelo boompies is met vier van die beste Beltsville sub-isolate geïmmuniseer en word vergelyk met die huidige twee isolate wat gebruik word vir preïmmunisasie. Die Marsh boompies is by Riversbend in die Nkwaleni Vallei geplant en die Star Ruby boompies by Tambuti Landgoed in Swaziland. Die boompies is nou 'n jaar oud en daar is aanduidings dat van die sub-isolate groei strem (afdeling 4.2.4). Die virulensie van 'n STV isolaat word hoofsaaklik bepaal deur die teenwoordigheid van strawwe rasse, die gasheer en omgewingsfaktore. Daarom is dit belangrik om isolate in die verskeidenheid van klimaatstreke waar pomelos verbou word te evalueer. 'n Proef is gevestig in die Oranje Rivier Vallei waar verskillende STV isolate in Star Ruby by die hoë droë somer temperature en lae winter temperature ge-evalueer sal word (afdeling 4.2.5). Na die glashuis evaluering van 'n groot aantal STV isolate wat in verskillende pomelo gebiede vanaf voortreflike bome versamel is, is vier isolate geïdentifiseer wat moontlik goeie kruisbeskermings-eienskappe besit. Die vier isolate kom toevallig uit vier verskillende pomelo gebiede nl. Nkwaleni vallei, Swaziland, Hoedspruit en Tshipise. Bome word voorberei om hulle verder onder boord toestande te evalueer en te vergelyk met ander isolate en sub-isolate (afdeling 4.2.6). In die proef waar verskillende STV isolate as kruisbeskermings-agente vir Star Ruby geëvalueer word, het bome met isolate GFMS 35 en GFMS 78 die beste produksie oor 'n periode van vyf jaar gelewer. Bome met hierdie twee isolate het betekenisvol beter geproduseer as bome wat virusvry geplant is, die wat met GFMS 12 (Nartia) en GFMS 67 geïmmuniseer is en die met die twee strawwe isolate. Die resultate toon dat STV isolaat GFMS 35, wat die huidige kruisbeskermings-isolaat vir rooi pomelos is, tesame met GFMS 78, superieur was bo die ander isolate. Die bome met die derde beste isolaat het 12% swakker presteer. Die doel van die proef is bereik, maar, dit sal voordelig wees om te sien tot hoe 'n mate boom leeftyd en ekonomiese produksie verleng sal word (afdeling 4.2.7). Met die evaluasie van STV isolate vir Star Ruby pomelo in twee klimatologiese gebiede, Malelane en Nelspruit, word die volgende gevolgtrekkings gemaak: 1. Die natuurlike druk van STV was strawwer in Malelane as in Nelspruit. Die bome wat virusvry geplant is in die twee gebiede, toon meer groei beperking, strawwer stamgleuf simptome en 'n laer produksie in Malelane. 2. Algemene stamgleuf ontwikkeling was strawwer op Malelane. Dit is 'n teenstrydige resultaat aangesien STV gewoonlik strawwer simptome ontwikkel by koeler temperature. 3. Behalwe vir bome wat met GFMS 35 geïmmuniseer was, was die reaksie van STV isolate in die twee gebiede dieselfde ten opsigte van groei, produksie en gesondheid. Die swak vertoning van bome op Malelane wat met GFMS 35 isolaat geïmmuniseer was is onverwags en kan nie verduidelik word nie. 4. Oor die algemeen het bome wat met LMS 6 (die huidige kruisbeskermings-isolaat vir soetlemoen en mandaryne) geïmmuniseer was, die beste resultate gelewer in beide gebiede. Die doel van die proef is bereik en sal gesluit word (afdeling 4.2.8). Ses-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red, het baie eenvormig gereageer met vier STV isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Daar is aanduidings van interaksies tussen sommige pomelo seleksies en die STV isolate. Betekenisvolle afnames in boom grootte het voorgekom by sekere

seleksie/isolaat kombinasies. Die kruisbeskermings-eienskappe van elk van die vier isolate vir die verskillende pomelo seleksies sal met tyd bekend word (afdeling 4.2.9). Die twee sub-isolate wat geïdentifiseer is om 'n superieure isolaat vir pomelos saam te stel word verder onder glashuis toestande geëvalueer. Hulle word ook in drie ander proewe (sien afdelings 4.2.4, 4.2.5 en 4.2.6) as afsonderlike sub-isolate in verskeie pomelo gebiede vergelyk met ander isolate en sub-isolate (afdeling 4.2.10).

Dit is reeds genoem dat kruisbeskerming voordelig is by die sogenaamde tolerante sitrus kultivars. Studies om geskikte STV isolate vir kruisbeskerming by die sitrusgroep te identifiseer, gaan voort. Vruggroottes is 'n groot probleem by Clementines in die Oos – en Wes Kaap en daar was 'n aanvraag om die invloed van kruisbeskerming op Clementines te bepaal. In 'n vorige Clementine proef is gevind dat die regte STV isolaat die oeswaarde (vruggroottes gekoppel aan markpryse) tot soveel as 40% kan verhoog (sien CRI Groep Navorsings-verslag van 2003, pp 249-254). Nietemin, 'n proef is by Addo Navorsingstasie gevestig waar virusvrye en gepreïmmuniseerde bome van sewe Clementine seleksies en 'n satsuma seleksie vergelyk word. Die proef is nou 'n jaar oud (afdeling 4.2.11). Verskillende STV isolate word in Palmer nawel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Die vyf-jaar oue bome met LMS 6 en SM 45 isolate was betekenisvol groter as bome wat met isolate SM 36 en die met die bekende strawwe isolaat geïnkuleer was. Die onderstamme het tans geen effek op boom grootte nie. Tans presteer bome met LMS 6 isolaat, die huidige kruisbeskermings-agent, die beste (afdeling 4.2.12). In 2003 se verslag is gerapporteer dat Turkey Valencia blykbaar meer gevoelig is vir STV as ander Valencia seleksies. 'n Proef om 'n geskikte STV isolaat te identifiseer vir hierdie seleksie word voorberei en is nog in die glashuis stadium (afdeling 4.2.13). Die effek van verskillende STV isolate word op drie Valencia bostamme (McClean, McClean Saadloos en Delta) geëvalueer. Die bome is nou vier jaar oud en van die bostamme het McClean saadloos betekenisvol beter geproduseer as McClean en Delta bome (44% en 36% respektiewelik). Bome wat gepreïmmuniseer is met SM 49 was betekenisvol groter en het die beste geproduseer. Dit blyk dat daar interaksies is tussen bostamme en STV isolate (afdeling 4.2.14). 'n Proef word voorberei om verskillende belowende STV isolate wat vanaf soetlemoen versamel is in verskillende kommersiële Valencia bostamme in die Oranje Rivier Vallei te evalueer. Die boompies is voorberei en die STV isolate word tans geïnkuleer (afdeling 4.2.15).

Die inokulasie van sitruskroei in Delta Valencia bome op 17 verskillende onderstamme induseer 'n afname in boom grootte en produksie in vergelyking met ongeïnkuleerde bome. Serologiese analises van die 12-kD proteïene wat slegs in sitruskroei besmette bome voorkom, is gebruik om bome te identifiseer wat met sitruskroei besmet is. Uitslae bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Die resultate van die verskillende toetse komplimenteer mekaar nie en maak die interpretasie van data moeilik. Sommige van die ongeïnkuleerde bome begin simptome van sitruskroei toon as gevolg van natuurlike besmetting. Onderstamme soos C35 citrange, Empress mandaryn en Carrizo citrange is die meeste ge-afgete deur sitruskroei terwyl bome op X639, Sun Chu Sha en Sunki mandaryn die meeste toleransie vertoon (afdeling 4.2.16).

Vergroeningsiekte bly 'n belangrike probleem in Suid Afrika en is 'n beperkende faktor in sekere produksie gebiede. Vinnige en akkurate opsporing is belangrik vir die beheer van die siekte. 'n Polimerase kettingreaksie tegniek is ontwikkel wat nie net die Afrika vergroening kan identifiseer nie maar ook die Asiatiese en die nuut ontdekte Amerikaanse tipes (afdeling 4.2.2). In 'n proef om die effek van snoei op die besmetting van vergroeningsiekte te bepaal is gevind dat snoei nie net die produksie met 30% verlaag het nie maar ook die persentasie vergroende vrugte met 169% verhoog het in vergelyking met ongesnoeide bome. 'n Tristeza isolaat het die persentasie vergroening by snoei verminder (van 169% na 79%). Dit is belangrik dat snoei nie die normale groei stuwings versteur nie sodat beheer van die vektor doeltreffend gehou kan word (afdeling 4.2.17).

Sitrus viroïed (SVd) isolate is ge-evalueer as verdwergings-agente en is vergelyk met die standaard preïmmuniserings STV isolaat (LMS 6). Die isolate is geïnkuleer in virusvrye Midnight Valencia op 13 verskillende onderstamme. Nege van die onderstamme was *Poncirus trifoliata* seleksies (vyf groot blom tipes en vier klein blom tipes) terwyl vier, *P. trifoliata* kruisings was. Na agt jaar in die boord het die SVd isolate minder uitwerking op bome met die trifoliaat kruising-onderstamme gehad as op bome met die *P. trifoliata* onderstamme ten opsigte van groei, produksie en "Gum Pocket" siekte ontwikkeling. Bome met die STV preïmmuniserings-isolaat was betekenisvol groter en 'n betekenisvolle hoër produksie gehad as die met die SVd isolate. Tesame met die trifoliaat kruisings, het Rubidoux *P. trifoliata* die meeste toleransie teen "Gum Pocket" siekte getoon (afdeling 4.2.18).

Project summary

Progress in *Citrus tristeza virus* (CTV) research enables a better understanding of the disease and therefore assists with the application of control measures. Quick, sensitive, accurate diagnosis and identification of pathogens are essential for successful management, control and research on the pathogens. Since CTV is endemic in southern Africa because of the abundance of aphids, the only control measure for the disease is mild strain cross-protection. The long-term assessment of new better cross-protecting isolates is a restriction that delays optimal control measures to obtain maximum production. Grapefruit forms an important part of the southern African citrus industry, however, production and fruit size are greatly affected by CTV. It was also proved that cross-protection is beneficial to the production of sweet oranges and mandarins. The search for better cross-protecting isolates is therefore a continuous process. Re-indexing of mother trees at the Citrus Foundation Block (CFB) at Uitenhage to assess the virulence of the CTV cross-protecting isolates is time consuming, based on the biological reaction of indicators and becomes more extensive every year. Primers that can discriminate between virulent and avirulent strains using reverse transcription nested polymerase chain reaction, were developed locally and can be used successfully. With the necessary adjustments of the technique, virulent strains that are harboured in mother trees at the CFB can be detected and the trees avoided for budwood supply (section 4.2.2). A virus-free gene bank was established with 213 cultivars and selections. New plant material that has been cleared of graft transmissible diseases through shoot-tip grafting shall be added to the collection every year (4.2.3).

Marsh and Star Ruby grapefruit trees were pre-immunized with four of the best Beltsville sub-isolates and will be compared with the present pre-immunizing isolates. The Marsh trees were planted at Riversbend in the Nkwaleni Valley and the Star Ruby trees at Tambuti Estates in Swaziland. The trees are now a year old and there are indications that some of the sub-isolates retard growth (section 4.2.4). The virulence of a CTV isolate is mainly determined by the presence of severe strains, the host and environmental conditions. Therefore, it is important to evaluate isolates in the diversity of climatic areas of grapefruit production. A trial has been established in the Orange River Valley where different CTV isolates will be evaluated in Star Ruby at the extreme temperatures of the area (section 4.2.5). After the glasshouse evaluation of a large number of CTV isolates that were collected from outstanding trees in the different production areas, four isolates were identified that may have good cross-protecting characteristics. The four isolates were derived from different areas namely, the Nkwaleni Valley, Swaziland, Hoedspruit and Tshipise. Trees are being prepared to evaluate these isolates in an orchard situation and compare them with other isolates and sub-isolates (section 4.2.6). In the trial where different new CTV isolates are evaluated as cross-protectors for Star Ruby grapefruit, trees pre-immunised with GFMS 35 and GFMS 78 gave the best production over a five-year period. These trees produced significantly better than trees that were planted virus-free, trees with mild isolates GFMS 12, GFMS 67 and those with the two severe isolates. The results show that CTV isolate GFMS 35, the present pre-immunizing isolate for red grapefruit, together with GFMS 78, were superior to the other isolates. The isolate that was third best was 12% poorer. The objective of the trial has been achieved, but it will be beneficial to observe to what extent tree life and economic production will be extended (section 4.2.7). With the evaluation of CTV isolates at two climatic areas, Malelane and Nelspruit, the following conclusions are made: 1. The natural pressure of CTV was more severe at Malelane than at Nelspruit. The control trees that were planted virus-free at the two sites show more stunting, a lower yield and more severe stem pitting at Malelane. 2. General stem pitting development was more severe at Malelane. This is a contradicting result since CTV is usually more severe in cooler climates. 3. Except for trees that were pre-immunized with GFMS 35, the reactions of the CTV isolates were similar in the two areas regarding growth, yield and health. The poor performance of trees with GFMS 35 at Malelane was unexpected and cannot be explained. 4. Generally, trees pre-immunized with LMS 6 (present pre-immunizing isolate for sweet orange and mandarin) gave the best results in both areas. The objective of the trial was achieved and it will be terminated (section 4.2.8). Six-year-old trees of seven red grapefruit selections (Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben, Oran Red) reacted very similarly to four mild CTV isolates (GFMS 12, GFMS 35, GFMS 67, GFMS 73). There are indications of interactions between some isolates and grapefruit selections. Significant reductions in tree sizes occurred where some selection/isolate combinations were used. The cross-protection abilities of each of the isolates in the different selections will be revealed over time (section 4.2.9). The two sub-isolates that were identified for construction of a superior isolate for grapefruit are still in a process of evaluation in the glasshouse. They are also evaluated in three field trials (see sections 4.2.4, 4.2.5, 4.2.6) as separate sub-isolates in different grapefruit areas and they are compared to other isolates and sub-isolates (section 4.2.10).

It was already mentioned that cross-protection has beneficial effects on the so-called tolerant citrus cultivars. Studies to identify suitable isolates for cross-protection are continuing. The lack of good fruit size is a big problem with Clementines in the Eastern and Western Cape and a request was made to evaluate the effect of pre-immunisation on the fruit size of Clementine. In a previous Clementine trial it was found that the crop value (fruit size coupled with market prizes) was increased by 40% in pre-immunised trees (see CRI Group

Research Report of 2003, pp 249-254). However, a trial was established at Addo Research Station to compare virus-free and pre-immunised trees of seven Clementine selections and a satsuma selection (section 4.2.11). Results on the evaluation of different CTV isolates in Palmer navel on four commercial rootstocks (Rough lemon, Troyer citrange, Swingle citrumelo, C35 citrange) for the Eastern Cape showed that trees with isolates LMS 6 and SM 45 were significantly larger than the other treatments after four years. The rootstocks have no influence on tree size at this stage. Presently trees with LMS 6 produces the best (section 4.2.12). In the 2003 annual report it was shown that Turkey Valencia is more sensitive to CTV than the other Valencia selections. A trial to identify a suitable isolate to pre-immunise this selection has been initiated and is still in the glasshouse stage where the different isolates are being inoculated (section 4.2.13). The effects of CTV on three Valencia scions (McClellan -, McClellan Seedless – and Delta Valencia) are evaluated at Malelane. The trees are now four years old and the McClellan Seedless Valencia yielded significantly better than McClellan and Delta Valencia trees (44% and 36% respectively). Trees with isolate SM 49 were the largest and produced the best. There appear to be interactions between the scions and CTV isolates (section 4.2.14). A trial is being prepared to evaluate different CTV isolates, which were derived from sweet orange, in commercial Valencia scions. The trees are currently inoculated by the isolates and will be planted in the Orange River Valley (section 4.2.15).

The inoculation of citrus Blight (CB) to Delta Valencia trees on 17 different rootstocks induced a decrease in tree size and production in comparison with un-inoculated trees. Serological analysis of the 12-kd protein that only occurs in CB-affected trees was used to identify infected trees. Results confirmed the visual symptoms in the orchard but also identified early infections where no symptoms could be observed. The results of the different test do not compliment each other and that makes the interpretation of the results difficult. Some of the uninoculated trees are starting to show symptoms of CB due to natural infection. Rootstocks such as C 35 citrange, Empress mandarin and Carrizo citrange were the most affected by CB while trees on X639, Sun Chu Sha and Sunki rootstocks show the most tolerance (section 4.2.16).

Greening disease remains an important disease in South Africa and is a limiting factor in some production areas. Quick and reliable detection is important for proper control of the disease. A polymerase chain reaction technique was established at Pretoria University that will detect African greening as well as Asian and the newly discovered American types (section 4.2.2). In a trial to establish the effect of pruning on greening infection, it was found that pruning not only reduced production by 30% but also increased the percentage greening infected fruit by 169%, in comparison with the control trees. A tristeza isolate reduced the percentage greening in pruned trees (from 169% to 79%). It is important that pruning does not disturb the normal flush rhythms so that vector control can be kept optimal (section 4.2.17).

Citrus viroid (CVd) isolates were evaluated as dwarfing agents and compared to the standard pre-immunizing CTV isolate (LMS 6). The isolates were inoculated into virus-free Midnight Valencia on 13 different rootstocks. Nine of the rootstocks were *Poncirus trifoliata* selections (five large flower types and four small flower types), while four were *P. trifoliata* hybrids. After eight years in the field, the CVd isolates had less effect on the hybrid rootstocks than on the *P. trifoliata* rootstocks regarding growth, yield and the development of Gumpocket disease. Trees with the CTV pre-immunizing isolate were significantly larger and had a higher yield than the trees with the CVd isolates. Together with the trifoliata hybrids, Rubidoux *P. trifoliata* showed the most tolerance to Gumpocket disease (section 4.2.18).

4.2.2 Research by new Virologist

By G. Pietersen (CRI at UP)

Opsomming

Vinnige, sensitiewe, akkurate diagnose en identifikasie van patogene is onderliggend tot hul suksesvolle bestuur, beheer en navorsing op hulle. Die doel van hierdie inleidende proef is om polimerase ketting reaksie (PKR) tegnieke te vestig teen die veroorsakende organismes van die twee belangrikste sitrussiektes in Suid-Afrika, naamlik sitrus tristeza en vergroening. Voorvoeders, ontwerp deur Sambade *et al.* (2003) is lokaal vervaardig en amplifikasie reagens en kondisies is aangepas vir lokale gebruik. Hierdie voorvoeders het die vermoë om Spaanse ligte en strawwe rasse van *Sitrus tristezavirus* (CTV) te kan opspoor en onderskei in 'n getru-transkribeerde "nested" PKR (RT-N-PCR). Dié PKR het getoon dat beide strawwe- sowel as ligte CTV rasse in die kruisbeskermende bronne GFMS 12 en GFMS 35 voorkom. Drie voervoeder stelle is plaaslik vervaardig en PKR'e gevestig om *Candidatus Liberibacter* sp. op te spoor. Hierdie bakterieë is die veroorsakende organismes van Huanglongbing (vergroening) siekte van sitrus. Twee van die PKR sisteme, onderskeidelik deur Harakava *et al.* (2000) en Hocquellet *et al.* (1999) ontwikkel, kan beide *L. asiaticus* (Asiatiese vergroening) en *L. africanus* (Afrika vergroening) sekwensie amplifiseer, weliswaar van verskillende dele van die genoom van die bakterieë. Die twee bakterieë word van mekaar onderskei deur restriksie ensiem vertering in die eerste geval of deur verskillende grootte amplikone in die tweede geval. *L.*

africanus se opsporing met altwee sisteme is bewys, maar die opsporing van *L. asiaticus* kon nie gedemonstreer word nie weens 'n gebrek aan 'n positiewe kontrole vir dié organisme. *L. africanus* is in monsters vanaf Nelspruit en Rustenburg gevind m.b.v. die PCR sisteme. Om die nut ontdekte *L. americanus* op te spoor, is die ongepubliseerde voorvoerders G1 en G3 van Bove (IOCV, 2004) verkry en die PCR gevestig teen *L. americanus* DNA verkry vanaf Fundecitrus, Brasilië. Vermeerdering van 'n 1027bp band is hiermee verkry. Hierdie proef se doelwitte is dus bereik deurdat beide CTV en *L. africanus* nou opgespoor kan word. Verder kan daar tussen verskeie CTV ligte en strawwe rasse onderskei word, *L. americanus* kan opgespoor word, en teoreties ook *L. asiaticus*.

Introduction

Rapid, sensitive and accurate diagnosis and identification of pathogens is fundamental to their management and control, whether by phytosanitary legislation, certification schemes, vector control, resistance selection and breeding, or cultivation practices. Development of such techniques is also an essential first-step in most research actions involving such pathogens. Because of a loss of virologists over the past few years the local citrus industry has a lack of such techniques ready for immediate use for the majority of graft-transmissible pathogens. For *Citrus tristeza virus* (CTV), the most important virus of citrus, ELISA's are performed with imported ELISA kits at high cost/test at CRI and ARC-ITSC, limiting the number of tests which can be performed within research projects, the certification scheme and routine diagnostic services. The capacity to detect CTV by PCR at ARC-ITSC was lost with the resignation of Mr. Luttig and cannot be considered to be available locally currently. PCR to the next important pathogen, *Liberibacter africanus*, the cause of African greening, was developed in the laboratories of Bové, France, established by the group of Prof. Lize Korsten, UP, and accredited for routine use a couple of years ago. However, this service has subsequently been discontinued, and is no longer available to the industry. The same test, also previously conducted at the University of Stellenbosch is also no longer available for routine use. The aim of this introductory experiment was therefore to establish a diagnostic capability (ELISA- or PCR-based) locally to detect the two most important major graft-transmissible pathogens of citrus, namely CTV and greening, and in the case of CTV also to be able to differentiate between mild and severe strains of the virus. Both researchers involved in this study are based at the University of Pretoria (UP). Prof. Gerhard Pietersen, employed by CRI since April, 2004, has been seconded to UP, where he mentored the CTV component of the experiment for an Honours student, Ms. Katherine Stewart, a CRI Prestigious Bursary holder.

Materials and Methods

Citrus tristeza virus:

One seedling each of CTV mild strain cross-protected (GFMS 12 & GFMS 35) Mexican limes were obtained from Mr. J.H.J. Breytenbach, CRI. Plants were maintained in a glasshouse at UP for the duration of the experiment.

CTV presence in samples was confirmed by Triple antibody sandwich (TAS) ELISA using sero reagents supplied by Prof. Richard Lee (USDA ARS National Clonal Germplasm Repository for Citrus, Riverside, California), and following the protocol of the supplier. These results were further supported by Immunoelectron microscopy (IEM) using the same sero reagents, and were performed by Mr. G. G. F. Kasdorf at ARC-Plant Protection Research Institute (ARC-PPRI).

Double stranded RNA (dsRNA) extraction was performed using the method of Dodds *et al.* (1987) as modified by Moreno *et al.* (1990). Total RNA extracts were prepared using the SV Total RNA Isolation Kit (Promega, Wisconsin, USA).

Complementary DNA (cDNA) was synthesized from total RNA extracts using PM51 viral p23 sequence specific primer (Sambade *et al.*, 2003) with M-MLV Reverse transcriptase, (USB).

Nested RT-PCR was performed using primer sets designed by Sambade *et al.* (2003) for the differentiation of CTV isolates into 3 groups (mild, severe, and atypical). PCR conditions however were modified. The first round PCR was performed in a 50 µl reaction mixture containing 200ng cDNA, 0.1% Triton-X-100, 10 µg BSA, 10mM 2-Mercaptoethanol, 2mM MgCl₂, 200µM of each dNTP, 500µM of each primer of a set, NH₄ buffer (Bioline) (16mM (NH₄)₂SO₄, 67mM Tris-HCl, pH 8.8, 0.1% Tween 20) and 2.5 U of *Taq* DNA polymerase (Bioline). Amplification was achieved under the following conditions: 5 min at 94°C, 40 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C with a final extension of 2 min at 72°C. During the second round 2 µl of 1st round product was utilized as template. The reagents used were the same as in the first round and cycling conditions were the same except that the annealing temperature was 60°C. The amplified product was detected by horizontal agarose electrophoresis followed by ethidium bromide staining using standard molecular techniques (Sambrook *et al.*, 1989).

The amplified product was purified from an electrophoresis gel by using a Wizard SV PCR purification kit (Promega, Wisconsin, USA) and the specificity of the PCR system evaluated by cycle sequencing of the amplified product using the Big Dye sequencing kit (ABI). The product from this reaction was sequenced at the sequencing facility of the Biochemistry department, UP.

Candidatus Liberibacter africanus

Source plants and pathogens: Valencia orange trees displaying typical greening symptoms were collected from the Nelspruit area by Dr. H.F. le Roux, CRI. *Candidatus Liberibacter americanus* primer GB1/GB3- (Bové, IOCV, 2004) amplified product was obtained, with thanks, from W. Cintra de Jesus Junior (Fundecitrus, Brazil). Total DNA was purified from plants using the CTAB (cetyl trimethyl ammonium bromide) method of Doyle and Doyle (1990) and used as templates in PCR.

Three PCR systems were established, based on published data. The first system utilizes primers CN 265 and CN 266 (Harakava *et al.*, 2000) and detects *Candidatus Liberibacter asiaticus* and *Candidatus L. africanus*. PCR conditions used for the CN 265/CN 266 system were those described by Jagoueix *et al.* (1996) except that Triton X-100 was used instead of W1 detergent, and the annealing step was done at 58°C. The amplified product was purified from an electrophoresis gel by using a Wizard SV PCR purification kit (Promega, Wisconsin, USA) and the specificity of the PCR system evaluated by cycle sequencing of the amplified product using the Big Dye sequencing kit (ABI). The product from this reaction was sequenced at the sequencing facility of the Biochemistry department, UP.

A second primer set, utilized in PCR was the A2 and J5 set of Hocquellet *et al.* (1999) directed against ribosomal protein genes of the β -operon of *L. asiaticus* and *L. africanus*. In this case the two bacteria can be differentiated by the size of the amplicon, with *L. asiaticus* yielding expected products of 703 bp while *L. africanus* yields expected products of 669 bp. Reagent concentrations were as described by Jagoueix *et al.* (1996) except that Triton X-100 was used instead of W1 detergent. Amplification conditions were as described by Hocquellet *et al.* (1999).

To be able to detect the recently discovered, *L. americanus* specifically, unpublished primers GB1 and GB3 (Bové, IOCV, 2004) were synthesized and used in a PCR using the same reagent concentrations as the other two PCR systems already established. Amplification was by 35 cycles of 92°C for 45 sec, 64°C for 45 sec, and 72°C for 60 sec.

Results and discussion

Citrus tristeza virus

Mild strain cross protecting isolates GFMS 12 and GFMS 35 were shown to contain high titres of CTV particles by ELISA (Table 4.2.2.1) and IEM (not shown). DsRNA and Total RNA extractions were successfully prepared from both sources, but Total RNA extracts proved more suitable as templates for PCR.

Table 4.2.2.1. The ELISA recorded absorbance averages and standard deviations of the triplicate wells.

Description of wells	Average reading		Standard deviation	
	30 min	60 min	30 min	60 min
Sample 1 – GFMS 12	0.994	1.786	0.057	0.089
Sample 2 – GFMS 35	0.969	1.741	0.031	0.053
Positive control T36	0.658	1.191	0.067	0.094
Healthy plant (R. Lee)	0.086	0.130	0.003	0.005
Virus-free plant (UP)	0.085	0.131	0.001	0.002
Uncoated control	0.099	0.108	0.009	0.050
Buffer control	0.073	0.162	0.053	0.047

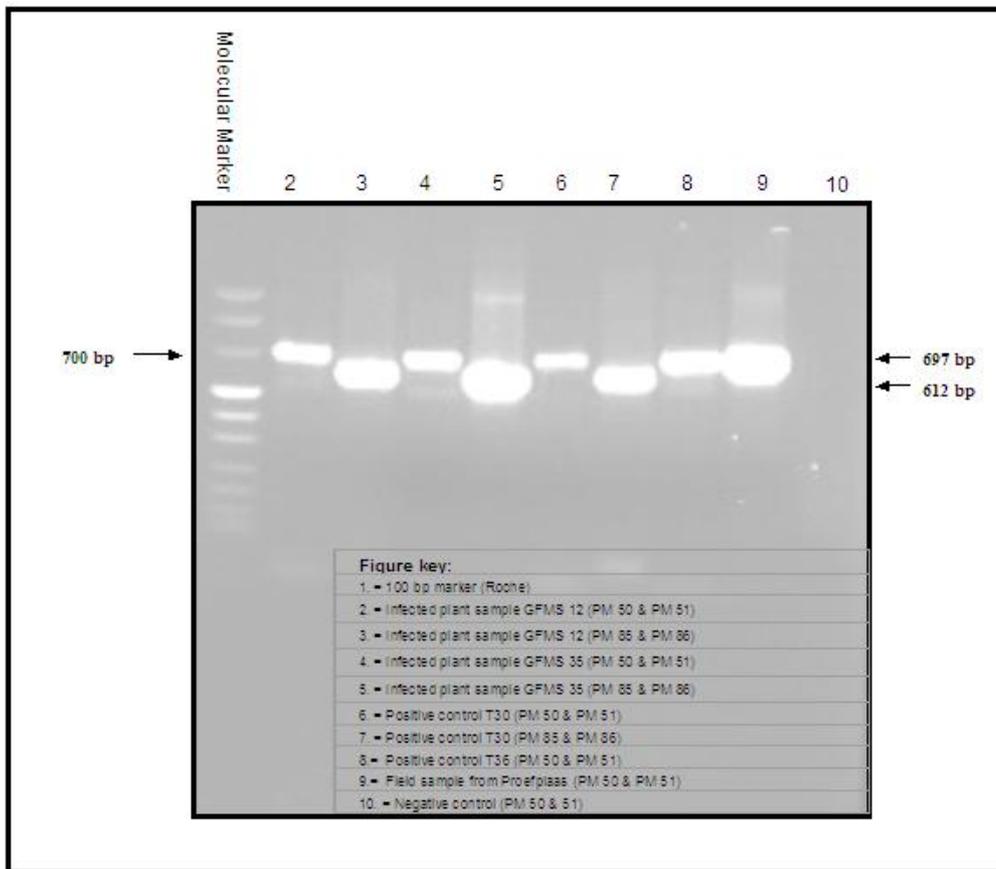


Figure 4.2.2.1. Amplification of the conserved regions of two pre-immunising isolates (GFMS 12 and GFMS 35) of the p23 gene of CTV using primer sets PM50 and PM51 and PM85 and PM88 (Sambade *et al.*, 2003).

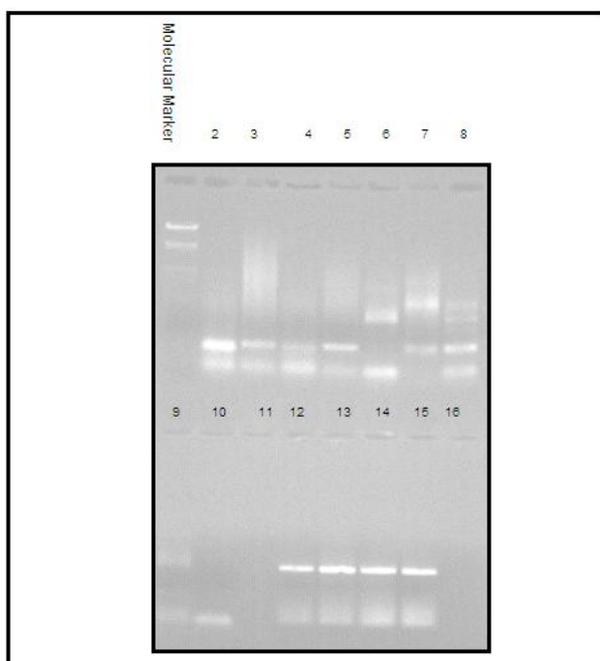


Figure 4.2.2.2. Amplification of CTV p23 gene sequences using internal primers PM82 and PM83 or PM82 and PM84 to differentiate mild and severe forms of CTV (Sambade *et al.*, 2003). 1 = 100bp marker (Roche), 2 = Infected plant sample GFMS 12 (PM82 & PM83) 3 = Infected plant sample GFMS 12 (PM82 & PM84), 4 = Infected plant sample GFMS 35 (PM82 & PM83), 5 = Infected plant sample GFMS 35 (PM82 & PM84), 6 = Positive control T30 (mild) (PM82 & PM83), 7 = Positive control T30 (mild) (PM82 & PM84), 8 = Positive control T36 (severe) (PM82 & PM83), 9 = Positive control T36 (severe) (PM82 & PM84), 10 = Negative control (PM82 & PM83)

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ATGGATGATACTATCGGACAACTTTCGTTTCTGTGAACCTTTCTGACGAAAGCAACACGGCAAGCACTAAAGTT
GAAAACGTAAAATCGGAAGCGGATCGCTTGAATTTTTACGTAAAATTAATCCCTTTATTGTTGACGCTCTGGTGC
GGAAAACCAATTATCAGGGTGCTCGCTTTCGCGCAAGAATAATAGGAGTGTGCGTGGATTGTGGTAGAAAACAC
GACAAGGCGCTCAAGACTGAACGTAAGTGAAGGTCAACAATACGCAATCTCAGAACGAGGTGGCGCATATGTT
GATGCACGATCCCGTTAAGTATTTGAACAAAAGAAAGGCTAGAGCCTTTTCTAACGCAGAGATGTTTGCGATTGA
ATTGGTTTTGTACCCAAGGAAAGGCAATTGGCGGTGATTTAGCCGCTGAAAGGGAGAAGACGAGACTGGCTC
GTAGACACCCAATACGTTCTCCGGAAGAACTCCGGAACATTATAAATTCGGTATGACTGCTAAGGCAATGTTAC
CGGACATCAACGCCGTAGACGTTGGTGATAACGAGGAACTTCGTCGGAGTACCCAGTGAGTCTGAGTGTTTCT
GGCGGAGTTCTCCGTGAACACCACTTCATCTGATT

```

Figure 4.2.2.3. The 631 bp Nucleotide sequence of the p23 gene of CTV from sample GFMS 12 using primers PM85-PM86. Highest level of identity (93%) recorded in pair-wise comparison with CTV p23 gene (GENBANK AJ579762) of a mild isolate from Spain.

Candidatus Liberibacter africanus

PCR systems targeting the three *Liberibacter* species currently known were successfully established.

The first system utilizes primers CN265 and CN266 (Harakava *et al.*, 2000) and targets the 16s ribosomal DNA of *Candidatus L. asiaticus* and *Candidatus L. africanus*. Single amplification product bands of c.448 bp were obtained with the total DNA extracts of the two Nelspruit sources (Figure 4.2.2.4). It is possible to differentiate *L. asiaticus* and *L. africanus* by restriction (*Xba*I) enzyme digestion of the 448 bp amplicon in a step subsequent to PCR amplification. As no *L. asiaticus* DNA was available at this stage, this could not be assessed. The PCR product obtained was sequenced, BLASTED against Genbank sequences and the data aligned to cogent regions of the two most identical archived sequences, that of *L. africanus* (Nelspruit) and that of *L. africanus* sp. *capensis* sequence to confirm the specificity of amplification (Figures 4.2.2.5 and 4.2.2.6).

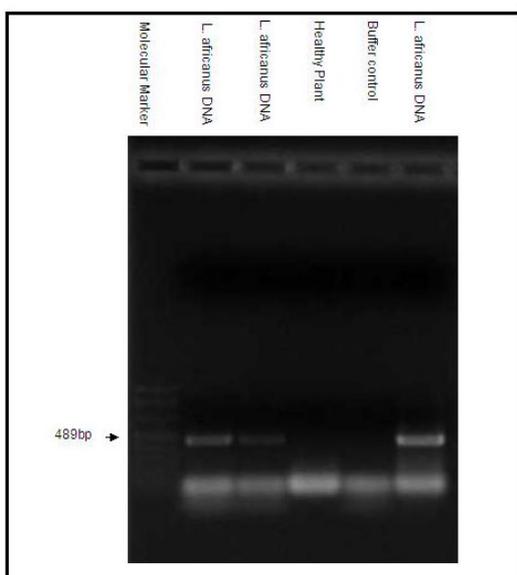


Figure 4.2.2.4. Agarose gel electrophoresis of the DNA amplified with primers CN265 and CN266 (Harakava *et al.*, 2000) for *L. asiaticus* or *L. africanus*. Molecular marker (very faint): Marker VIII (Roche).

Liberibacter africanus 16S ribosomal RNA gene, partial sequence,
Length = 1454, Score = 529 bits (267), Expect = e-147 Identities = 321/344 (93%), Gaps = 1/344 (0%) Strand = Plus / Plus

Query: 51 caagattaaactcanaggaattgacggggggccgcacaagcggtggagcatgtggtta 110
|||||
Sbjct: 813 caagattaaactcaaaggaattgncggggggccgcacaagc-gtggagcatgtggtta 871

Query: 111 attcgatgcaacgcgcanaacctnccagccctgacatatgttgacgatatcagagat 170
|||||
Sbjct: 872 attcgatgcaacgcgcagaacctaccagccctgacatatgttgacgatatcagagat 931

Query: 171 gatatttctttcngagacttctacaggtgctgcntggctgctgcagctcgtgtnn 230
|||||
Sbjct: 932 gatatttctttcngagacttctacaggtgctgcntggctgctgcagctcgtgctg 991

Query: 231 ngagatgtgggtaagtcccgaacnagcgcaaccctacctctagtggccatcnagnt 290
|||||
Sbjct: 992 tgagatgtgggtaagtcccgaacnagcgcaaccctacctctagtggccatcaagt 1051

Query: 291 nagatttatctagatgttgggtactttatagggactgccgntgataagccnngaagg 350
|||||
Sbjct: 1052 tagatttatctagatgttgggtactttatagggactgccgntgataagccggaggaagg 1111

Query: 351 tggggatgacntctagtcctcatggcccttatgggctgggctac 394
|||||
Sbjct: 1112 tggggatgacntcaagtcctcatggcccttatgggctgggctac 1155

Figure 4.2.2.5. Sequence and pair-wise alignment of amplification product of PCR using CN265 and CN266 (Harakava *et al.*, 2000) for *L. asiaticus* or *L. africanus* using DNA from a Valencia orange sample from Nelspruit vs. the known sequence of *L. africanus* (Nelspruit) (GENBANK L22533.1).

Liberibacter africanus subsp. *capensis* 16S ribosomal RNA gene, partial sequence
Length = 1179 Score = 436 bits (220), Expect = e-119 Identities = 313/344 (90%), Gaps = 7/344 (2%)
Strand = Plus / Plus

Query: 51 caagattaaactcanaggaattgacggggggccgcacaagcggtggagcatgtggtta 110
|||||
Sbjct: 766 caagattaaactcaaaggaattgacgggggn--gcacaagcggtggagcatgtggtta 823

Query: 111 attcgatgcaacgcgcanaacctnccagccctgacatatgttgacgatatcagagat 170
|||||
Sbjct: 824 attcgatgcaacgcgcagaccctaccagctcttgacatatga--gacgatatcagagat 881

Query: 171 gatatttctttcngagacttctacaggtgctgcntggctgctgcagctcgtgtnn 230
|||||
Sbjct: 882 gatatttctttc-gagacttctacaggtgctgcntggctgctgcagctcgtgctg 940

Query: 231 ngagatgtgggtaagtcccgaacnagcgcaaccctacctctagtggccatcnagnt 290
|||||
Sbjct: 941 tgagatgtgggtaagtcc-gcaacgagcgcaaccctacctctagtggccatcaagt 999

Query: 291 nagatttatctagatgttgggtactttatagggactgccgntgataagccnngaagg 350
|||||
Sbjct: 1000 tagatttatctagatgttgggtactttatagggactgccgntgataagccggaggaagg 1059

Query: 351 tggggatgacntctagtcctcatggcccttatgggctgggctac 394
|||||
Sbjct: 1060 tggggatgacntcaagtcctcatcg-cgttatgggctgggctac 1102

Figure 4.2.2.6. Sequence and pair-wise alignment of amplification product of PCR using CN265 and CN266 (Harakava *et al.*, 2000) for *L. asiaticus* or *L. africanus* using DNA from a Valencia orange sample from Nelspruit vs. the known sequence of *L. africanus* sp. *capensis* (GENBANK AF137368).

A second PCR, based on primer set A2 and J5 of Hocquellet et al. (1999) and directed against ribosomal protein genes of the beta-operon of *L. asiaticus* and *L. africanus* was established successfully, with single highly amplified PCR product bands of about 670 bp obtained (Figure 4.2.2.7). In this case the two bacteria can be differentiated by the size of the amplicon, with *L. asiaticus* yielding expected products of 703 bp while *L. africanus* yielding expected products of 669 bp. As no *L. asiaticus* DNA was available at this stage this could not be assessed.

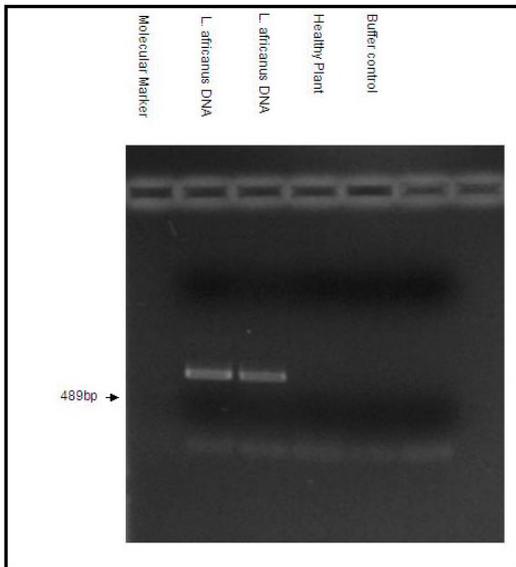


Figure 4.2.2.7. Agarose gel electrophoresis of the DNA amplified with primers A2 and J5 (Hocquellet *et al.*, 1999) for *L. asiaticus* or *L. africanus*. Molecular marker (very faint): Marker VIII (Roche).

L. africanus infections could be demonstrated using the above two PCR systems in citrus samples from Nelspruit and Rustenburg.

To be able to detect the recently discovered, *L. americanus* specifically, PCR using unpublished primers GB1 and GB3 (Bové, IOCV, 2004) was established and tested against DNA obtained from Brazil (Fundecitrus). A highly amplified single PCR product band of the expected size c. 1027 bp was obtained (Figure 4.2.2.8).

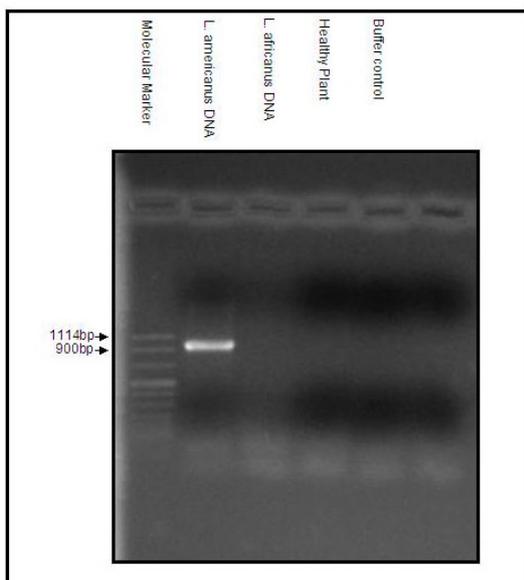


Figure 4.2.2.8. Agarose gel electrophoresis of the DNA amplified with primers GB1 and GB3 (Bové, IOCV, 2004) for *L. americanus*. Molecular marker (very faint): Marker VIII (Roche).

Conclusion

PCR methods to detect citrus tristeza virus (CTV) as well as to differentiate between mild and severe isolates (Sambade *et al.*, 2003) has been established. Using this it was shown that both GFMS 12 and GFMS 35 contain both mild and severe forms of CTV.

PCR to detect *L. asiaticus*, *L. africanus* and *L. americanus* –specific were established. The two PCR systems capable of detecting and differentiating *L. asiaticus* and *L. africanus* could not be tested for their ability to detect *L. asiaticus* as positive controls were not available for this exotic pathogen.

Future research

To address the control of CTV by cross-protection, and to confirm and exploit the probable underlying mechanism of RNA-silencing, it is imperative that information on the variability of this virus be obtained locally. As variable regions between isolates are generally unknown, these areas must be identified first before obtaining sequence information. Micro-array analysis is ideally suited to this. Further research on CTV is therefore aimed at assessing the variability of the local mild-strain cross-protecting CTV isolates used in South Africa using micro-array analysis and genotyping PCRs, to develop molecular markers for the individual strains and to confirm the mechanisms of cross-protection .

Methods must be developed to improve sample preparation for PCR to *Liberibacter* species in order to shorten the test and to allow greater samples to be tested. Sampling methods, taking cognisance of the uneven distribution of the pathogen within trees, must be developed. The ability to differentiate *L. asiaticus* and *L. africanus* in the CN 265/CN266 and A2/J5 PCR systems must be confirmed.

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4.2.3 Citrus virus-free gene source

Experiment 790 by S.P. van Vuuren and J.H.J. Breytenbach (CRI)

Opsomming

Groeipunt enting word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene. Virusvrye boompies van veskillende cultivars en seleksies word in 'n insekvrue tonnel by CRI bewaar. Die virusvrye

bron by die LNR-ITSG is gedupliseer by CRI en 'n totaal van 213 cultivars en seleksies is gevestig. Virusvrye materiaal word gepre-immuniseer met 'n geskikte *Sitrus tristeza virus* isolaat voordat dit aan die Sitrus Grondvesblok te Uitenhage verskaf word.

Introduction

The overall objective of the southern African Citrus Improvement Programme is to enhance the productivity of the industry by making the highest quality propagation material available. Graft transmissible agents have detrimental effects on the growth and production of citrus trees since they are responsible for stunting, decline and small fruit. 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).

Materials and methods

In vitro cultured rootstocks

The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the testa of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile deionized water. Three to four seeds are planted in growth tubes containing sterile MS agar medium (Murashige and Skoog, 1962). Germination takes place at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they can be stored at 4°C in darkness.

Scion preparation

Method 1. Buds of the plant that should go through STG are budded on a standard rootstock in the glasshouse. After the buds have grown and matured (approximately 3 – 4 months), the contaminated plants are defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested, and surface sterilised on a flowbench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile deionized water.

Method 2. Budsticks from the mother plant are cut in 50 mm lengths and surface sterilised in 70% ethanol for 5 seconds followed by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile deionized water the budsticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32°C and exposed to 16 h light. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG

The seedling rootstock is aseptically decapitated about 10 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. 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 under a stereo microscope. The growth point with primordia is placed on the horizontal cut of the incision. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light.

STG plant care

The shoot tip will start growing three to four weeks after STG. The seedling rootstock with the shoot tip is now grafted onto a vigorously-growing virus-free rootstock in the glasshouse. After grafting it is enclosed in a plastic bag for 8 days.

Virus indexing

Elimination of graft transmissible pathogens is confirmed by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Virus-free plants are multiplied and kept in an aphid-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunized (van Vuuren and Collins, 1990) with suitable *Citrus tristeza virus* (CTV) isolates prior to release to the Citrus Foundation Block at Uitenhage.

Results and discussion

For STG

Contaminated cultivars and selections were transferred from the ARC-ITSC to CRI. In addition 12 new cultivars and selections were received from clients. STG has been initiated on the latter additions.

For enzyme-linked immunosorbent assay (ELISA) (Bar-Joseph *et al.* 1979)

Cultivars and selections that went through STG previously but of which the CTV status is unknown were transferred from a tunnel at the ARC-ITSC to a glasshouse at CRI. ELISA has to be performed on these 103 cultivars and selections to establish if CTV is present (Table 4.2.3.1). For those where the virus is present, STG will have to be redone and those that are negative will be incorporated in the virus-free source.

Establishing and maintaining a virus-free gene source

The virus-free gene source at the ARC-ITSC was duplicated in an aphid-free tunnel at CRI. The cultivars and the number of selections of each cultivar are listed in Table 4.2.3.1.

Table 4.2.3.1. The following virus-free and contaminated cultivars have been successfully established at CRI.

Cultivar	Number of selections		
	Virus-free	Possibly contaminated	Total
Clementine	15	15	30
Diverse (Citron, Sour orange, etc.)	1	2	3
Ellendale	4	0	4
Grapefruit	16	3	19
Kumquat	1	1	2
Lemon	20	3	23
Lime	1	3	4
Midseason	22	2	24
Navel	33	19	52
Pummelo	7	1	8
Reticulata	31	33	64
Rootstock	20	14	34
Satsuma	8	2	11
Valencia	34	5	39
Total	213	103	316

Conclusion

A total of 213 virus-free cultivars and selections have been successfully established in an insect-free tunnel. Additions to the source are a continuous process. Control of insects in the tunnel is crucial.

Future research

Apply STG and ELISA on the current contaminated sources of CRI and clients.
Receive and maintain new additions.
Maintain the virus-free source in the insect-free tunnel.
Pre-immunise virus-free sources that are required by the Citrus Foundation Block.
Keep records of each source.

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4.2.4 **Cross-protection of Marsh and Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain**

Experiment 679 by JHJ Breytenbach (CRI)

Opsomming

Daar is gevind dat die Nartia isolaat (GFMS 12) wat huidiglik gebruik word vir pre-immuisering van wit pomelos en pompelmoese, gekontamineer is met 'n strawwe *Sitrus tristeza virus* ras. Twintig sub-isolate van die oorspronklike isolaat is in Beltsville MD, VSA, voorberei deur middel van enkel plantluis oordragings. Ses van die 20 sub-isolate wat 'n potensiaal as kruisbeskermings-agente getoon het nadat dit deur biologiese indeksering in die glashuis ge-evalueer is, is gebruik om hul beskermingsvermoëns teen strawwe rasse in die boord te bepaal. Virusvrye Star Ruby en Marsh pomelo boompies is gepreïmmuniseer met die ses Beltsville sub-isolate asook twee enkel plantluis oordraging sub-isolate van die ITSG (GFMS 12/7, GFMS 12/9), GFMS12 en GFMS35. Boompies is virusvry gelaat as kontrole. Preïmmunisasie is bevestig deur middel van ELISA. ELISA het uitgewys dat twee van die Beltsville sub-isolate nie voldoen aan sekere vereistes vir 'n goeie kruisbeskermings-isolaat nie deurdat hulle 'n lae persentasie oordraagbaarheid het, en stadig vermeerder en beweeg in die plant. Die twee sub-isolate word nie verder ge-evalueer nie. Die Marsh boompies is uitgeplant by Riversbend in die Nkwaleni vallei en die Star Ruby is uitgeplant by Tambuti landgoed in Swaziland. Die boompies se stamdeursneë is gemeet ses maande na uitplant. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate. Nietemin, daar is aanduidings dat van die sub-isolate groei strem. Met tyd sal dit egter duidelik word of van die sub-isolate beter beskermers is vir pomelo as die huidige twee isolate, GFMS 12 en GFMS 35.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagative material and various aphid species of which *Toxoptera citricida* is the most efficient. Many strains of CTV occur and may co-exist as mixtures in individual field trees and even in single buds on a tree. Symptoms produced 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 disease at present is by mild strain cross-protection (van Vuuren, *et al.*, 1993). A breakdown in the protection offered by the 'nartia' (GFMS 12) isolate owing to the presence of a severe strain within the complex (van Vuuren, *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained and in this study they are being evaluated for mildness and their potential as cross-protecting isolates in the field.

Materials and methods

The 20 SAT sub-isolates of the 'nartia' isolate were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. They were bud-inoculated separately to CTV sensitive Mexican lime indicator plants which are differential hosts that develop symptoms characteristic of the biological activity of a sub-isolate. Growth and stem pitting were determined and the virus titre was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate six months after inoculation. The six mildest sub-isolates (B389-1, B389-3, B389-4, B390-3, B390-4 and B390-5) were bud-inoculated (pre-immunization) to virus-free Marsh and Star Ruby grapefruit on MxT rootstocks. They will be compared with GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), GFMS 12/7 and GFMS12/9 (ITSC single aphid transfer sub-isolates) and trees that are planted virus-free. Pre-immunization has been confirmed by ELISA six months after inoculation. The Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates and the Marsh grapefruit trees at Riversbend in the Nkwaleni valley.

Results and discussion

Although there are significant differences between the isolates and sub-isolates in the Marsh grapefruit trees (Table 4.2.4.1), it is still too early to draw any conclusion from these data as these trees were only planted in September 2003. However, there are indications that some of the sub-isolates (B389/1, B390/5) retard growth at an early age.

Table 4.2.4.1 Tree circumference of different CTV isolates and sub-isolates in Marsh and Star Ruby grapefruit six months after planting.

Treatment	Trunk circumference (mm) [*]	
	Marsh (Riversbend)	Star Ruby (Tambutiti)
B389/1	13.0 C	19.5 a
B389/4	15.8 Ab	19.7 a
B390/3	15.6 Ab	22.5 a
B390/5	13.7 Bc	20.9 a
GFMS12/7	16.2 Ab	18.0 a
GFMS12/9	15.5 Abc	17.4 a
GFMS12	16.9 A	18.4 a
GFMS35	15.9 Ab	17.5 a
Virus free	17.1 A	17.2 a

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

Conclusion

The trees are still young and no conclusions can be made at this stage, however, there are indications that growth is retarded by some sub-isolates.

Future research

Evaluate the horticultural performance of trees over a 10-year cycle using the following parameters:

- Canopy volume,
- Stem pitting,
- Yield and fruit size.

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4.2.5 Cross-protection of Marsh and Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Experiment 738 by JHJ Breytenbach (CRI)

Opsomming

As gevolg van die teenwoordigheid van 'n strawwe ras in die Nartia isolaat (GFMS 12) was dit nodig om die isolaat te verdeel in sub-isolate deur middel van enkel plantluis oordragings. Hierdie sub-isolate is voorberei in 'n kwarantyn fasaliteit in Beltsville, VSA. Nadat die sub-isolate deur biologiese indeksering ge-evalueer is om tussen die ligte en strawwe rasse te onderskei, is gevind dat slegs vier uit die 17 potensiaal het vir verdere evaluering. Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG is ingesluit in die proef asook GFMS 12 (standaard vir wit pomelos) en GFMS 35 (standaard vir rooi pomelos). Virusvrye Star Ruby boompies is in 'n glashuis voorberei en gepre-immuniseer met die isolate en sub-isolate. 'n Virusvrye behandeling is ingesluit as kontrole. Omdat *Sitrus tristeza virus* deur die gasheer en klimaat beïnvloed word, is dit nodig om isolate in die verskillende sitrus produserende streke te evalueer. Nadat pre-imunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing uitgeplant gedurende September 2004, en sal jaarliks ge-evalueer word vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte.

Introduction

A breakdown in the CTV protection offered by the GFMS12 (Nartia) isolate, owing to the presence of severe strains within the complex, motivated the separation of the strains in the isolate by single aphid transmission (Marais, *et al.*, 1996). Seventeen single aphid transferred (SAT) sub-isolates were prepared at the quarantine facility in Beltsville MD, USA. These sub-isolates have undergone biological indexing to differentiate between the severe and mild forms. Some sub-isolates have no potential since virus

concentration and movement of the virus were poor as well as unacceptable severe stem pitting (Breytenbach, *et al.*, 2002). Four of these 17 sub-isolates will be evaluated as cross-protectors. Promising SAT sub-isolates of Nartia obtained from the ARC-ITSC will also be included in this experiment (van Vuuren, *et al.*, 2000). As CTV exhibits host and geographical specificity, it is imperative that mild protective isolates be tested in the different production areas (da Graça, *et al.*, 1984).

Materials and methods

Virus-free Star Ruby budwood was budded to virus-free MxT rootstocks. When the scions had developed to approximately 5 mm thickness they were bud inoculated with the sub-isolates of GFMS 12 (Beltsville sub-isolates: B389-1, B389-4, B390-3, B390-5; ITSC sub-isolates GFMS 12/7, GFMS 12/9) and compared to the two standards (GFMS 12 for white grapefruit; GFMS 35 for red grapefruit) and trees that were left virus-free. After pre-immunization is confirmed by ELISA, they will be planted in the Kakamas area according to a randomized block with five replications.

Results and discussion

Pre-immunization has been confirmed by ELISA and the plants were planted at Kromhout boerdery in the Kakamas area during September 2004.

Conclusion

These plants have now been six months in the field and there are no results yet.

Future research

Evaluate horticultural performance i.e. growth (tree size) health (stem pitting) and harvest data (fruit size and kg/tree).

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4.2.6 Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa Experiment 742 by JHJ Breytenbach (CRI)

Opsomming

Enthout is gesny van 108 uitstaande pomelo bome in die verskillende pomelo gebiede wat moontlik ligte isolate van *Sitrus tristeza virus* (STV) huisves. Die enthout is terug gebring na die glashuis by CRI en geïnkuleer op Meksikaanse lemmetjie wat 'n indikator plant is vir STV (biologiese indeksering), om te bepaal of hulle wel so lig en beskermend is as wat hulle in die boord wys. Na die eerste biologiese indeksering is slegs 19 isolate gekies vir verdere evaluering. Hierdie 19 isolate is 'n tweede keer geïnkuleer op Meksikaanse lemmetjie en vergelyk met GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate. Die geïnkuleerde plante is ge-evalueer vir groei, stamgleuf en virus titre wat deur middel van ELISA bepaal is. Hierdie isolate en sub-isolate is ook geïnkuleer op Etrog citron om te bepaal of daar enige viroiede teenwoordig is. Die mees belowendste van hierdie 19 veld isolate, wat vry is van viroiede (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, Tshipise 19/5 – versamel vanaf Marsh in Tshipise), word gebruik om virusvrye Marsh en Star Ruby boompies mee te preïmuniseer. Die isolate word vergelyk met GFMS 12 (standard vir wit pomelos), GFMS 35 (standard vir rooi pomelos), asook die vier beste Beltsville (B389-1, B389-4, B390-3, B390-5) en LNR sub-isolate (GFMS 12/7, GFMS 12/9). Hierdie bome sal in twee pomelo produksie streke uitgeplant word.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources (de Lange, *et al.*, 1981). In South Africa, the benefit of optimum growth and production of virus-free trees cannot be utilized because of the abundance of the aphid insect vector of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClellan, 1963). Factors such as the type of strain, host and environment will determine dominance of a specific strain and it may change if any of the factors change *viz.* co-infection of a new strain or extreme temperatures (da Graça, *et al.*, 1984; Oberholzer, 1959). The only method to overcome this problem is by cross protection, a deliberate infection of virus-free material with a known CTV isolate. The first step in searching for mild isolates for cross protection purposes is to look for old trees which are healthy and produce good quality fruit (Müller & Costa, 1987).

This experiment is a follow-up of the glasshouse trial (exp. 49) where 108 CTV isolates were collected in different grapefruit production areas from productive old grapefruit trees. After an initial screening in the glasshouse, 19 isolates showed potential as cross protectors. These 19 isolates were then compared to the present pre-immunizing isolates. The most promising of these 19 field isolates, that are free of citrus viroids, will be bud inoculated to virus-free Star Ruby and Marsh grapefruit trees, the Beltsville and ITSC sub-isolates of Nartia and compared to the current GFMS 12 (standard for white grapefruit) and GFMS 35 (standard for red grapefruit) isolates as controls. The trees will be planted in two grapefruit production areas.

Materials and methods

Virus-free Troyer citrange rootstocks were propagated and budded with virus-free Star Ruby and Marsh grapefruit budwood. When the scions have developed to approximately pencil thickness, they were inoculated with the selected CTV isolates in the scions. The following CTV isolates were used: the four most promising isolates selected from the original 108 field isolates (Tabankulu 1 – derived from Star Ruby in Swaziland, New Venture 41/2 – derived from Star Ruby in the Nkwaleni Vally, ORE 8 – derived from Marsh in the Hoedspruit area, Tshipise 19/5 – derived from Marsh in Tshipise), the four best Beltsville sub-isolates (B389-1, B389-4, B390-3, B390-5) and two ITSC sub-isolates (GFMS 12/7, GFMS 12/9). They will be compared to GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit) isolates and trees that will be left virus-free. After three months, positive pre-immunization will be confirmed by ELISA whereafter the trees will be planted in two grapefruit production areas according to a randomized block design with five replicates. Growth, production and tree health will be monitored.

Results and discussion

Virus-free rootstocks were budded with virus-free Marsh and Star Ruby grapefruit.

Conclusion

No conclusion yet.

Future research

Pre-immunize virus-free Marsh and Star Ruby trees with the different isolates and sub-isolates. Plant trees in two grapefruit production areas. Evaluate horticultural performance *i.e.* growth (tree size), production (fruit size and kg/tree) and health (stem pitting).

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4.2.7 The response of Star Ruby to different CTV isolates

Experiment 561015: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Die onvermoë van GFMS 12 (Nartia) *Sitrus tristeza virus* (STV) isolaat as 'n kruisbeskermings-agent vir Star Ruby pomelo, het gemoedlik dat GFMS 35 isolaat as 'n kruisbeskermer gebruik word in die *interim*. Ses nuwe STV isolate (GFMS 65, GFMS 67, GFMS 71, GFMS 73, GFMS 77, GFMS 78) wat verkry is van Star Ruby en Rosé pomelobome, is ge-evalueer en vergelyk met GFMS 12, GFMS 35, twee isolate wat vanaf Star Ruby bome by die Sitrus Grondvesblok (SG) versamel is (GFMS 12a, GFMS 12b), twee strawwe isolate (GFSS 1, GFSS 5) en bome wat virusvry geplant is. Bome wat die beste produksie gehad het oor 'n vyf-jaar tydperk was gepre-immuniseer met isolate GFMS 35 en GFMS 78. Hierdie bome het betekenisvol beter geproduseer as bome wat virusvry geplant is, bome met isolate GFMS 12, GFMS 67 en die met die twee strawwe isolate. Berekening van die oeswaarde (vruggrootheid gekoppel met mark pryse), was bome met GFMS 78, 1% beter as bome met GFMS 35. Bome met GFMS 12a was derde beste en was 12% swakker as die beste. Laasgenoemde isolaat is versamel van 'n goeie moederboom by die SG wat met GFMS 12 gepre-immuniseer was. Die resultate toon dat STV isolaat GFMS 35, wat die huidige isolaat is vir pre-immunisering van rooi pomelos, tesame met GFMS 78, superieur is bo die ander isolate. Nietemin, dit sal voordelig wees om te sien of die twee isolate se superioriteit volgehou sal word en tot watter mate boom leeftyd en ekonomiese produksie verleng sal word.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources in South Africa (Fourie and van Vuuren, 1993). However, the benefit of optimum growth and production of virus-free trees cannot be utilised because of the abundance of the aphid vector, *Toxoptera citricida* (Kirkaldy) of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClellan, 1963). Factors such as the type of strain, host plant and environment will determine dominance of a specific strain and it may change if any of the factors change *viz.* co-infection of a new strain or extreme temperatures (da Graça, *et al.*, 1984; Oberholzer, 1959). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV isolate (Müller and Costa, 1987).

Of the commercial citrus cultivars grown in southern Africa, grapefruit is the most sensitive to the disease which causes stem pitting, decline and production of small fruit. With the initiation of the southern African Citrus Improvement Program (CIP), all grapefruit selections are pre-immunised with the GFMS 12 CTV isolate (von Broembsen and Lee, 1988). This isolate originated from a 50-year-old Nartia (Marsh type) grapefruit tree in the Western Cape Province. Bud-wood source trees at the Citrus Foundation Block (CFB) at Uitenhage, which is the only source for propagating certified trees, are evaluated visually each year for decline and stem pitting symptoms. In addition, each tree of every selection is biologically indexed on an annual basis to establish the CTV severity. When there are indications of severe CTV, such a tree is terminated as a bud-wood source.

In 1993 it was found that 6-year-old Star Ruby bud-wood source trees varied in stem pitting development and fruit size (unpublished data). Testing the CTV stem pitting severity revealed that severe strains were dominating in four of the five bud-wood source trees. At first it was thought that GFMS 12 did not protect against co-infection of severe strains. However, subsequent research showed the presence of a severe strain in the original isolate and that segregation of the strains, where the severe strain became dominant, may be the cause of the problem (van Vuuren, *et al.*, 2000). The unsuitability of GFMS 12 as a protector for Star Ruby was also confirmed in a field trial (van Vuuren and van der Vyver, 2000). Consequently, GFMS 35 (derived from a Rosé grapefruit tree) has been approved for the pre-immunisation of all red grapefruit on the interim (Luttig, *et al.*, 2002).

The first step in searching for mild isolates for cross protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller and Costa, 1987). The Star Ruby grapefruit industry in South Africa started in the late 1970s and therefore no trees older than 15 years existed at the time. To overcome this problem, the best producers in the oldest plantings at Malelane, Mpumalanga Province, and Swaziland

were selected. Isolates from these trees were evaluated in glasshouse tests and those with the best potential were evaluated in the field.

This report is on the field evaluation of the best isolates identified, and the objective of the study was to find superior CTV isolates for pre-immunisation that will maximise the profitability (productive live and quality) of Star Ruby grapefruit.

Materials and methods

Virus-free Star Ruby trees on Swingle citrumelo rootstock were grown under aphid-free conditions using standard nursery practices. When the scions had developed to approximately 5 mm diameter, they were bud-inoculated with different CTV isolates. These isolates were selected from healthy-looking trees and showed potential as cross-protecting isolates in glasshouse tests. The following treatments were applied in replicates of five:

1. GFMS 12 (derived from Nartia grapefruit A – standard at the time);
2. GFMS 12a (derived from Star Ruby mother tree at CFB, showing mild stem pitting – pre-immunised with GFMS 12);
3. GFMS 12b (derived from GFMS 12 pre-immunised mother tree at CFB, displaying severe stem pitting and small fruit);
4. GFMS 35 (derived from Rosé grapefruit at Komatipoort. Marsh grapefruit trees pre-immunised with this isolate performed better than trees with GFMS 12 over a 12-year period (van Vuuren and da Graça, 2000). Present pre-immunising isolate for red grapefruit);
5. GFMS 65 (derived from a Star Ruby tree at Tambankulu Estates, Swaziland);
6. GFMS 67 (similar than 5);
7. GFMS 71 (derived from old budwood source Star Ruby, Esselen Nursery, Malelane);
8. GFMS 73 (similar than 7);
9. GFMS 77 (similar than 7);
10. GFMS 78 (derived from 10-year-old planting, F. Esselen, Malelane);
11. GFSS 1 (derived from 5-year-old Marsh grapefruit tree with severe stem pitting, Nkwaleni Valley);
12. GFSS 5 (derived from 5-year-old Star Ruby grapefruit with severe stem pitting, F. Esselen, Malelane).
13. Control. Trees planted virus-free.

After ELISA confirmed infection, a field trial was planted at Nelspruit in a randomised block.

The following data was taken:

- Tree size was measured and calculated as a cylinder and half sphere according to Burger *et al.* (1970).
- Fruit were harvested, graded in export sizes (Anon, 1990) and weighed.
- Tree health was monitored by evaluating stem pitting and decline.

The approximate monetary value of the fruit of each CTV isolate was calculated and a projection of income was made for a hectare planting (6 X 3 spacing). The average production over three years of such a planting was determined and the value calculated according to fruit size distribution of each treatment. The value of the crop in relation to fruit size was determined by calculating the average value per export box for ten years. The highest price equalled a value of ten while the other values were calculated accordingly. The value of the crop per hectare for each treatment was determined by multiplying the production of a specific fruit size (export box) by the value for that size.

Results and discussion

Growth, production and disease rating of the 7-year-old Star Ruby trees on Swingle citrumelo rootstock are given in Table 4.2.7.1.

Tree size. The canopy volumes of the trees of all the treatments were even, except for trees pre-immunized with GFMS 65 and the two severe isolates. Canopy sizes of these trees were significantly smaller when compared to trees pre-immunized with GFMS 12a, GFMS 12b, GFMS 35, GFMS 73, GFMS 76, as well as those that were planted virus-free.

Yield at year six. Trees pre-immunized with GFMS 35 produced the largest crop and were significantly larger than trees pre-immunized with GFMS 12, GFMS 67, GFMS 71, GFMS 73, the trees that were planted virus-free, and those with the two severe isolates.

Yield efficiency. The highest yield efficiency (kg/canopy volume) was achieved by trees pre-immunized with GFMS 65 (the smallest trees with a mild isolate) but was not significantly better than those of trees pre-immunized with isolates GFMS 35, GFMS 67, GFMS 77 and GFMS 78.

Stem pitting. Trunks of trees with isolates GFMS 35 and GFMS 78 showed no external pitting. Except for the trees with the two severe isolates, severe pitting occurred in trees pre-immunized with GFMS 12 (Nartia), GFMS 12b, GFMS 65 and GFMS 67. No decline occurred in any treatment but one tree with GFSS 5 died at an early stage.

Cumulative yield. The highest cumulative yield over five seasons was produced by trees pre-immunized by GFMS 35 but this was not significantly better than those of trees pre-immunized with GFMS 12a, GFMS 12b, GFMS 65, GFMS 76, GFMS 77 and GFMS 78.

Crop value. High yields can reduce fruit size and therefore the value of the crop. However, trees with GFMS 35 also had the best crop value, 5% better than that of trees with GFMS 78, which was second best.

Table 4.2.7.1. The average tree size, production, yield efficiency and stem pitting rating of 7-year-old Star Ruby trees pre-immunized with different CTV isolates. The cumulative yield for 5 years and the relative monetary value is presented. The control trees were planted virus-free

Treatment	Tree canopy size (m ³)	2003 yield (kg)	Yield efficiency (kg/m ³)	Stem pitting rating	Cumulative yield (kg) 1999 - 2003	Relative crop value R/ha/yr
Control	7.2 a	39 c	5.4 ef	0.6 abc	87 d	6658
GFMS 12	5.9 abc	46 bc	7.8 bcde	2.8 e	111 cd	8426
GFMS 12a	7.6 a	64 ab	8.4 bcd	0.2 ab	158 abc	11696
GFMS 12b	7.3 a	63 ab	8.6 bcd	2.5 e	153 abc	11534
GFMS 35	7.1 a	76 a	10.7 abc	0.0 a	174 a	13588
GFMS 65	4.0 bcd	57 abc	14.3 a	2.5 e	134 abcd	10147
GFMS 67	5.4 abc	55 bc	10.2 abcd	2.2 de	118 bcd	8862
GFMS 71	6.5 ab	53 bc	8.2 bcde	1.4 cd	123 bcd	9186
GFMS 73	7.6 a	51 bc	6.7 de	1.1 bc	123 bcd	9133
GFMS 76	7.5 a	59 abc	7.9 cde	1.2 bcd	136 abcd	10240
GFMS 77	5.5 abc	56 abc	10.2 ab	0.9 abc	148 abc	11197
GFMS 78	6.3 ab	65 ab	10.3 abcd	0.0 a	164 ab	12877
GFSS 1	3.4 cd	11 d	3.2 f	3.0 e	26 e	1533
GFSS 5	1.9 d	11 d	5.8 ef	2.6 e	22 e	1461

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

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

- Trees pre-immunised with GFMS 35, the present cross-protecting isolate for Star Ruby in the CIP, gave the best results. This isolate was followed by isolates GFMS 78 and GFMS 12a. The latter was derived from the original mother tree for Star Ruby budwood at the CFB.
- Trees with GFMS 35 produced significantly better than trees with mild isolates GFMS 12, GFMS 67, those that were planted virus-free, and the two severe isolates.
- Calculating the total crop value (fruit size and market prices), the crop value of trees with GFMS 35 and GFMS 78 averaged 23% better than the rest.
- GFMS 35 is recommended for use as a pre-immunising isolate for Star Ruby grapefruit in the southern Africa Citrus Industry. This isolate contains no strains that cause severe stem pitting (Luttig, *et al.*, 2002) and the chances of detrimental strain shifts within Star Ruby trees, which are planted in different climatic areas, are minimised.

Future research

The objective of this trial has been achieved and it will therefore be terminated. Data will be published in the Proc. 16th Conf. of the International Organization of Citrus Virologists.

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4.2.8 Field evaluation of promising mild isolates for Star Ruby in two climatic areas

Experiment 561015: Trial 2 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Die vergelyking van Star Ruby pomelo bome op Swingle citrumelo onderstam, wat met verskillende *Sitrus tristeza virus* (STV) isolate (GFMS 35, GFMS 67, GFMS 71, GFMS 73, LMS 6, GFSS 5 (straf), Virusvry (kontrole)) geïnkuleer is, in twee klimatologiese gebiede, toon die volgende verskille:

- Die natuurlike druk van STV was strawwer in Malelane as in Nelspruit. Die bome wat virusvry geplant is in die twee gebiede, toon meer groei beperking, strawwer stamgleuf simptome en 'n laer produksie in Malelane.
- Algemene stamgleuf ontwikkeling was strawwer op Malelane. Dit is 'n teenstrydige resultaat aangesien STV gewoonlik strawwer simptome ontwikkel by koeler temperature.
- Behalwe vir bome wat met GFMS 35 gepre-immuniseer was, was die neiging tussen STV isolate in die twee gebiede dieselfde ten opsigte van die drie parameters (groei, produksie, gesondheid) wat gebruik is. Die swak vertoning van bome op Malelane wat met GFMS 35 isolaat gepre-immuniseer was is onverwags en kan nie verduidelik word nie.
- Oor die algemeen het bome wat met LMS 6 (die huidige kruisbekerings-isolaat vir soetlemoen en mandaryne) gepre-immuniseer was, die beste resultate gelever in albei gebiede.

Introduction

(Refer to section 4.2.7).

The aim of the study is to find a better CTV isolate for Star Ruby grapefruit that will be stable in different climatic conditions.

Materials and methods

Virus-free Star Ruby grapefruit, budded onto Swingle citrumelo rootstock, were inoculated with five mild isolates of CTV (GFMS 35, GFMS 67, GFMS 71, GFMS 73, LMS 6). These trees were compared to trees with GFMS 12 (standard at the time) and GFSS 5 (severe) isolates as well as trees planted virus-free. Subsequent to the initiation of this trial, GFMS 35 was approved to replace GFMS 12 as the pre-immunizing isolate for red grapefruit and LMS 6 showed promise as a pre-immunizing isolate for grapefruit in another trial (van Vuuren & van der Vyver, 2000).

The trees were planted at Nelspruit (1996) and Malelane (1998) in randomized blocks with five replications.

Results and discussion

Growth, stem pitting rating and production of the trees in the two areas are compared in Table 4.2.8.1.

Nelspruit. No yield data was obtained at this site since the trees were harvested accidentally by the farm manager without taking records. The largest trees were those pre-immunized by GFMS 73 and LMS 6. They were significantly larger than trees with GFMS 35, those that were planted virus-free and those with the severe isolate.

Malelane. Trees pre-immunised with isolates GFMS 73 and LMS 6 were the largest. They were significantly larger than trees with GFMS 35, GFMS 71, those that were planted virus-free and those with the severe isolate. Stem pitting in trees with isolates GFMS 67, GFMS 71, GFMS 73 and LMS 6 were milder than that in trees with GFMS 12, GFMS 35, those that were planted virus-free and those with the severe isolate. Except for trees with GFMS 35 and the severe isolate, the production of trees with the other isolates did not differ.

Table 4.2.8.1. Comparison of tree size, stem pitting rating and production of Star Ruby trees pre-immunized with different CTV isolates at two different climatic sites, Nelspruit (N) seven years after planting and Malelane (M) six years after planting*.

Treatment	Tree size (m ³)		Stem pitting rating**		Yield (kg)	
	N	M	N	M	N	M
Control	14.2 a	8.7 b	1.6 a	3.2 c	122.3 a	85.8 b
GFMS 12	10.3 b	10.9 ab	3.8 c	3.2 c	101.8 ab	93.6 ab
GFMS 35	11.5 ab	8.2 b	2.4 b	3.0 c	118.8 a	64.1 c
GFMS 67	12.8 ab	10.6 ab	2.2 ab	2.0 ab	98.8 ab	92.9 ab
GFMS 71	10.1 b	8.3 b	1.8 a	2.0 ab	87.9 b	80.4 b
GFMS 73	14.8 a	12.2 a	1.6 a	2.2 b	111.1 ab	115.3 a
LMS 6	14.1 a	13.2 a	1.6 a	1.4 a	123.0 a	121.9 a
GFSS 5 (sev)	4.8 c	2.5 b	4.0 c	4.2 d	30.7 c	32.3 d

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

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

When comparing the results at the two sites, the following observations are made:

- The challenge by natural CTV strains at Malelane was more severe than at Nelspruit. The trees that were planted virus-free at the two sites show more growth restriction and stem pitting and a lower yield at Malelane.
- Generally stem pitting development was more severe at Malelane. This is a contradicting result since CTV usually develops more severe symptoms at low temperatures.
- Except for trees with GFMS 35, the trends between the different isolates at the two sites regarding the three parameters were the same. The poor performance of trees with GFMS 35 at Malelane is unexpected and cannot be explained.
- Overall, trees with LMS 6, the present cross protecting isolate for sweet orange and mandarin, performed the best at both sites.

Future research

The objective of this trial has been achieved and therefore the trial will be terminated.

References cited

(Refer to section 4.2.7).

4.2.9 The response of different red grapefruit cultivars to *Citrus tristeza virus* Experiment 561015: Trial 3 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Ses-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte *Sitrus tristeza virus* (STV) isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Daar is aanduidings van interaksies tussen sommige STV isolate en pomelo seleksies, bv. betekenisvolle afnames in boom groottes het voorgekom by die volgende kombinasies: Rio Red en Oran Red bome met GFMS 12, Hendersen, Flame en Oran Red met GFMS 35, Hendersen en Oran Red met GFMS 67, Star Ruby en Hendersen met GFMS 73. Dit is moontlik dat Oran Red 'n genetiese dwerg eienskap het. Produksie was gekoppel aan boom grootte en daar was geen verskil in die voorkoms van stamgleuf tussen die seleksies of isolate nie.

Introduction

(Refer to section 4.2.7).

The objective of this study is to evaluate new CTV isolates in different red grapefruit selections.

Materials and methods

Seven red grapefruit selections viz. Star Ruby, Flame, Rio Red, Nelruby, Henderson, Ruben and Oran Red were budded as scions on Swingle citrumelo rootstocks. Tristeza isolates GFMS 35, GFMS 67, GFMS 71 and GFMS 73 are evaluated in each scion and compared to the standard (GFMS 12) and a severe isolate (GFSS 5). Infection was confirmed by ELISA before they were planted in a randomized split plot with five replications at Malelane during December 1998.

Results and discussion

Tree size, yield and stem pitting ratings of the red grapefruit selections that were pre-immunized with different CTV isolates are presented in Table 4.2.9.1, Table 4.2.9.2 and Table 4.2.9.3 respectively.

Tree size: Overall, the canopy sizes of the trees pre-immunized with the different mild CTV isolates did not differ from each other. Trees with the severe isolate were significantly smaller. Of the selections, tree sizes of Nel Ruby and Ruben were significantly larger than those of Oran Red (Table 4.2.9.1).

In the body of the table, the results indicate interactions between the mild isolates and the grapefruit selections. Significant reductions in size occurred with the following combinations: GFMS 12 with Rio Red and Oran Red; GFMS 35 with Henderson, Flame and Oran Red; GFMS 67 with Henderson and Oran Red; GFMS 73 with Star Ruby and Henderson. However, it is possible that Oran Red has a genetic dwarfing characteristic.

Production: No significant difference occurred between the mild CTV isolates. With the grapefruit selections, the Rio Red and Nel Ruby trees yielded significantly better than the Star Ruby trees. The interactions is mainly couple with tree size.

Stem pitting: Overall the stem pitting did not differ among trees pre-immunized with the different mild CTV isolates. The Ruben and Oran Red trees had less stem pitting than trees of the other selections. Generally the trunks of the trees are smooth with occasional pits. Even where the severe isolate was inoculated only the Star Ruby and Rio Red trees showed moderate pitting.

Table 4.2.9.1. The effect of different CTV isolates on tree size (canopy volume = m³) of 6-year-old red grapefruit selections*

Grapefruit selections	CTV Isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	12.6 ab	14.6 abc	14.3 ab	10.7 b	4.6 c	11.4 wx
Rio Red	11.3 bc	18.0 a	15.5 ab	13.2 ab	6.0 bc	12.8 wx
Henderson	11.8 ab	10.8 c	13.0 bc	10.8 b	7.8 b	10.8 wx
Nel Ruby	14.5 ab	17.0 ab	16.3 a	15.0 a	6.9 bc	13.9 w
Flame	14.5 ab	13.8 bc	14.2 ab	13.1 ab	7.8 b	12.7 wx
Ruben	14.8 a	14.3 abc	16.7 a	11.8 ab	11.0 a	13.7 w
Oran Red	7.9 c	10.4 c	10.2 c	10.2 b	5.1 bc	8.8 x
Mean	12.5 y	14.1 y	14.3 y	12.1 y	7.0 z	

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

Table 4.2.9.2. The effect of different CTV isolates on the production (kg/tree) of 6-year-old red grapefruit selections

Grapefruit selections	CTV isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	135 ab	107 cd	129 ab	122 c	62 b	111 z
Rio Red	126 bc	169 a	136 ab	163 a	77 b	134 y
Henderson	134 abc	101 d	131 ab	129 bc	84 ab	116 yz
Nel Ruby	135 abc	147 b	150 a	145 ab	92 ab	134 y
Flame	151 a	108 cd	140 a	156 a	93 a	130 yz
Ruben	152 a	137 b	115 b	132 bc	106 a	129 yz
Oran Red	114 c	125 bc	130 ab	123 c	89 ab	116 yz
Mean	135 x	128 x	133 x	139 x	86 y	

* Figures in each row in the body of the table that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.3. The effect of different CTV isolates on stem pitting (rating^{**}) of 6-year-old red grapefruit selections*

Grapefruit Selections	CTV Isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	2.2 b	2.2 c	1.6 ab	2.0 bc	3.2 c	2.2 x
Rio Red	2.4 bc	2.0 bc	1.8 b	2.2 c	3.6 d	2.4 x
Henderson	1.8 a	2.2 c	2.8 d	2.2 c	2.0 a	2.2 x
Nel Ruby	2.4 bc	2.4 cd	1.4 a	2.6 d	2.4 b	2.2 x
Flame	2.2 b	2.4 cd	2.4 c	1.8 b	2.6 b	2.3 x
Ruben	2.0 ab	1.4 a	1.8 b	1.0 a	2.0 a	1.6 v
Oran Red	2.6 c	1.8 b	1.8 b	1.2 a	2.2 a	1.9 w
Mean	2.2 yz	2.1 y	1.9 y	1.9 y	2.6 z	

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

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

The reaction of the different grapefruit selections to the different CTV isolates were very similar at this stage and the cross-protection ability of each isolate for the selections can only be measured over time.

Future research

Measure trees, rate disease occurrence, harvest and grade fruit.

References cited

(Refer to section 4.2.7).

4.2.10 Constructing a superior *Citrus tristeza virus* isolate for cross-protection of grapefruit selections

Experiment 561022: Trial 1 by S.P. van Vuuren (CRI)

Opsomming

Gedurende 2003 is sewe sub-isolate van GFMS 12 (Nartia), vier enkel plantluis oordragings en drie gasheer skeidings, ge-evalueer vir hul kruisbeskermings-eienskappe (virulensie, vermenigvuldiging, beweeglikheid) in vyf pomelo seleksies (Marsh, Star Ruby, Flame, Rio Red, Henderson) en vergelyk met die twee isolate wat tans vir pomelos in die bedryf gebruik word. Twee sub-isolate wat deur plantluis skeiding verkry is het goeie eienskappe vir kruisbeskerming getoon en was beter as beide die huidige kruisbeskermings-isolate. Sub-isolate 12/7 en 12/9 was hoogs oordraagbaar, vertoon eenvoudige enkel-string konformasie polimorfism (SSCP) profiele ('n aanduiding van 'n enkel ligte ras) en het geen negatiewe effek op groei van al die pomelo seleksies gehad nie. Evaluasies van hierdie twee sub-isolate word voortgesit, afsonderlik en in kombinasie. Plante vir die glashuis evaluasie is voorberei en gereed om geïnokuleer te word. Vir boord evaluasie is die twee sub-isolate in eksperimente 679 en 738 gevestig te Tambuti Estates (Swaziland), Riversbend (Nkwalení Vallei) en die Oranje Rivier Vallei. Verdere evaluasie in nog twee pomelo gebied is in voorbereiding (eksperiment 742).

Introduction

The traits (biological activity (severity), multiplication, movement) for good cross protecting *Citrus tristeza virus* (CTV) isolates have been described (Lee, *et al.*, 1987). Many CTV strains exist in South Africa and, because of the abundance of the aphid vectors, occur naturally as mixtures in trees (McClellan, 1963; Racca, *et al.*, 1978). Vastly different biological activities have been reported for trees propagated from the same bud wood source but planted under different growing conditions (da Graça, *et al.*, 1984). Müller (1980) has proposed that the biological activity expressed by an isolate depend on which strain predominates. Some CTV strains are better adapted to warm conditions, whereas some prefer cooler temperatures (van Vuuren, 1982). Extremely hot growing conditions reduce CTV titre and can result in temporary thermotherapy (Garnsey, *et al.*, 1980). It has been shown that the host can favour the multiplication and movement of specific strains (Moreno, *et al.*, 1963). This will lead to different symptom expressions when isolates are constituted of several strains. When a severe strain is present and becomes the prevailing strain, it will result in serious consequences.

Because of the diversity of climatic conditions in which different grapefruit selections are cultivated in South Africa (Barry, 1996), it is important for a good cross-protector to adapt to all these variable factors. If that is not possible, it appears that it will be necessary to identify specific isolates for each grapefruit selection (Star Ruby, Flame, Rio Red, Marsh, etc.) as well as for the different production areas (*i.e.* for hot humid coastal areas, hot and humid inland areas, hot and dry inland areas, etc.).

Several sub-isolates were obtained from the GFMS 12 isolate by single aphid transfers as well as by host passage (van Vuuren, *et al.*, 2000; unpublished data). Apart from the difference of aphid transmissibility between the two groups, biological and molecular differences were also established among sub-isolates within each group (van Vuuren, *et al.*, 2000; Luttig, *et al.*, 2001; unpublished data). Each of these sub-isolates may have special cross-protecting properties and in combination may support or complement each other so that such an isolate is adaptable to different conditions. The traits of these sub-isolates regarding the two main factors viz. host and environment, should be established to enable the construction of an isolate that may be suitable for different hosts and environmental conditions.

It was found that sub-isolates from isolates GFMS 12 and GFMS 35 differ in transmissibility and movement. Where the aim was to identify single strains for cross-protection to minimise variability, it appeared that movement of the sub-isolates varied among hosts and in specific environmental conditions. The incomplete invasion of the whole plant by the protecting strain leaves the plant vulnerable to infection by severe strains introduced by aphid vectors. The sub-isolates of GFMS 12 appear to be more stable in variable conditions than those of GFMS 35 (unpublished data).

Two aphid-transmitted sub-isolates GFMS 12/7 and GFMS 12/9 exhibited good qualities for a cross-protecting isolate. They were highly transmissible, revealed simple single-strand polymorphism profiles

(indication of a single strain) and had no negative effects on growth of seven grapefruit selections. Evaluations of these two isolates are being continued, singly and in combination.

Materials and methods

Five virus-free grapefruit selections (Marsh, Star Ruby, Flame, Rio Red, Henderson) were established on Troyer citrange rootstock under aphid-free conditions according to normal nursery practices. When the scions had developed to a thickness of approximately 5 mm, they will be bud-inoculated by two buds containing selected CTV sub-isolates GFMS 12/7 and GFMS 12/9. The sub-isolates are compared to GFMS 12 (standard isolate for white grapefruit), GFMS 35 (standard isolate for red grapefruit) and un-inoculated plants as controls. Each scion/CTV combination will be replicated five times. After inoculation the plants were cut back at two buds above the inoculation point to force new growth. The plants were kept at a temperature regime of 28-32°C in a glasshouse with additional lighting to exclude the effect of short daylight on growth (Roistacher and Nauer, 1985).

To establish multiplication and movement of the CTV in each host, enzyme-linked immunosorbent assay (ELISA) will be performed on the subsequent flush at six weeks after inoculation to establish the presence and titre of the virus. The top two leaves of the flush will be sampled for the test. The procedure was repeated at 12, 24 and 28 weeks after inoculation.

At six months the growth since inoculation will be removed, measured and the bark stripped to evaluate stem pitting.

Results and discussion

Glasshouse

The grapefruit selections (Marsh, Star Ruby, Flame, Rio Red, Henderson) were established on Troyer citrange rootstock and are ready for inoculation with the isolates and sub-isolates.

Field

The two sub-isolates (GFMS 12/7 and GFMS 12/9) were incorporated in three field experiments (experiments 679, 738, 742) for evaluation in Star Ruby and Marsh grapefruit in several climatic conditions. Trials at Tambuti Estates (Swaziland), Riversbend (Nkwaleni Valley) and the Orange River Valley have been established and another that will be planted at two sites is in preparation.

Conclusion

Evaluation of sub-isolates GFMS 12/7 and GFMS 12/9 in the glasshouse is in progress. For field evaluation, they were established in three trials and they are included in another two trials that are in preparation.

Future research

Inoculate five grapefruit selections on Troyer citrange rootstock with the sub-isolates and do ELISA tests to establish titre and movement of the virus. For field evaluation, they are incorporated in trials where they will be compared with other isolates and sub-isolates regarding growth, production and tree health

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4.2.11 The effect of CTV pre-immunization on the fruit size of Clementine and Satsuma Experiment 561004: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Vruggrootte is 'n groot probleem by Clementines in die Oos – en Wes Kaap. Om die invloed van pre-immunisering op vruggrootte te bepaal, word ongepre-immuniseerde en gepre-immuniseerde bome van sewe Clementine seleksies (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) en een Satsuma seleksie (Miho Wase) op Addo Navorsingstasie vergelyk. Die bome is 'n jaar oud en nog nie in drag nie.

Introduction

All citrus propagation material is pre-immunized with a mild isolate of *Citrus tristeza virus* (CTV). Cross protection is specific with regard to the citrus type, i.e. the most effective protecting strain for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Programme, all citrus, including mandarin types, was pre-immunized with an isolate originating from grapefruit for the interim until suitable isolates was found for the different types (von Broembsen & Lee, 1988). Subsequently a suitable isolate, LMS 6, has been identified for lime (van Vuuren, *et al*, 1993). LMS 6 contains a mild form of seedling yellows which the grapefruit isolate does not have, and therefore it was approved to replace GFMS 12 as the pre-immunizing isolate for the mandarin types. The suitability of LMS 6 as a protector for Clementines has not been confirmed and evaluations are currently being done (van Vuuren & Maritz, 2002).

Fruit size of Clementine is a major problem in the Western and Eastern Cape citrus production regions. Production costs associated with fruit size improvement cultural practices are high. Since mandarins have a lower sensitivity to CTV, it may not be essential to pre-immunize mandarin cultivars to protect them against severe strains of CTV. The prospect to improve size of fruit borne on virus-free trees needs to be investigated.

Materials and Methods

This trial was initiated by Prof. E. Rabe and was taken over by S.P. van Vuuren when Prof. Rabe left South Africa. Virus-free and LMS 6 pre-immunized trees of seven Clementine selections (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) and one satsuma selection (Miho Wase) were prepared on Swingle citrumelo rootstock in a commercial nursery (rootstocks may have got infected with CTV prior to budding).

When the scions had developed they were planted at Addo Research Station according to a randomized block design in 2003. Since there was a variation in the number of trees available, they were split in three separate trials. Trial one consisted of selections Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated four times. Trial two consisted of selections Clementine late, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated five times. Trial three was Miho Wase satsuma and each treatment was replicated eight times. Growth, production and fruit size will be the main criteria in the trials.

Results and Discussion

The trunk circumference of the trees was measured one year after planting and is presented in Table 4.2.11.1. The trials are too young to interpret the results.

Table 4.2.11.1. Trunk circumference (mm) of virus-free (VF) and pre-immunised (PI) Clementine selections and Miho Wase Satsuma one year after planting.

Cultivar or selection	Trial 1		Trial 2		Trial 3	
	VF	PI	VF	PI	VF	PI
Oronules	63	56	-	-	-	-
Clementine late	61	64	65	56	-	-
Esbal	65	55	56	58	-	-
Orogrande	70	63	67	66	-	-
Guillermína	50	51	57	58	-	-
Nour	62	59	68	55	-	-
Clemenpons	55	49	55	59	-	-
Miho Wase	-	-	-	-	70	67

Conclusion

No conclusion yet.

Future research

Measure tree size and harvest fruit when they start bearing.

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4.2.12 Evaluation of CTV isolates in navel

Experiment 561004: Trial 4 by S.P.van Vuuren (ARC-ITSC)

Opsomming

Verskillende *Sitrus tristeza virus* (STV) isolate word in Palmer navel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Die vyf-jaar oue bome met LMS 6 en SM 45 isolate was betekenisvol groter as bome wat met isolate SM 36 en die bekende strawwe isolaat geïnkuleer is. Die onderstamme het tans geen effek op boom grootte nie. Nietemin, daar was interaksies tussen die trifoliaat tipe ondertamme en die ligte STV isolate, bv. die grootste bome op Swingle ondertam is met SM 41 gepre-immuniseer terwyl bome met dieselfde isolaat op Troyer en C 35 betekenisvol kleiner was. Tans presteer bome met LMS 6, die huidige kruisbeskermings-agent, die beste.

Introduction

The failure of sour orange as a rootstock for most citrus cultivars in South Africa in 1896, is probably the earliest recorded evidence for the presence of citrus tristeza virus (CTV), although it does not necessarily mean that South Africa is the country of origin (Oberholzer, 1959; Webber, 1925). The sensitive sour orange rootstock was abandoned because of CTV quick decline, and replaced by tolerant rootstocks such as rough lemon (Marloth, 1938). This practice is no solution for sensitive scion cultivars such as grapefruit and cross protection with mild isolates is the most successful approach to reduce the effect of the disease (Müller & Costa, 1972; van Vuuren, *et al.*, 1993a, 1993b).

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that tristeza virus has no effect on sweet oranges and mandarins. This situation can be partly ascribed to nurserymen who unwittingly applied cross protection by selecting parent trees showing the best health and production. These trees carried virus that had the least effect on growth and production. With the implementation of shoot-tip grafting, the virus-free material is vulnerable to infection by various strains occurring in nature, which are transmitted by aphids. Evidence of the presence of severe strains that can affect sweet orange exist in foreign countries (Barkley, 1991; Roistacher, 1988) as well as in South Africa (Marais, 1994).

All citrus cultivars in the Southern African Citrus Improvement Programme are freed from viruses by shoot-tip grafting (de Lange, *et al.*, 1981). The abundance of the most effective aphid vector, *Toxoptera citricida* (Kirk.) will result in virus-free trees becoming naturally infected with various strains (Schwarz, 1965) including virulent strains (Barkley, 1991; Calavan, *et al.*, 1980; Müller, *et al.*, 1968). It is therefore necessary to protect the virus-freed plants from severe CTV strains by deliberately infecting them with mild strains (de Lange, *et al.*, 1981; von Broembsen & Lee, 1988). The interaction of mild CTV isolates with regard to cross protection is specific with regard to biological activity (Müller & Costa, 1987; Van Vuuren, *et al.*, 1993b) and therefore, mild CTV isolates specifically for tolerant cultivars should be identified.

The aim of this research is to obtain suitable isolates to cross protect navel in the Eastern Cape production area.

Materials and methods

CTV isolates are evaluated in Palmer navel on four commercial rootstocks for that area *viz.* Rough lemon, Troyer citrange, Swingle citrumelo and C35 citrange (J. Miller, personal communication). Three tristeza virus isolates, with the seedling yellows component, (SM 36, SM 41, SM 45) are being evaluated and compared to trees with LMS 6 (standard), a severe isolate (SOSS 2) and trees that were left un-inoculated. The trees were prepared according to standard nursery practices in an aphid-free environment.

The trees were planted at Addo in November 1999 according to a split plot design with five replications.

The effect of the CTV isolates on growth, production, fruit size and tree health will be determined.

Results and discussion

Tree size, production and yield efficiency are presented in Table 4.2.12.1, Table 4.2.12.2 and Table 4.2.12.3 respectively. Overall, trees with isolate LMS 6 (present pre-immunizing isolate) and SM 45 were the largest but not significantly than those with isolate SM 41 and the control trees (Table 4.2.12.1). Trees with SM 36 and the severe isolate were significantly smaller than trees with isolates LMS 6 and SM 45. Overall the rootstocks did not affect tree size. There were interactions between the trifoliate type rootstocks and some of the mild CTV isolates, *viz.* the largest trees on Swingle rootstock were pre-immunized with SM 41 while trees with the same isolate on Troyer and C 35 were significantly smaller. Not only were trees with LMS 6 and SM 45 the largest, they also had the highest yield efficiency (Table 4.2.12.3), with the result that they had a significant higher production per tree than those with the other mild isolates as well as the trees that were planted virus-free (control) (Table 4.2.12.2).

Table 4.2.12.1. The effect of different CTV isolates on the growth (canopy volume = m³) of 5-year-old Palmer navel on different rootstocks¹.

CTV Isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	6.6 a	5.5 ab	5.3 ab	7.1 a	6.1 x
SM 36	4.8 ab	3.8 b	3.9 b	4.6 bc	4.3 yz
SM 41	6.1 ab	7.2 a	3.6 b	3.5 c	5.1 xy
SM 45	6.6 a	5.0 ab	6.4 a	6.3 ab	6.1 x
SOSS 2	3.8 b	3.1 b	3.2 b	3.1 c	3.3 z
Control	6.2 ab	3.5 b	4.8 ab	5.4 abc	5.0 xy
Mean	5.7 z	4.7 z	4.5 z	5.0 z	

¹ Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

² Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Table 4.2.12.2. The effect of different CTV isolates on the production (kg/tree) of 5-year-old Palmer navel on different rootstocks¹.

CTV isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	37.6 a	45.1 a	27.3 ab	47.5 a	39.4 x
SM 36	22.0 a	17.5 b	22.6 ab	29.7 a	23.0 z
SM 41	36.6 a	19.7 b	13.7 b	35.7 a	26.4 z
SM 45	31.4 a	30.3 b	39.9 a	42.6 a	36.1 xy
SOSS 2	28.2 a	21.8 b	28.4 ab	32.2 a	27.7 yz
Control	26.0 a	13.7 b	23.1 ab	41.2 a	26.0 z
Mean	30.3 xy	24.7 y	25.8 y	38.2 x	

¹ Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

² Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Table 4.2.12.3. The effect of different CTV isolates on the yield efficiency (kg/m³) of 5-year-old Palmer navel on different rootstocks¹.

CTV Isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	5.9 a	8.8 a	6.5 ab	6.9 a	7.0 xy
SM 36	4.8 a	5.7 b	7.0 ab	7.3 a	6.2 y
SM 41	6.3 a	2.3 b	3.7 b	10.9 a	5.8 y
SM 45	5.3 a	7.4 ab	6.6 ab	6.8 a	6.5 y
SOSS 2	8.1 a	6.0 b	11.4 a	11.0 a	9.1 x
Control	4.0 a	2.5 b	4.5 b	7.9 a	4.7 y
Mean	5.7 y	5.5 y	6.6 xy	8.5 x	

¹ Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

² Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Conclusion

Overall the rootstocks did not affect tree size, however, the CTV isolates affected size. Trees with SM 36 and the severe isolate (SOSS 3) were significantly smaller than trees with LMS 6, SM 45 and the control trees. Interactions occurred between the trifoliate type rootstocks and some of the mild CTV isolates. Currently trees with LMS 6, the present pre-immunising CTV isolate, are performing the best.

Future research

Measure trees, harvest and grade fruit.

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4.2.13 Identification of suitable *Citrus tristeza virus* isolates for pre-immunizing Turkey Valencia Experiment 561023: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig is vir *Sitrus tristeza virus* (STV) as ander Valencia tipes (CRI Groep Navorsings-jaarverlag, 2003). Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte STV isolaat te vind om Turkey Valencia te pre-immuniseer. Virusvrye Turkey Valencia op Troyer citrange onderstam word in 'n glashuis voorberei en met STV isolaat (LMS 6 (standaard), SM 45, SM 46, SM 47, SM 48, SM 49, GFMS 12 (positiewe kontrole), virusvry (negatiewe kontrole), wat vanaf soetlemoene versamel is, geïnkuleer om die beste ligte isolaat te identifiseer vir kruisbeskermings-doeleindes. Die proef is nog in die glashuis stadium waar die isolaat geïnkuleer word waarna preïmmunisasie bevestig sal word met ELISA en die bome in 'n boord uitgeplant sal word.

Introduction

Cross protection to control *Citrus tristeza virus* (CTV) is specific with regard to the citrus type, i.e. the most effective protecting CTV isolate for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Programme, all citrus, including sweet oranges, was pre-immunized with a CTV isolate originating from grapefruit for the interim until suitable isolates was found for the different types (von Broembsen & Lee, 1988). Subsequently a suitable isolate, LMS 6, has been identified for lime (van Vuuren, *et al.*, 1993). LMS 6 contains a mild form of seedling yellows which the grapefruit isolate does not have, and therefore it was also approved to replace GFMS 12 as the pre-immunizing isolate for sweet oranges (van Vuuren, *et al.*, 2000).

The suitability of LMS 6 as a protector for sweet oranges and mandarins has not been confirmed and evaluations are currently being done in Clementine, navel and Valencia. Midseasons and other sweet oranges are known to be affected by a transmissible factor causing abnormal bud-union on Rough lemon rootstock (McClellan, 1974). The transmissible factor is not insect or seed transmissible and was not correlated to any known citrus disease. It has been shown that it can be removed by shoot tip grafting (Navarro, *et al.*, 1993).

Recently it was found that Turkey Valencia trees on Rough lemon and Volckameriana rootstocks developed bud-union creasing symptoms (personal observation)(Beeton, *et al.*, 2000). Various sources of Turkey Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other

Valencia cultivars (CRI Group Annual Research Report, 2003). Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV isolate for cross-protection remains a high priority.

The objective of this study is to evaluate CTV isolates to identify a suitable cross protecting isolate without the bud-union crease factor for Turkey Valencia.

Materials and methods

Virus-free Turkey Valencia scions on Troyer citrange rootstocks are prepared in the glasshouse according to normal nursery practices. When the scions have developed to approximately 5 mm, they will be inoculated with different mild CTV isolates by budding two buds containing the required CTV isolate into the scions (Table 4.2.13.1). After three months the trees will be tested for the presence of the CTV isolates by ELISA. When pre-immunisation is confirmed, they will be planted in the field where they will be subjected to normal CTV challenge by aphids. Each treatment will be replicated five times and uninoculated virus-free trees will serve as controls. Evaluations will be on growth, production and tree health.

Table 4.2.13.1. Treatments for Turkey Valencia on Troyer citrange rootstock to identify a suitable CTV isolate for pre-immunisation

Treatment	Origin and comments
LMS 6	Mexican lime, Tzaneen. Present pre-immunising isolate for sweet orange
SM 45	Portsgate Valencia, Hoedspruit. Show promise in current cross protecting trials
SM 46	Shamouti Midseason, Messina.
SM 47	Valencia, Grahamstown. Tree > 100 years old
SM 48	Midseason, Citrusdal. First planting of citrus in the area
SM 49	Valencia, Nelspruit. Induce greening tolerance
GFMS 12	Grapefruit, Nartia. Possitive control
Control	Virus-free. Negative control

Results and discussion

The trees have been made and are ready for inoculating the treatments.

Conclusion

No conclusion.

Future research

Inoculate trees with different CTV isolates and confirm infection by ELISA. Plant in field. Take data of growth, production and tree health.

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4.2.14 Evaluation of CTV isolates in Valencia

Experiment 561004: Trial 2 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Die effek van verskillende ligte *Sitrus tristeza virus* (STV) isolate (LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49) word in drie Valencia bostamme (McCleen, McCleen Saadloos en Delta) op Troyer citrange onderstam ge-evalueer. Boom grootte van die drie Valencia seleksies het nie van mekaar verskil nie. Bome met die SM 49 STV isolaat was betekenisvol grootter as die bome met die ander isolate. Interaksies tussen bostamme en STV isolate het voorgekom. So was bome McCleen saadlose Valencia met isolate SM 41 en SM 45 betekenisvol grootter as McCleen Valencia bome met dieselfde isolate. Van die bostamme, het McCleen saadloos bome 'n betekenisvol beter produksie gelewer as McCleen en Delta bome (44% en 36% respektiewelik). Bome wat gepreïmmuniseer is met SM 49 het die beste geproduseer.

Introduction

Refer to section 4.2.12.

The objective of this trial is to evaluate promising CTV isolates in three Valencia scions and identify suitable cross-protecting isolates.

Materials and methods

McCleen -, McCleen seedless - and Delta Valencia trees on Troyer citrange rootstock were grown according to normal nursery practices under aphid-free conditions. When the scions have developed to approximate five mm in diameter, they were inoculated with isolates derived from sweet orange and showed promise in glasshouse tests. The isolates are LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49. Trees with these isolates will be compared to trees with a severe isolate (SOSS 2) as well as un-inoculated virus-free (VF) plants.

The trees were planted in 2000 according to a split plot design with five replications at Malelane.

The effect of the CTV isolates on growth, production, fruit size and tree health will be determined.

Results and discussion

Tree size

Tree sizes were measured and the canopy volumes calculated according to Burger *et al.* (1970) (Table 4.2.14.1). Overall the canopy volumes of the three Valencia selections did not differ significantly. With the CTV isolates however, trees with the SM 49 isolate had the largest canopies and they were significantly larger than those of trees with isolates LMS 6, SM 34, SM 36 and the trees that were planted virus-free. The smallest trees were pre-immunized with SM 36. Although this isolate has a mild effect on Mexican lime indicator plants, it causes stem pitting in sweet orange.

Comparing the effects of the CTV isolates on the growth of the three scions, show that mild isolates LMS 6, SM 36, SM 49 were stable in all the scions. With the other isolates, isolate SM 34 was significantly better in McCleen Seedless Valencia than in both the other scions, SM 41 and SM 45 were significantly better in McCleen Seedless Valencia than in McCleen Valencia.

Production and yield efficiency

The production of the four-year-old trees of each treatment is shown in Table 4.2.14.2 and the yield efficiency in Table 4.2.14.3. Overall, the McCleen Seedless Valencia trees yielded significantly better than McCleen and Delta Valencia trees. Of the CTV isolates, trees with isolate SM 49 produced significantly better than trees with all the other mild isolates, the severe isolate SOSS 3 and the trees that were planted virus-free.

The McCleen Seedless trees with isolates SM 36, SM 45 and those that were planted virus-free produced significantly lower than trees with SM 34 and SM 49. With McCleen, trees with the different isolates yielded similarly except for trees with SM 49 which was significantly better than trees with SM 36. The Delta Valencia trees with SM 49 were significantly better than trees with all the other treatments except those with SM 41.

McClea Valencia and Delta Valencia trees had significantly lower yield efficiencies than that of McClea Seedless trees.

Table 4.2.14.1. Tree size (canopy volume = m³) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free, four years after planting*.

CTV Isolate	Scion**			Mean
	McC	McC SL	Delta	
LMS 6	7.5 bc	9.1 ab	7.5 c	8.0 v
SM 34	6.7 cd	10.3 a	7.3 c	8.1 v
SM 36	4.3 d	3.9 c	4.6 d	4.3 w
SM 41	8.0 abc	11.1 a	9.3 abc	9.5 uv
SM 45	8.0 abc	11.5 a	9.3 abc	9.6 uv
SM 49	9.5 a	11.1 a	11.4 a	10.7 u
SOSS 3	8.1 abc	10.1 a	8.7 bc	9.0 uv
VF	7.6 bc	7.2 b	7.6 c	7.5 v
Mean	7.5 w	9.3 w	8.2 w	

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

** Scions: McC = McClea Valencia; McC = McClea seedless Valencia; Delta = Delta Valencia.

Table 4.2.14.2. The production (kg/tree) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free, four years after planting*.

CTV Isolate	Scion**			Mean
	McC	McC SL	Delta	
LMS 6	37.9 ab	62.9 abc	31.3 bc	44.0 v
SM 34	30.4 ab	67.8 ab	34.8 bc	44.3 v
SM 36	27.2 b	28.0 d	27.1 c	27.4 w
SM 41	28.8 ab	59.0 bc	42.2 ab	43.3 v
SM 45	36.0 ab	55.1 c	39.3 bc	43.5 v
SM 49	41.9 a	72.9 a	49.1 a	54.6 u
SOSS 3	28.3 ab	62.4 abc	33.0 bc	41.2 v
VF	24.6 ab	49.8 c	35.0 bc	36.5 vw
Mean	31.9 z	57.2 y	36.5 z	

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

** Scions: McC = McClea Valencia; McC = McClea seedless Valencia; Delta = Delta Valencia.

Table 4.2.14.3. The yield efficiency (kg/m³ canopy) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free, four years after planting*.

CTV Isolate	Scion**			Mean
	McC	McC SL	Delta	
LMS 6	5.1 ab	6.9 ab	4.2 ab	5.4 uv
SM 34	4.5 bc	6.6 ab	4.8 ab	5.3 uv
SM 36	6.3 a	7.2 a	5.9 a	6.5 u
SM 41	3.6 bc	5.3 b	4.5 ab	4.5 v
SM 45	4.5 bc	4.8 c	4.2 ab	4.5 v
SM 49	4.4 bc	6.6 ab	4.3 ab	5.1 uv
SOSS 3	3.5 bc	6.2 ab	3.8 b	4.5 v
VF	3.2 c	6.9 ab	4.6 ab	4.9 uv
Mean	4.4 z	6.3 y	4.5 ab	

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

** Scions: McC = McClea Valencia; McC = McClea seedless Valencia; Delta = Delta Valencia.

Conclusion

Of the scions, McClean Seedless Valencia yielded significantly better than McClean and Delta Valencia trees. At this stage CTV isolate SM 49 shows the most promise.

Future research

Harvest, size and weigh fruit. Determine tree size.

References cited

Refer to section 4.2.12.

4.2.15 The effect of different CTV isolates in Valencias on different rootstock combinations for the Orange River Valley

Experiment 739 by JHJ Breytenbach (CRI)

Opsomming

Omdat *Sitrus tristeza virus* gasheer - en klimaat spesifiek is, is dit nodig om isolate wat potensiaal het vir kruisbeskerming, in die verskillende sitrus produserende streke te evalueer. Ligte isolate wat versamel is van goeie soetlemoenbome (SM 36, SM 45, SM 46, SM 47, SM 48, SM 49) sal gebruik word om virusvrye Delta, Midnight, McClean seedless en Turkey Valencia boompies op Troyer citrange, C35 citrange en Minneola x Trifoliaat hibried (MxT) te preïmuniseer. Hierdie isolate sal vergelyk word met LMS 6 (standaard vir soetlemoene) en boompies wat virusvry geplant word. Nadat preïmunisering bevestig is deur middel van ELISA, sal die boompies in die Kakamas omgewing uitgeplant word en jaarliks ge-evalueer word vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte.

Introduction

Refer to section 4.2.12.

The objective of this experiment is to evaluate selected CTV isolates in four Valencia selections on three different rootstocks in order to identify a suitable cross-protecting CTV isolate for specific rootstock/scion combinations in the Orange River Valley. The experiment will give horticultural information on the use of the most suitable Valencia selection on a specific rootstock as well.

Materials and methods

Virus-free budwood of four Valencia scions (Delta, Midnight, McClean seedless, Turkey) was budded to three rootstocks (Troyer citrange, C 35 citrange, Minneola x Trifoliate hybrid (MxT)). When the scions had developed to approximately 5 mm, each Valencia selection was bud-inoculated with six selected CTV isolates originating from sweet orange (SM 36, SM 45, SM 46, SM 47, SM 48, SM 49). These isolates will be compared to trees inoculated with LMS 6 (standard) and trees planted virus-free. Successful pre-immunisation will be confirmed with ELISA whereafter the trees will be planted in the field.

Results and discussion

Virus-free rootstocks were budded with the different virus-free scions.

Conclusion

This experiment is still in the phase of applying all the different treatments.

Future research

Trees will be pre-immunized with the different mild isolates and as soon as pre-immunisation is confirmed by ELISA, they will be planted in the field. Annual evaluations for horticultural performance ie. growth (tree size), health (stem pitting ratings) and yield (kg/tree and fruit size).

References cited

Refer to section 4.2.12.

4.2.16 Screening of rootstocks for Citrus Blight tolerance

Experiment 32 by JHJ Breytenbach (CRI)

Opsomming

Die inokulasie van sitruskroei in Delta Valencia bome op 17 verskillende onderstamme induseer 'n afname in boom grootte en produksie in vergelyking met ongeïnokuleerde bome. Serologiese analises van die 12-kd proteïene wat slegs in sitruskroei besmette bome voorkom, is gebruik om bome te identifiseer wat met sitruskroei besmet is. Uitslae bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Die resultate van die verskillende toetse komplimenteer mekaar nie en maak die interpretasie van die data moeilik. Sommige van die ongeïnokuleer bome begin simptome van sitruskroei toon as gevolg van natuurlike besmetting. Onderstamme soos C35 citrange, Empress mandaryn en Carrizo citrange is die meeste ge-afgekeer deur sitruskroei. Bome op X639, Sun Chu Sha en Sunki mandaryn toon die meeste toleransie teen die siekte terwyl bome op C36 citrange die vatbaarste was.

Introduction

Citrus blight (CB) affects most commercially grown scion cultivars in the citrus production areas of the world where this disease occurs. CB is primarily a disease affecting the rootstock, the most sensitive rootstock cultivars appear to be Rough lemon, Volckameriana and Rangpur lime. These are followed by trifoliolate orange and its citrange hybrids, Cleopatra mandarin, sweet orange and sour orange.

The symptoms of trees with CB are similar to those of a number of other declines of citrus. The finding of distinctive proteins in leaves and roots of infected trees has led to the development of serological tests that are useful in distinguishing trees with CB from those declining from other disorders. Two CB-associated proteins (35 and 12-kd) were purified by preparative electrofocusing and SDS-PAGE. Polyclonal antisera were produced to both proteins, and a monoclonal antibody was produced to the 12-kd protein. Both proteins were readily detected in crude extracts from CB trees by immuno spot and western blot assays. In several experiments, trees with symptoms of CB that were positive by water uptake tests and zinc wood analyses were also positive in the serological tests. Some bearing trees were found to contain the two proteins up to one year before CB symptoms developed. The 12-kd protein was detected in young trees three months after root-graft inoculations (Derrick, *et al.*, 1993).

Until the inception of the Citrus Improvement Programme in South Africa in 1973, practically all commercial citrus orchards were established on Rough Lemon rootstock. Rough Lemon remained the most popular rootstock up until 1990 and in 1991 was superseded by Volckameriana, Swingle citrumelo, Carrizo Citrange and Troyer Citrange. The presence of CB in South Africa has influenced the rootstock choice in affected areas. Rootstocks such as X639 (*Poncirus trifoliata* x Cleopatra mandarin), M&T (Minneola tangelo x *P. trifoliata*) and Swingle citrumelo are being used to establish new plantings. Trees on Swingle citrumelo are being used to replace trees that have succumbed to CB in existing orchards.

This investigation is to identify rootstocks that can be used successfully in CB affected areas.

Materials and methods

A rootstock experiment to test the tolerance of various rootstocks to CB has been established in Letsitele at Bosveld Sitrus. The trial comprises Delta Valencia on Rough lemon, Empress mandarin, Troyer citrange, *P. trifoliata*, Volckameriana, Gou Tou, Orlando tangelo, Sampson tangelo, M&T, X639, Marsh grapefruit, Swingle citrumelo, Troyer citrange, Zhu Luan, Sweet Orange and Carrizo citrange, that were planted in 1990. In 1995 trees on Cleopatra mandarin, C35 and Sun Chu Sha were added, and during 1996 trees on Benton citrange and Sunki mandarin were included.

Virus-free Delta Valencia scion material was used for all the rootstocks. Trees on the different rootstocks were planted in pairs as receptor trees equidistant from a CB infected donor tree. Three to four roots, 5-6 mm in diameter, of one of the pair of receptor trees were approach grafted to the roots of the donor tree. Six pairs of each rootstock were planted and grafted. The non-grafted trees constituted as the controls. The donor trees were selected using standard diagnostic techniques such as water uptake and zinc accumulation in the xylem. The trees were treated with granular formulations of Temik and Ridomil and trunk paint applications of Aliette, every three months to exclude the effects of *Phytophthora* and citrus nematode infections.

The following data are taken each year:

- Tree sizes are measured;
- Yield and fruit size are determined;
- Water uptake tests;
- The presence of the 12-kd protein is determined.

Results and discussion

Since CB is a disease that develops mostly after eight years or more after planting, the results of the canopy size and yield are presented according to their planting dates (Table 4.2.16.1 and Table 4.2.16.2).

Tree size

In the 1990 planting, trees on Volckameriana and Sampson tangelo rootstocks show the least effect on tree size reduction after inoculation. The tree sizes of the 1992 planting show a severe reduction only where M&T rootstock was used. Trees on X639 rootstock were the least affected with trees on Gou Tou and Orlando tangelo rootstocks also showing no or very little tree size reduction. Results of the 1995/96 planting indicate that tree sizes on C 35 citrange rootstock are severely reduced with trees on Sun Chu Sha and Cleopatra rootstocks the least affected. To summarize, trees on X639 rootstock show tolerance in a CB situation while trees on C35 citrange rootstock are the most susceptible.

Yield

Trees on Empress mandarin rootstock had the highest yield of the 1990 planting. However, fruit size was poor (36% < count 105) and it was increased in the CB inoculated trees (64%) with a lower yield (60% reduction). Comparing the percentage decrease in production and the presence of small fruit in control and inoculated trees, Swingle rootstock performed the best in this planting. In the 1992 planting, trees on X639 and Zhu Luan show the least effect on production and the presence of small fruit. Inoculated trees on Sunki mandarin were the least affected in the 1995/96 planting.

Water uptake

The water uptake test shows a decrease in the water uptake ability, due to the presence of occlusions by amorphous plugs, of CB inoculated trees. Trees on Swingle citrumello (early planting) and Gou Tou (later planting) show the least effect and C35 citrange the most affected (Table 4.2.16.3).

CB protein

The presence of the 12-kd protein was much higher in the root-grafted trees than in the control non-grafted trees (Table 4.2.16.4). The latter can get infected by natural means. The 12-kd protein was higher in control trees on X639, C35 citrange and Sunki mandarin rootstocks. The high 12-kd protein in the trees on X639 rootstock does not correlate with the growth of trees on this rootstock, but correlates with the water uptake.

Generally the results are difficult to interpret since water uptake and protein tests do not complement the growth and yield data, *i.e.*, trees on X639 were least affected in growth and yield after inoculation but the control trees had a poorer water uptake and a higher percentage trees with the 12-kd protein of CB.

Table 4.2.16.1. Comparison of tree size (canopy volume) of CB-inoculated and un-inoculated (control) Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Tree volume (m ³)		% Difference
		Control	Inoculated	
<i>P. trifoliata</i>	1990	38.6	29.8	-23
Swingle citrumelo	1990	63.7	44.8	-30
Empress mandarin	1990	45.4	27.1	-40
Carrizo citrange	1990	35.6	20.8	-42
Volckameriana	1990	61.5	55.0	-11
Sampson tangelo	1990	59.2	55.2	-7
Average 1990		50.7	38.8	-23.5
MxT	1992	48.8	38.9	-20
X639	1992	44.8	58.4	30
Gou Tou	1992	59.2	61.6	4
Orlando tangelo	1992	46.3	44.5	-4
Zhu Luan	1992	26.3	23.2	-12
Marsh grapefruit	1992	23.7	28.2	19
Average 1992		41.5	42.5	+2.4
Cleopatra mandarin	1995	21.3	21.5	1
Sun Chu Sha	1995	37.5	40.1	7
C35 citrange	1995	26.5	10.8	-59
Sunki mandarin	1996	15.3	11.8	-23
Benton citrange	1996	11.3	6.7	-41
Average 1995/96		22.4	18.2	-18.3
Mean		39.1	34.0	-13.0

Table 4.2.16.2. Comparison of yield (kg) and % small fruit (< count 105) of control and CB-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year planted	Control		Inoculated	
		Production (kg)	% Small fruit	Production (kg)	% Small fruit
<i>P. trifoliata</i>	1990	106.4	17.1	94.1	27.5
Swingle citrumelo	1990	97.9	3.9	77.7	6.4
Empress mandarin	1990	146.0	35.9	58.6	63.9
Carrizo citrange	1990	113.5	6.6	30.2	4.1
Volckameriana	1990	134.8	23.4	92.4	10.2
Sampson tangelo	1990	22.3	17.6	50.5	9.2
Average 1990		103.5	17.4	67.3	20.2
MxT	1992	82.6	25.1	65.4	21.3
X639	1992	109.8	13.9	83.3	11.7
Gou Tou	1992	48.5	24.4	45.3	20.9
Orlando tangelo	1992	49.1	14.0	73.1	35.2
Zhu Luan	1992	73.0	8.0	75.4	10.2
Marsh grapefruit	1992	33.6	6.9	27.2	8.1
Average 1992		66.1	15.4	61.6	17.9
Cleopatra mandarin	1995	31.1	20.5	27.3	16.0
Sun Chu Sha	1995	43.1	28.5	29.2	22.2
C35 citrange	1995	45.9	7.8	31.1	7.5
Sunki mandarin	1996	66.0	16.5	53.0	12.9
Benton citrange	1996	70.3	5.5	23.5	3.6
Average 1995/96		51.3	15.8	32.8	12.4
Mean		74.9	16.2	55.1	17.1

Table 4.2.16.3 Comparison of water-uptake (seconds/10 ml) through the trunk xylem of CB-inoculated and uninoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Uninoculated	Inoculated	% Difference
<i>P. trifoliata</i>	1990	55.1	140.2	155
Swingle citrumelo	1990	84.0	75.3	-10
Empress mandarin	1990	95.6	96.4	-1
Carrizo citrange	1990	78.3	168.5	115
Volckameriana	1990	81.8	83.6	2
Sampson tangelo	1990	108.0	115.5	7
MxT	1992	56.8	110.8	95
X639	1992	151.0	57.8	-62
Gou Tou	1992	45.8	81.8	79
Orlando tangelo	1992	82.3	78.5	-5
Zhu Luan	1992	85.2	170.5	100
Marsh grapefruit	1992	92.4	162.8	76
Cleopatra mandarin	1995	102.6	172.0	68
Sun Chu Sha	1995	17.2	200.0	1063
C35 citrange	1995	124.2	200.0	61
Sunki mandarin	1996	116.0	115.3	-1
Benton citrange	1996	153.5	20.0	-87

Table 4.2.16.4. Comparison of protein (12-kd) serological tests of CB-inoculated and control Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	% Infected with 12-kd protein	
		Inoculated	Control
<i>P. trifoliata</i>	1990	33.3	16.6
Swingle citrumelo	1990	33.3	16.6
Empress mandarin	1990	50.0	16.6
Carrizo citrange	1990	83.3	16.6
Volckameriana	1990	50.0	16.6
Sampson tangelo	1990	0	16.6
MxT	1992	66.6	0
X639	1992	16.6	33.3
Gou Tou	1992	16.6	16.6
Orlando tangelo	1992	0	16.6
Zhu Luan	1992	33.3	33.3
Marsh grapefruit	1992	16.6	0
Cleopatra mandarin	1995	50.0	50.0
Sun Chu Sha	1995	16.6	0
C35 citrange	1995	66.6	83.3
Sunki mandarin	1996	16.6	50.0
Benton citrange	1996	50.0	0

Conclusion

The results of the different tests do not compliment each other and it is therefore not possible to draw a meaningful conclusion to transfer to growers. Since some of the un-inoculated trees of the first planting are developing CB symptoms due to natural spread, they cannot be compared to the inoculated trees regarding the effect of CB on growth and production any longer. It is therefore suggested that these trees will be evaluated finally in 2005. Evaluations of trees of the other plantings will also be terminated when they reached the same age. Trees on X639, Sun Chu Sha and Sunki mandarin appear to exhibit some tolerance while trees on C35 citrange are the most sensitive.

Future Research

Continue to monitor disease development, measure canopy volumes and take yield data. A final evaluation of the 1990 planting will be made in 2005, 15 years after planting. Final evaluations will be made on the other plantings accordingly.

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4.2.17 The effect of pruning and graft transmissible isolates on Huanglongbing (greening) infection Experiment 561005: Trial 1 by S.P. van Vuuren (CRI)

Opsomming

Hoëdigtheid aanplantings is 'n algemene praktyk in Suid Afrika. Om verdigtheid in die boord te voorkom moet bome gesnoei word. Die effek van hierdie praktyk op die besmetting van vergroeningsiekte in 'n gebied waar die siekte voorkom is ondersoek. Virusvrye Delta Valencia op Troyer citrange onderstam is onder insekvrue toestande gekweek en gepre-immuniseer met LMS 6 (kontrole), CD 6 ('n sitrus viroid verdwergings-agent) en CD 4 ('n Tristeza virus isolaat wat vergroenings-besmetting verminder) voordat die bome in 'n boord geplant is in 1996. Die kontrole behandeling se plantafstand was 6x3 meter terwyl die ander behandelings by 6x2 meter geplant is. Boomvorming en snoei is toegepas by bome wat gepre-immuniseer is met LMS 6 en CD 4 by hoëdigtheid. Die standaard vektorbeheer is toegepas. Oor 'n 4-jaar periode het ongesnoeide bome met CD 4 by hoëdigtheid die hoogste produksie gelewer maar dit was nie betekenisvol beter as die van die kontrole bome nie. Snoei het produksie met 30% en 43% verminder by bome wat respektiewelik met LMS 6 en CD 4 gepre-immuniseer was. Produksie van die bome met die sitrus viroid isolaat (CD 6), was 23% laer as die van die kontrole maar het die laagste persentasie vergroening gehad (18%). Vergroening het toegeneem waar snoei toegepas is in vergelyking met ongesnoeide bome met dieselfde oordraagbare isolate (169% met LMS 6 en 79% met CD 4). CD 4 het vergroening betekenisvol verminder in vergelyking met LMS 6.

Introduction

Escalating production costs of citrus in South Africa necessitate an increase in production per unit area. Improved genetic material together with high density planting are presently the key factors to meet the goal. High density planting at 1000 trees/ha is currently common practice (Rabe, *et al.*, 1996). Better management practices are needed to overcome the disadvantages of high density planting. Overcrowding, which develops within the first five years, makes vehicle access to control diseases and pests difficult (Aubert, 1990; Bevington and Bacon, 1977; Wheaton, *et al.*, 1978) and it should be prevented without a loss of production (Rabe, *et al.*, 1996). In the absence of suitable genetic dwarfing material, and the adverse effects of dwarfing by transmissible agents (van Vuuren and da Graça, 1996a; 1996b), the only practical option in South Africa to control overcrowding is by pruning or tree shaping (Joubert, *et al.*, 1999). However, the adverse affects of pruning on yield (Boswell, *et al.* 1975) and greening infection (Aubert, 1990) have been shown.

A *Citrus tristeza virus* (CTV) isolate that reduced greening infection was identified (van Vuuren and da Graça, 2000; van Vuuren, *et al.*, 2000). In this trial the effectiveness of the isolate was evaluated in a pruned and un-pruned situation, in an area where greening is present.

Materials and methods

Virus-free Delta Valencia on Troyer citrange rootstock was grown under aphid-free conditions in a greenhouse according to normal nursery practices. When the scions had developed to approximately 5 mm thickness, they were inoculated with three different isolates of graft transmissible agents (Table 4.2.17.1). Six months were allowed for the agents to establish in the plants before planting in the field.

Table 4.2.17.1. Graft transmissible agents and pruning treatments.

Treatment	Comments
LMS 6 (Control)	Standard pre-immunising <i>Citrus tristeza virus</i> (CTV) isolate for sweet orange in South Africa (van Vuuren, <i>et al.</i> , 2000).
CD 4	CTV dwarfing isolate that also gave some protection against Huanglongbing (van Vuuren and da Graça, 2000; van Vuuren, <i>et al.</i> , 2000).
CD 6	CD-III dwarfing isolate (van Vuuren, <i>et al.</i> , In Press).
LMS 6 + Prune	Pruning according to Joubert <i>et al.</i> (1999).
CD 4 + Prune	Pruning according to Joubert <i>et al.</i> (1999).

The trees were planted at a spacing of 6 x 2 m (6 x 3 for controls) according to a randomised block design in 1996. Each treatment was replicated five times and consisted of 12-tree plots (8 for controls). The site was situated in the Nelspruit district, which is in a greening area. The standard control measures for greening in South Africa i.e. planting of healthy material, control of the vector *Trioza erytae*, and the removal of infected branches, were followed in the field (Buitendag and von Broembsen, 1993). Pruning and tree shaping started in the first year after planting (Joubert, *et al.*, 1999). The effect of the treatments on tree size, production and Huanglongbing infection were monitored over a 7-year period.

Results and discussion

Pruning and tree shaping was terminated after four years in the field since yield was drastically reduced by the treatment and the aim of obtaining a high production as soon as possible after planting, was not being achieved.

High infections of greening occurred during 2001 and 2002 when unexpectedly high populations of the psylla vector were present despite control measures. Tree size, production and percentage greening infection of each treatment are shown in Tables 4.2.17.2 and 4.2.17.3 respectively.

The control trees were significantly larger than trees in all the other treatments. Pruning reduced tree size significantly in both treatments but more where the dwarfing CTV isolate (CD 4) was used. The size of the CVD-III treatment was similar to the CD 4 pruning treatment and the canopy height of both treatments were significantly reduced in comparison with the other treatments (Table 4.2.17.3).

The control trees (LMS 6) and CD 4 (un-pruned) with 282 and 284 kg of fruit per tree respectively, achieved the highest production. The former had 25.6% greening in comparison with 23.2% of the CD 4 trees. This result does not confirm a previous finding (van Vuuren, *et al.*, 2000). The two pruning treatments (LMS 6 + prune, CD 4 + prune) showed that pruning decreased production by 30% and 43% and increased greening infection by 118% and 46% respectively. Between the pruning treatments, CD 4 trees had 67% less greening than the LMS 6 trees. This shows that the CD 4 CTV isolate gave some protection against Huanglongbing infection, which supports the previous finding (van Vuuren, *et al.*, 2000) (Table 4.2.17.3). The CVd-III isolate (CD 6) had a lower yield than the control trees and although the percentage greening was lower, it was not significant (Table 4.2.17.3).

Table 4.2.17.2. The effect of graft transmissible agents and pruning on tree size (trunk circumference and canopy height) of 7-year-old Delta Valencia trees.

Treatment	Circumference (cm)	Height (m)
LMS 6 (Control)	33.6 a	3.5 a
CD 4	30.3 b	3.4 a
CD 6	27.5 c	2.9 b
LMS 6 + Prune	30.8 b	3.4 a
CD 4 + Prune	26.8 c	2.8 b

Table 4.2.17.3. The effect of graft transmissible agents and pruning on the cumulative yield (kg/tree) and greening infection (%) of 7-year-old Delta Valencia trees over a 4-year production period.

Treatment	Yield (Kg/tree)	% Greening
LMS 6 (Control)	282 a	25.6 ab
CD 4	284 a	23.2 a
CD 6	218 b	18.0 a
LMS 6 + Prune	197 bc	55.9 c
CD 4 + Prune	161 c	37.5 b

Conclusion

- Two main disadvantages of pruning, a reduction of yield (Boswell, *et al.* 1975) and an increase in greening infection (Aubert, 1990), were confirmed with this study.
- To maintain the benefits of high density planting, tree shaping and pruning should not commence before the trees start touching each other.
- Selective pruning of unproductive branches is recommended.

- Pruning should be done after harvest so that the flush rhythms will not be disturbed during which time vector control measures are taken.

Future research

The objective of this trial has been achieved and will therefore be terminated. The results will be published in the Proceedings of the 16th Conference of the International Organization of Citrus Virologists.

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4.2.18 The Association of Group III Citrus Viroids with Gum Pocket Disease in South Africa Experiment 561005, Trial 2 by S.P. van Vuuren (CRI)

Opsomming

Vier sitrus viroïed (SVd) isolate (CD 6, CD 12, CD 22, CD 47), sowel as 'n *Sitrus tristeza virus* (STV) isolaat (CD 4) is ge-evalueer as verdwergings-agente en is vergelyk met die standaard pre-immuniserings STV isolaat (LMS 6). Die isolate is geïnkuleer in virusvrye Midnight Valencia op 13 verskillende onderstamme. Nege van die onderstamme was *Poncirus trifoliata* seleksies (vyf groot blom tipes en vier klein blom tipes) terwyl vier *P. trifoliata* kruisings was. Tydens biologiese indeksering het die SVd isolate tipiese SVd-Groep III simptome ontwikkel maar die intensiteit van die simptome het tussen die isolate verskil. Sekwensies van klone van elk van die SVd isolate is bepaal en, met klein afwykings, was tipies die van SVd-Groep IIIb. Na agt jaar in die boord het die SVd isolate minder uitwerking op bome met die trifoliaat kruising- onderstamme gehad as op bome met die *P. trifoliata* onderstamme ten opsigte van groei, produksie en

“Gum Pocket” siekte ontwikkeling. Bome met die STV preïmmuniserings-isolaat was betekenisvol grootter en ’n betekenisvolle hoër produksie gehad as die met die SVd isolate. Tesame met die trifoliaat kruisings, het Rubidoux *P. trifoliata* die meeste toleransie teen “Gum Pocket” siekte getoon. Die CD 12 en CD 47 isolate het meer virulent voorgekom as die ander twee SVd isolate.

Introduction

Gum Pocket disease in South Africa affects *Poncirus trifoliata* rootstocks of sweet orange trees and was reported in 1969 by Schwarz and McClean. With the use of certified budwood, the disease “disappeared” until citrus viroids (CVd) were experimentally used to reduce tree size for high-density plantings (Van Vuuren and da Graça, 1996a; 1996b). An apparently similar disease, also associated with CVd dwarfing, was described in Australia as Gummy Pitting (Fraser, *et al.*, 1976). Similar disease symptoms were later found in Italy and Turkey (Azeri, 1985; Cartia, *et al.* 1984). Marais *et al.* (1996) associated CVd-III with gum pocket disease in South Africa, however, because the CVd was not fully characterized, the association of CVd-III was questioned (Duran-Villa, *et al.*, 2002). Since a CVd-III isolate is successfully used for CVd dwarfing in Australia (Broadbent, *et al.*, 1992), and an isolate was selected in California that does not induce disease symptoms (Semancik, *et al.*, 1997), attempts were made in South Africa to find a similar isolate. This paper describes the search for such an isolate.

Materials and methods

Isolates

CVd: Four citrus dwarfing (CD) isolates (CD 6, CD 12, CD 22, CD 47) were selected from a collection, maintained in sweet orange on Rough lemon rootstock, and obtained in commercial orchards from trees on various rootstocks. The sources were slash inoculated to healthy Etrog citron (Arizona 861-S-1) to remove the *Citrus tristeza virus* (CTV) present in the isolates and kept in a greenhouse at 28-32°C. These plants served as source plants for the research project. Symptom expression on Etrog citron was compared with CVd-III isolates obtained from California and Spain but the latter two isolates were not used in the field evaluation (obtained from J.S. Semancik, California and N. Duran-Villa, Spain – personal communication).

CTV: Two CTV isolates were included in the trial. CD 4 was collected from a dwarfed Valencia tree on *P. trifoliata* rootstock (van Vuuren and da Graça, 2000) and LMS 6 (standard) is the isolate being used to pre-immunize all virus-free commercial sweet orange material for cross-protection before it is released to the industry.

Isolation of double-stranded RNA (dsRNA) from infected tissue

Pooled samples of bark and midrib tissue (4 g) were frozen with liquid nitrogen and pulverized, and dsRNA was extracted by the phenol-detergent method of Dodds *et al.* (1987) with minor modifications. The aqueous phase containing nucleic acids was adjusted to 35% ethanol, incubated at –20°C for 1 hour and then centrifuged at 8000 g for 20 min to remove impurities. Nucleic acid preparations enriched in dsRNA were obtained by non-ionic cellulose column chromatography (CF-11; Whatman International, Maidstone, England). Cellulose contamination was prevented by an additional phenol extraction step. Finally dsRNA was concentrated by ethanol precipitation and resuspended in 50 µl sterile distilled water.

Reverse transcription-polymerase chain reactions (RT-PCR)

For molecular characterization and differentiation of CVd-III isolates, complementary DNA was synthesized from dsRNA by reverse transcription and PCR amplification using the Titan One Tube RT-PCR System (Roche Diagnostics, GmbH) and primers based on the sequence of CVd-IIIb (Rakowski, *et al.*, 1994). Primer CV-IIIIL contained an *EcoRI* restriction site (5-GGCGGAATTC~~ACTCTCCGTCTTTACTCCA~~-3), while CV-IIIIR had a *HindIII* restriction site (5-TATAAAGCTT~~CTCCGCTAGTCGGAAAGACTCCGC~~-3).

Two microlitre dsRNA was heat-denatured in the presence of 0,4 µM of each primer for 5 min, chilled on ice for 5 min and annealed at room temperature for 30 min. One-step RT-PCR was performed in a 25 µl reaction mixture containing 1 x RT-PCR reaction buffer (with 1.5 mM MgCl₂ and DMSO; Roche Diagnostics, GmbH), 0.2 mM of each dNTP, 5mM DTT, and 0.5 µl enzyme mix (AMV reverse transcriptase and Expand High Fidelity enzyme mix; Roche Diagnostics, GmbH) in addition to the dsRNA-primer mix.

Thermocycling conditions were: 30 min at 50°C for RT, 3 min at 94°C, and 30 cycles of 30 s at 94°C, 20 s at 60°C and 60 s at 68°C. In all cases, a final extension of 5 min at 68°C was used. RT-PCR products were visualized in 1% agarose gels stained with ethidium bromide.

SSCP analysis

For SSCP analysis, a modified procedure described by Yap and McGee (1994) was followed. One microlitre of the RT-PCR product was mixed with 9 µl dH₂O and 1 µl denaturing solution (500 mM NaOH, 10 mM EDTA pH 8.0). The mixture was heated for 10 min at 42°C and 1 µl loading dye added (0.5% xylene [w/v] and 0.5% bromophenol blue [w/v] in deionized formamide). Denatured DNA of the CVd-III RT-PCR products were separated by electrophoresis in a non-denaturing 12% polyacrylamide minigel without glycerol, using 0,5 x TBE (44.5 mM Tris-Borate, 1 mM EDTA, pH 8.0) as electrophoresis buffer and 200 V for 4 h at 8°C. Gels were stained with silver nitrate (Beidler, *et al.*, 1982).

Cloning

RT-PCR products with different SSCP profiles were separated on a 1% agarose gel and eluted by using the Qiaex gel extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The recovered full-length cDNA was digested with *EcoRI/HindIII*, cleaned and ligated to *EcoRI/BamHI* restricted pGEM-3Z vector (Promega). Transformation of *Escherichia coli* JM109 (Promega, Wisconsin, USA) cells and plasmid isolation were done according to standard procedures (Maniatis, *et al.*, 1989).

Sequencing

Sequencing of both strands was performed using dye terminator sequencing kits from ABI and an ABI 310 genetic analyzer.

Preparation of plants for field evaluation

Virus-free Midnight seedless Valencia was budded on nine *Poncirus trifoliata* selections (five large flower types and four small flower types (Shannon, *et al.*, 1960), and four *P. trifoliata* hybrids (Table 4.2.18.1). After the scions had grown to approximately 30 cm, the four CVd and two CTV isolates were bud-inoculated in the scions. Six months were allowed for the agents to establish in the plants whereafter they were planted in the field according to a split plot design with four replications during 1997 in the Nelspruit area.

Table 4.2.18.1. *Poncirus trifoliata* selections and hybrids used to assess the effect of CVd-III isolates on tree size and production of Midnight seedless Valencia.

Rootstock	Type description
Rich	<i>P. trifoliata</i> , large flower.
Argentina	<i>P. trifoliata</i> , large flower.
Kryder 55-1	<i>P. trifoliata</i> , large flower.
Christian	<i>P. trifoliata</i> , large flower.
Yamaguari	<i>P. trifoliata</i> , large flower.
Rich 22-2	<i>P. trifoliata</i> , small flower.
English	<i>P. trifoliata</i> , small flower.
Rubidoux	<i>P. trifoliata</i> , small flower.
Jacobsen	<i>P. trifoliata</i> , small flower.
Benton citrange	<i>P. trifoliata</i> /sweet orange hybrid.
Troyer citrange	<i>P. trifoliata</i> /sweet orange hybrid.
Yuma citrange	<i>P. trifoliata</i> /sweet orange hybrid.
Swingle citrumelo	<i>P. trifoliata</i> /grapefruit hybrid.

Data collection

Biological indexing: The symptoms induced by the four dwarfing isolates were compared with the symptoms induced by the isolates from California and Spain. The two CTV isolates were indexed for the presence of CVd previously and was CVd-free.

Tree size: Canopy volumes were determined according to Burger *et al.* (1970), where the canopy was calculated as a cylinder and half sphere.

Yield: The fruit were picked annually and weighed.

Tree health: The trees were inspected annually for abnormalities.

Results and discussion

Isolate characteristics: With the exception of CD 47, the symptoms induced on Etrog citron by the CD isolates were similar to that of the two CVd-III isolates from California and Spain. Minor differences occurred and the isolate from Spain appeared to give the mildest symptoms. The plants that were inoculated with CD 47 displayed severe vein browning as well as leaf chlorosis and appeared to be more virulent.

Molecular cloning and sequence analysis of CVd-III: Differences among the isolate variants and a comparison with the type sequence of CVd-IIIb (Rakowski, *et al.* 1994) are shown in Table 4.2.18.2. The degree of sequence homology varied only slightly or not at all, ranging from 0 - 5 nucleotide changes. The sequence of the clones in respect of homology and size are those of group CVd-IIIb.

Table 4.2.18.2. Number of nucleotide differences among CVd-III sequence variants.

	CD 6/ 1	CD 6/ 6	CD 6/ 8	CD 6/ 9	CD 12/ 1	CD 12/ 5	CD 12/ 10	CD 22/ 1	CD 22/ 4	CD 22/ 7	CD 47/ 1	CD 47/ 3	CD 47/ 5	CD 47/ 6	CD 47/ 9
CD 6/1	-	1	2	3	1	3	1	3	1	2	1	6	2	2	2
CD 6/6		-	3	5	2	4	2	4	2	3	2	7	3	3	3
CD 6/8			-	3	1	3	3	3	1	2	1	6	2	2	2
CD 6/9				-	2	4	4	4	2	3	2	7	2	3	3
CD 12/1					-	2	2	2	0	1	0	5	1	1	1
CD 12/5						-	4	4	2	4	2	7	3	3	3
CD 12/10							-	4	2	3	2	7	3	3	3
CD 22/1								-	2	3	2	6	3	3	3
CD 22/4									-	1	0	5	1	1	1
CD 22/7										-	1	6	2	2	2
CD 47/1											-	5	1	1	1
CD 47/3												-	6	6	6
CD 47/5													-	2	2
CD 47/6														-	2
CD 47/9															-
IIIb ^z	99.7	99.3	99.7	99.3	100	99.3	99.3	99.7	99.7	99.7	100	98.3	99.7	99.7	99.7

^z Percentage similarity to CVd-IIIb (Rakowski, *et al.*, 1994).

Tree size: The average tree size for each isolate is presented in Table 4.2.18.3. Overall, trees on the *P. trifoliata* selections were significantly smaller than those on the trifoliolate hybrids, except Yuma citrange that is affected by CTV (van Vuuren and da Graça, 1997). There was no difference between trees sizes that were on large flower and small flower *P. trifoliata* types but the trees on hybrid rootstocks were significantly larger. The CVd isolates also reduced tree size significantly in comparison with the control (LMS 6). Among the CVd isolates, trees with CD 6 were significant smaller than those inoculated with CD 22. Tree size of the latter did not differ significantly from those with the CTV dwarfing isolate (CD 4), which were marginally larger, and from those with CD 12 and CD 47, which was smaller. However, trees with CD 4 were significantly larger than those with CD 12 and CD 47.

Table 4.2.18.3. The effect of different rootstocks, with the presence of different graft transmissible isolates on tree size (canopy volume m³) of 7-year-old Midnight Valencia trees*.

Rootstock	Graft Transmissible Isolates						Mean
	LMS 6	CD 4	CD 6	CD 12	CD 22	CD 47	
Rich	4.3 a	3.7 ab	2.1 b	2.6 ab	3.4 ab	1.9 b	3.0 z
Argentina	4.7 a	4.5 a	3.0 ab	2.0 b	2.4 b	2.5 b	3.2 yz
Kryder 55-1	4.5 ab	4.8 a	1.8 c	2.4 c	3.1 bc	2.6 c	3.2 yz
Christian	4.4 a	3.6 ab	1.1 c	1.9 bc	3.7 ab	2.4 bc	2.8 z
Yamaguari	3.2 ab	4.4 a	1.8 c	1.7 c	2.5 bc	1.8 c	2.6 z
Rich 22-2	5.1 a	3.4 b	2.5 bc	1.7 c	3.8 ab	1.3 c	3.0 z
English	4.3 a	3.8 a	1.5 b	1.9 b	3.7 a	1.6 b	2.8 z
Rubidoux	3.0 bc	5.0 a	2.3 c	2.1 c	4.0 ab	2.1 c	3.1 z

Jacobsen	4.3 a	3.7 ab	1.6 cd	1.2 d	3.3 abc	2.0 bcd	2.7 z
Benton citrange	6.1 a	5.2 ab	2.9 c	4.5 abc	4.7 ab	4.0 bc	4.6 y
Troyer citrange	9.6 a	7.2 ab	5.9 b	7.0 b	7.6 ab	5.8 b	7.2 x
Yuma citrange	5.2 a	2.4 b	2.8 ab	2.9 ab	4.1 ab	5.0 ab	3.7 yz
Swingle citrumelo	9.1 a	5.9 b	5.6 b	6.0 b	5.2 b	5.7 b	6.2 x
Mean	5.2 w	4.4 wx	2.7 z	2.9 yz	4.0 xy	3.0 yz	

* Figures in each row of the table that are followed by the same letter do not differ significantly at the 5% level. Rootstock and treatment means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Yield: The cumulative yield over 4 years and the yield efficiency of the last year are presented in Table 4.2.18.4 and Table 4.2.18.5 respectively. In general, the trees on the *P. trifoliata* rootstocks produced less fruit than those on the hybrid rootstocks, however, the yield efficiency (kg/m³) of trees on the *P. trifoliata* rootstocks was 13% higher (Table 4.2.18.5). Three CD isolates (CD 6, CD12, CD 47) enhanced the productivity of the trees with a 25-33% increase in yield efficiency. The increase in yield efficiency confirms a previous report (Semancik, *et al.*, 1997). The yield efficiency of CD 22 was similar to that of the two CTV isolates.

Table 4.2.18.4. The effect of different rootstocks, with the presence of different graft transmissible isolates, on the cumulative yield (kg/tree) of 7-year-old Midnight Valencia trees*.

Rootstock	Graft Transmissible Isolate						Mean
	LMS 6	CD 4	CD 6	CD 12	CD 22	CD 47	
Rich	97.8	82.6	58.6	98.8	113.0	73.0	87.3 bc
Argentina	129.5	114.3	95.8	86.8	62.0	98.5	97.8 b
Kryder 55-1	118.0	99.7	56.7	87.1	110.4	97.2	94.9 b
Christian	126.1	70.5	45.9	60.4	94.4	74.0	78.5 c
Yamaguari	76.2	70.5	45.0	53.5	59.2	63.3	61.3 d
Rich 22-2	121.9	74.2	54.1	52.1	87.2	57.6	74.5 cd
English	134.9	118.9	49.2	77.5	113.2	68.3	93.7 b
Rubidoux	68.2	91.7	65.4	57.2	110.9	50.1	73.9 cd
Jacobsen	98.8	91.0	58.6	39.0	96.9	52.3	72.7 cd
Benton citrange	182.2	148.2	82.6	140.0	174.0	121.9	141.5 a
Troyer citrange	155.8	143.7	75.2	161.0	128.7	111.5	129.3 a
Yuma citrange	144.4	55.5	87.8	85.2	111.1	126.7	101.8 b
Swingle citrumelo	167.0	134.5	140.9	155.1	101.9	124.6	137.3 a
Mean	124.7 a	99.6 b	70.5 d	88.7 c	104.8 b	86.1 c	

* LSD: Rootstocks, 15.0; Graft Transmissible agents, 10.2; Rootstocks/Graft Transmissible agents, 36.8. Rootstock and treatment means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.18.5. The effect of different rootstocks with the presence of different graft transmissible isolates on the yield efficiency (kg/ m³) of 7-year-old Midnight Valencia trees.

Rootstock	Graft Transmissible Isolate						Mean
	LMS 6	CD 4	CD 6	CD 12	CD 22	CD 47	
Rich	10.1	8.3	9.8	14.4	9.7	10.7	10.5
Argentina	10.7	9.4	12.9	15.8	6.0	11.3	11.0
Kryder 55-1	10.9	9.9	12.8	12.2	10.8	11.2	11.3
Christian	10.2	9.4	15.1	14.0	10.5	12.3	11.9

Yamaguari	8.4	7.9	12.1	14.8	9.5	11.1	10.6
Rich 22-2	9.4	8.6	7.2	13.2	9.8	15.0	10.5
English	11.1	11.9	17.7	14.8	10.7	16.9	13.9
Rubidoux	6.1	8.8	9.7	8.0	10.1	10.2	8.8
Jacobsen	8.9	9.6	14.5	10.9	11.0	9.3	10.7
Benton citrange	7.3	10.7	10.6	9.7	12.0	11.3	10.3
Troyer citrange	8.5	8.8	9.0	10.1	8.5	9.9	9.1
Yuma citrange	10.0	12.6	9.5	10.8	11.0	9.1	10.5
Swingle citrumelo	6.6	7.4	10.4	8.7	9.5	9.6	8.7
Mean	9.1	9.5	11.6	12.1	9.9	11.4	

Tree health: Except for Huanglongbing (greening), no other disease symptoms were observed on the scions. The rootstocks, however, displayed several abnormal symptoms. The main abnormal symptom was similar to Gum Pocket disease (Marais, *et al.*, 1996; van Vuuren and da Graça, 1996a and 1996b) (Figure 4.2.18.1 A). A pitting symptom, similar to the report of Duran-Villa, *et al.* (2002), was also observed (Figure 4.2.18.1 B). Sometimes the two symptom types occurred together on a rootstock (Figure 4.2.18.1 C). The occurrence of the symptoms is summarized in Table 4.2.18.6. When both the symptoms occurred on a rootstock, it was regarded as Gum Pocket positive and the pitting symptom disregarded. All the *P. trifoliata* selections showed Gum Pocket and/or pitting symptoms. The Argentina, Kryder 55-1, Rich 22-2 and Jacobsen selections showed the most Gum Pocket symptoms while Jacobsen also showed the most pitting symptoms. No Gum Pocket symptoms developed on Rubidoux trifoliolate but three trees showed pitting symptoms. Gum Pocket symptoms on this rootstock were reported previously (van Vuuren and da Graça, 1996a and 1996b). Yamaguari and English selections also showed some tolerance to Gum Pocket disease and pitting. None of the trifoliolate hybrids displayed any symptoms. However, in a previous study gum-pocket symptoms were noted on Swingle citrumelo (van Vuuren and da Graça, 1996a).

None of the trees with the CTV isolates showed disease symptoms. Of the CVd isolates, all induced Gum Pocket and/or pitting symptoms with CD 12 and CD 47 the most virulent.

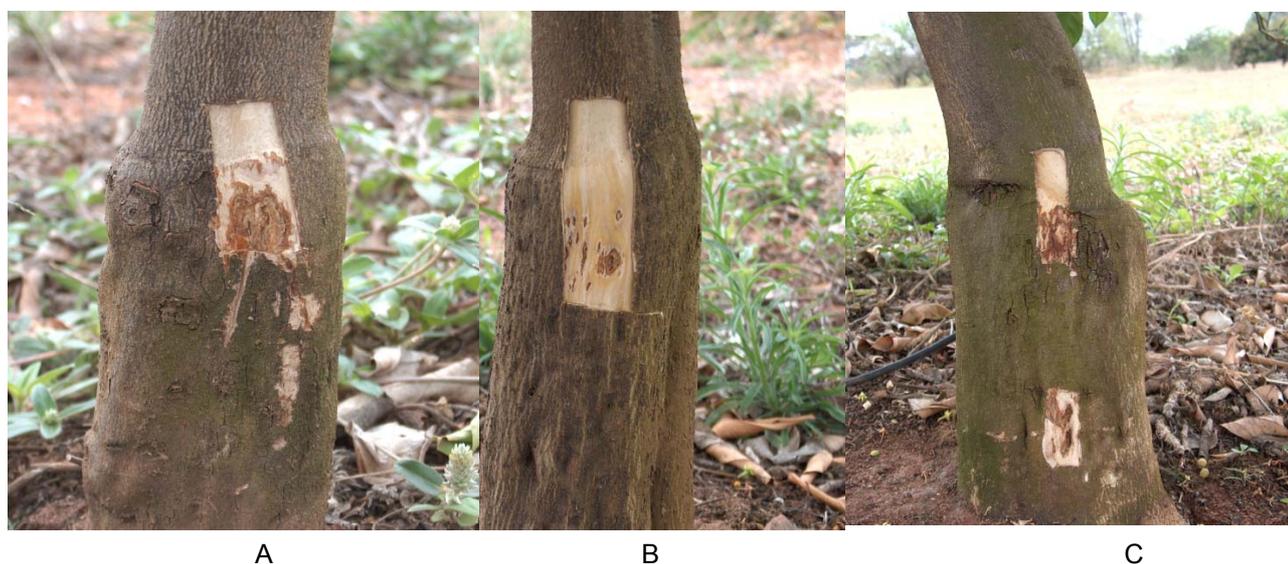


Figure 4.2.18.1. Symptoms displayed by *P. trifoliata* rootstocks seven years after inoculation with CVd-III isolates. A: Typical Gum Pocket disease symptoms. Note dead bark patches. Rootstock: Jacobsen; isolate: CD 47. B: Pitting symptoms showing no dead outer bark but necrosis at the cambium. Rootstock: Christian; isolate: CD 6. C: Gum Pocket and pitting symptoms together on the same rootstock. Note the girdling effect at the budunion. Rootstock: Kryder 55-1; isolate CD 6.

Table 4.2.18.6. The presence of Gum Pocket (GP) and pitting (P) symptoms on different trifoliolate rootstocks (+/4) that were inoculated with different graft transmissible isolates.

Rootstock	Graft Transmissible Isolate												Total	
	LMS 6		CD 4		CD 6		CD 12		CD 22		CD 47			
	GP	P	GP	P	GP	P	GP	P	GP	P	GP	P	GP	P
Rich	0	0	0	0	0	2	2	0	0	0	2	0	4	2
Argentina	0	0	0	0	0	1	3	1	0	1	3	0	6	3
Kryder 55-1	0	0	0	0	1	1	2	1	1	2	2	1	6	5
Christian	0	0	0	0	2	0	1	2	0	2	0	1	3	5
Yamaguari	0	0	0	0	1	0	1	0	0	1	0	0	2	1
Rich 22-2	0	0	0	0	0	0	2	2	0	1	4	0	6	3
English	0	0	0	0	0	0	0	2	0	0	2	0	2	2
Rubidoux	0	0	0	0	0	2	0	1	0	0	0	0	0	3
Jacobsen	0	0	0	0	0	3	4	0	0	4	3	1	7	8
Benton citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Troyer citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yuma citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swingle citrumelo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	4	9	15	9	1	11	16	3		

Conclusion

- The CD isolates were similar to CVd-IIIb (sequence size and homology) and induced Gum-pocket disease.
- The virulence of the CVd-IIIb isolates, although with only minor sequence differences, differed significantly in regard to induce Gum-pocket disease symptoms.
- *P. trifoliata* selections differed in their sensitivity to Gum-pocket disease but there was no difference between large flower and small flower types.
- The trifoliolate hybrids were more tolerant to gum-pocket disease but tree size control was less efficient for the CVd-III isolates.
- None of the CVd-IIIb isolates that were evaluated are suitable for tree size control in high-density plantings on a commercial scale.

Future research

The objective of the trial has been achieved and therefore it is terminated. The results will be published in the Proceedings of the 16th Conference of the International Organization of Citrus Virologists.

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4.3 PROJECT: FRUIT AND FOLIAR DISEASES

Project Co-ordinator: G.C. Schutte (CRI)

4.3.1 Project summary

Various new and old mandarin cultivars were tested for their susceptibility/resistance for *Alternaria* brown spot. Of all the cultivars that were investigated, only Primasol, Cami, M26, Fremont and Bay Gold, showed resistance against *Alternaria* brown spot. Hadass, Cami, Roma, C27, A25, I22, Mor, Michal, Seminole, O7, Lee and Nova were susceptible for *Alternaria* brown spot. A list consisting of susceptible and resistant mandarin cultivars was compiled (4.3.2). Koch's postulates were successfully applied with a *Phytophthora citrophthora* isolate on glasshouse trees (Troyer x Nules) to simulate the die-back of Clementines in the Eastern-Cape. The isolate originates from the orchard in Knysna where the field trial is being conducted. The glasshouse trees showed excretion of gumming within 7 days after inoculation and died within a month. The rootstock was not affected. Control programmes to control the disease are underway. The micro-irrigation in the experimental orchard was also adjusted from a 180° to 90° projection to limit the wetting of the trunks (4.3.3). Monoclonal antibodies (MAb) manufactured for use against *Botrytis cinerea* on grapes, worked well on a spore trap disc coated with Vaseline as mycelium was detected under a fluorescent microscope. Conidia could not be detected. The fungal structures were not embedded within the Vaseline coating and could easily be treated with the MAb. Polyclonal antibodies for *Phytophthora* and *Pythium* were obtained for further work on speedy detection of these fungi in Diagnostic Centre samples (4.3.4).

Projekopsomming

Verskeie nuwe en ou mandaryn kultivars is ondersoek vir hulle vatbaarheid/weerstand teen *Alternaria* bruinvlek. Van al die kultivars wat ondersoek is, het Primasol, Cami, M26, Fremont (tot 'n mate) en Bay Gold, weerstand teen *Alternaria* bruinvlek getoon. Hadass, Cami, Roma, C27, A25, I22, Mor, Michal, Seminole, O7, Lee en Nova was almal met *Alternaria* bruinvlek besmet. Lyste van vatbare- en weerstandbiedende mandaryn kultivars is saamgestel as verwysingsraamwerk en vir gebruik deur planttelers (4.3.2). Koch se

postulate is suksesvol toegepas met 'n *Phytophthora citrophthora* isolaat op glashuisboompies (Troyer x Nules) om die terugsterwing van Clementines in die Oos-Kaap te simuleer. Die isolaat is afkomstig uit die boord in Knysna waar die eksperimente uitgevoer word. Die glashuisboompies het binne sewe dae gomuitskeidings getoon en binne 'n maand het die boompies doodgegaan nadat die hele stam bo die entlas geringuleer was. Die onderstam was nie geaffekteer nie. Spuitprogramme vir die beheer van *Phytophthora* is toegepas en resultate behoort net voor oes in Mei 2005 verkry te word. Die mikrobeproeining is ook in die betrokke boord verander van 'n 180° na 'n 90° projeksie (4.3.3). Die monoklonale teenliggaampies wat vir *Botrytis cinerea* uit Skotland verkry is, was net effektief teen miselium en nie teen konidia soos gehoop is nie. Nogtans het die monoklonale uitstekend gewerk op miselium wat van 'n reinkultuur in 'n spoorvanger ingeblaas is en kon dit maklik waargeneem word onder 'n fluoresensie mikroskoop. Vaseline wat as kleefmiddel op die spoorvangerskyf gebruik is, het geen invloed op die monoklonale teenliggaam se werking en kleefvermoë gehad nie. Poliklonale teenliggaampies is verkry vir *Phytophthora* en *Pythium* vir verdere werk vir die spoedige opsporing van dié swamme in die Diagnostiese Sentrum (4.3.4).

4.3.2 **Screening of new mandarin and mandarin hybrids for their susceptibility/resistance towards Alternaria brown spot**

Experiment 767 by G.C. Schutte (CRI)

Opsomming

Verskeie nuwe en ou mandaryn kultivars is ondersoek vir hulle vatbaarheid/weerstand teen *Alternaria* bruinvlek. Van al die kultivars wat ondersoek is, het Primasol, Cami, M26, Fremont (tot 'n mate) en Bay Gold, weerstand teen *Alternaria* bruinvlek getoon. Hadass, Cami, Roma, C27, A25, I22 (Honey Gold), Mor, Michal, Seminole, O7, Lee en Nova (positiewe kontrole) was almal met *Alternaria* bruinvlek besmet. Lyste van vatbare- en weerstandbiedende Mandaryn kultivars is saamgestel as verwysings raamwerk en vir gebruik deur planttelers.

Introduction

Alternaria brown spot is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all the citrus production areas of the world. Simmons (1999) has described the South African strains to be a different species from that in the USA, viz. *A. turkisafrica*. Susceptible cultivars include Minneola and Orlando tangelo, Murcott tangor, Dancy tangerine and most hybrids of Dancy such as Nova, Sunburst, Rishon, Nouvelle and Lee. *Alternaria* brown spot attacks young flush, twigs and fruit causing small black/brown necrotic spots after a 24 to 36-hour incubation period. The pathogen's conidia produce a host-specific toxin in the presence of free water that can be used to screen new mandarin cultivars for their susceptibility/resistance towards the disease.

Materials and methods

Due to the importance of the project, the procedure of obtaining small trees, inoculating them first and then keeping them in a glasshouse was considered too laborious and time consuming. The trees have to grow to a certain age first and then be pruned to stimulate new growth on which the spore suspension could be placed to do the detached screening of the different cultivars.

The best and fastest method was to go to the ARCs experimental farm at Addo in the Eastern Cape and inspect the selected cultivars and do isolations from the visible lesions and confirm if it is *A. turkisafrica* or not.

The following cultivars were initially selected for evaluation:

- a) Primosole
- b) Bay Gold
- c) Hadass
- d) Cami
- e) Roma
- f) C27
- g) A25
- h) M26
- i) I22 (Honey Gold)
- j) Nova (positive control)

The following cultivars were also inspected for their resistance/susceptibility

- k) Fremont

- l) Mor
- m) Michal
- n) Seminole
- o) O7
- p) Lee

Results and discussion

Isolates were made from and identified as *Alternaria* from all the cultivars that were susceptible to *Alternaria* brown spot except for Primasol, Cami, M26, Fremont and Bay Gold. Fremont also had some lesions, but not that intense as the other cultivars listed and was one of the most promising new cultivars inspected. All the other cultivars had *Alternaria* brown spot infections (Fig. 4.3.2.1).

According to the literature, cultivars such as the Willow leaf and the King mandarin, are resistant to *Alternaria* brown spot. Inspection of these cultivars confirmed this finding and should be considered as ideal parents in the breeding of mandarin cultivars resistant to *Alternaria* brown spot.

Conclusion

Mandarin cultivars can be classified for susceptibility according to their parents as compiled in the following Tables.

Table 4.3.2.1. Relative susceptibility of citrus cultivars to *Alternaria turkisafrina*.

Cultivar	Sensitivity rate
<u>Tangelo</u>	
(<i>Citrus reticulata</i> cv. Dancy mandarin x <i>C. paradisi</i> cv. Duncan grapefruit)	+ +
Minneola (Duncan grapefruit x Dancy tangerine hybrid)	+ +
Orlando (Duncan grapefruit x Dancy tangerine hybrid)	+ +
Sampson (Duncan grapefruit x Dancy tangerine hybrid)	+ +
San jacinto (Duncan grapefruit x Dancy tangerine hybrid)	+ +
Seminole (Duncan grapefruit x Dancy tangerine hybrid)	+ +
Thornton (Duncan grapefruit x Dancy tangerine hybrid)	+ +
Yalaha (Duncan grapefruit x Dancy tangerine hybrid)	+ +
<u>Tangelo x grapefruit hybrids</u>	
Wekiwa (Sampson tangelo x Grapefruit)	+ +
Roma (Robinson mandarin x Marsh grapefruit)	+ +
<u>Clementine mandarin x tangelo hybrids</u>	
Ambersweet (Clementine mandarin x Orlando tangelo hybrid) x 15-3 seedling midseason sweet orange)	?
Fairchild (Clementine mandarin x Orlando tangelo hybrid)	+ +
Fortune (Clementine mandarin x Dancy tangerine hybrid)	+ +
Fremont (Clementine mandarin x Ponkan mandarin)	+
Lee (Clementine mandarin x Orlando tangelo hybrid)	+ +
Michal (Clementine mandarin x Dancy tangerine hybrid)	+ +
Nova (Fina Clementine mandarin x Orlando tangelo hybrid)	+ +
Osceola (Clementine mandarin x Orlando tangelo hybrid)	+ +
Page (Clementine mandarin x Minneola tangelo hybrid)	+ +
Robinson (Clementine mandarin x Orlando tangelo hybrid)	+ +
O 7 (Clementine mandarin x Ellendale tangelo hybrid)	+ +
Sunburst (Clementine mandarin x Orlando tangelo hybrid) x Osceola	?
<u>Mandarins</u>	
(<i>Citrus reticulata</i> & <i>C. reticulata</i> x soft citrus & soft citrus hybrids)	
Calamondin (<i>C. reticulata</i> var. <i>austere</i> x <i>Fortunella japonica</i>)	+ +
Cleopatra (<i>C. reshni</i>)	+
Dancy tangerine (<i>C. reticulata</i> hybrid)	+ +
Frua (King mandarin x Dancy tangerine)	+ +

Pixie (F2: King mandarin x Dancy tangerine)	+ +
Edit (Wilking mandarin x Michal)	+ +
Emperor (<i>C. reticulata</i> hybrid)	+ +
Ponkan (Batangas mandarin/Nagpur santara)	+ +
Satsuma (<i>C. unshiu</i>)	+ +
Tangor	
<i>(Citrus reticulata x C. sinensis)</i>	
Murcott (Honey mandarin)	+ +
Tankan (Ponkan mandarin x sweet orange)	+ +
1610 (Murcott x Clementine)	+ +
Grapefruit	
<i>(Citrus paradisi)</i>	
Marsh	+ +
Redblush	+ +
Star Ruby	+ +
Oroblanco	+ +
Wheeny	+ +
Other cultivars with unknown parents	
I 22	+ +
Cami	+ +
A25	+ +
C27	+ +

+ = mild stippling
++ = severe stippling

Table 4.3.2.2. Citrus cultivars resistant to *Alternaria turkisafria*.

CULTIVAR
Mandarins <i>(C. reticulata)</i> Cardivo di Ciaculli Mandarini Tachibana Unshiu Clementine <i>(Citrus nobilis)</i> King mandarin
Mandarin hybrids Encore (King mandarin x Willowleaf mandarin) Kara (King mandarin x Owari Satsuma) Kinnow (King mandarin x Owari Satsuma) Palazzelli (King mandarin x Clementine mandarin) Wilking (King mandarin x Willowleaf mandarin)
Tangor <i>(Citrus sinensis x C. reticulata)</i> Ortanique (mandarin x sweet orange) Kiyomi (Miyagawa Satsuma x Trovita orange)
Miscellaneous cultivars in Japan Hassaku Hyaganatsu Kawachi bankan Kinkojo Mikan kobayashi Sanbotan

Yama mikan

Other Bay Gold M 26

Future research

Breeders can in future use these tables to determine if new Mandarin cultivars are susceptible for *Alternaria* brown spot. A prerequisite is that they should know what their parents are and then these tables can be used as a guide for predetermining if they are resistant before growers purchase them. The project will be repeated and more new cultivars will be included in this project.

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Fig. 4.3.2.1. Alternaria brown spot lesions on a new Mandarin cultivar (I22) at the ARC's experimental farm at Addo in the Eastern Cape.

4.3.3 Investigation into die-back of Clementines in the Eastern-Cape Experiment 736 by G.C. Schutte (CRI)

Opsomming

Koch se postulate is suksesvol toegepas met 'n *Phytophthora citrophthora* isolaat op glashuis boompies (Troyer x Nules). Die isolaat is afkomstig uit die boord in Knysna waar die eksperimente uitgevoer word. Die glashuisboompies het binne sewe dae gomuitskeidings getoon en binne 'n maand het die boompies doodgegaan nadat die hele stam bo die entlas geringuleer was. Spuitprogramme vir die beheer van *Phytophthora* is toegepas en resultate behoort net voor oes in Mei 2005 verkry te word. Die mikrobeproeing is ook in die betrokke boord verander van 'n 180° na 'n 90° projeksie.

Introduction

Tree die-back was observed in the Knysna region of the Eastern Cape on the Nules Clementine selection. Other Clementine selections such as Marisol, SRA and Oroval seem to not be affected by this unknown disease complex. The die-back starts right on the scion (30 cm above ground level) as white fluffy mycelial growth and is accompanied by anthracnose and *Diplodia* fungal growth (both are secondary wound pathogens). To identify the fungus/fungi involved, isolates were collected and used to fulfil Koch's postulates using the same rootstock and cultivar. For the time being, the trees have to be protected using different fungicides to determine the efficacy of each and to eliminate those that are not effective.

Materials and methods

Isolations

During a visit to the farm "Candlewood" near Knysna on 24 September 2003, bark samples were taken from infected trees and taken to CRI in Nelspruit. From these isolates, 6 sub-samples with two replicates each were aseptically removed and placed onto PDA to secure a pure culture. One of the sub-cultures of each isolate was sent to the Plant Protection Research Institute in Pretoria for identification. The other sub-samples were kept for later use to fulfil Koch's postulates.

Koch's postulates

Thirty nursery trees (Troyer x Nules) were obtained from the CFB in Uitenhage and taken to CRI in Nelspruit where they were kept in a glasshouse. On the stems of these trees (above the scion), a 5x5 cm² area was surface sterilized with ethanol using three trees as replicates for each fungus. After a while, a 1x1 cm² within this larger area was wounded by means of a sterile scalpel where only the bark was slightly scraped to allow a site for entry into the stem. The following fungal isolates were evaluated:

- a) *Colletotrichum gloeosporioides*
- b) *Glomerella cingulata*
- c) *Fusarium solani*
- d) *Fusarium cf. oxysporum*
- e) *Diplodia natalensis*
- f) *Phytophthora nicotianae var. parasitica*
- g) *Phytophthora citrophthora*

Fungicidal treatments

The selected orchard at Candlewood farm was sub-divided into three groups consisting of three rows each. These rows were marked and the following treatments were applied at the following rates:

- a) Ridomil Gold (2 ml/m²)
- b) Fighter (1 l/hl water)
- c) Captan (300 g/hl water)

Tree rating

Prior to each treatment, all the trees were rated using two evaluation techniques, viz. the tree condition (according to a 10 point index where 1 = healthy and 10 = defoliated canopy) and lesion size on the trunk (according to a 10 point index where 0% = no lesion and 100% = trunk completely covered with fungal growth and gum and ring barked).

Results and discussion

Of all the isolates evaluated to fulfil Koch's postulates, only the two *Phytophthora* isolates gave similar symptoms to those observed in the orchard. Of these, only *Phytophthora citrophthora* resulted in the death of the tree while both isolates resulted in the excretion of gum after 7 days. White hyphal growth was observed soon afterwards, growing upwards on the bark away from the gum excretion (Figs. 4.3.3.1 & 2). From this fungal growth, *P. citrophthora* was successfully isolated onto PDA and Koch's postulates were fulfilled. *Phytophthora citrophthora* also made out 100% of all the isolations made from soil samples at Candlewood farm near Knysna. An isolate from this orchard was also deposited at the PPRI collection in Pretoria.

Conclusion

Now that the primary cause of the sudden decline of the Nules Clementines in Knysna has been established, the means of controlling this disease will surely be the most important factor. Trials are underway but results were not available at the time of reporting, but work will continue.

Future research

Reports were also received of similar problem in the Gamtoos Valley as well as certain Clementine groves in the Western Cape. These farms should be inspected to determine the extent of the problem on Clementines. An electron microscope study is also planned.

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Fig. 4.3.3.1. Close-up of typical foot rot symptoms on Nules caused by *Phytophthora citrophthora* showing large amounts of excreted gum and extensive dark, water-soaked artificial inoculated lesions on the trunk growing away from the gumming part.



Fig. 4.3.3.2. Typical foot rot symptoms on Nules caused by *Phytophthora citrophthora* showing large amounts of excreted gum and extensive, dark, water-soaked lesions on the trunk.

4.3.4 The pros and cons of a monoclonal antibody developed for *Botrytis cinerea* to be used with the Quest spore trap

Experiment 752 by G.C. Schutte and Gerhard Pietersen (CRI)

Opsomming

Die monoklonale teenliggaampies wat vir *Botrytis cinerea* uit Skotland vekry is, was net effektief teen miselium en nie teen konidia soos gehoop is nie. Nogtans het die monoklonale uitstekend gewerk op miselium wat van 'n reinkultuur wat in 'n spoorvanger ingeblaas is en kon dit maklik waargeneem word onder 'n fluoresensie mikroskoop. Vaseline wat as kleefmiddel op die spoorvangerskyf gebruik is, het geen invloed op die monoklonale teenliggaam se werking en kleefvermoë gehad nie.

Introduction

Currently, professional people are required to identify and count the laborious task of fungal spores on any spore trap disk. The South African manufactured spore trap with its unique spore trap monitoring disk can easily be used to apply mono-/poly clonal antibodies which will react with specific fungi. Growers, students, extension officers or researchers then easily count the fluorescent spores under a fluorescent microscope. According to a catalogue, a monoclonal antibody (MAb) exists for the fungus, *Botrytis cinerea*, on grapes.

The aim of this study is to determine the pros and cons of an existing monoclonal antibody such as the one mentioned above to determine whether it can be used with the existing sticking agents currently used to treat the monitoring disc, whether the thickness of the sticking agent will have an effect on the binding ability of the MAb to the fungal cell wall. If there is no problem with the existing system currently employed in disease monitoring (such as used for CBS).

Materials and methods

A MAb for *Botrytis cinerea* was purchased from Scotland. A Quest spore trap was mounted in the laboratory at the University of Stellenbosch. Conidia from a pure 7-day-old culture of *B. cinerea* was blown into the air allowing the spore trap to collect the conidia on the spore trap disc which was coated with aerosol Vaseline. The monitoring disc was then microscopically investigated to determine the efficacy of how the MAb bound to the surface of *B. cinerea*. If Vaseline did not give the desired results, a suitable sticking agent would have to be found.

Results and discussion

The conidia caught on the spore trap disc, were not highlighted under the fluorescent microscope as expected. This finding was confirmed by the manufacturer. According to them, the MAb is only effective for use on mycelium and not conidia. However, when tested with bits of mycelium, the MAb worked well and the mycelium could be easily detected under the fluorescent microscope. Aerosol Vaseline used as the sticking agent in this study, worked well without any problems such as embedding the fungal structures deep in the Vaseline.

Conclusion

The property that would allow one to differentiate *G. citricarpa* from *G. mangiferae* would be the presence of an epitope (a piece of a protein capable of eliciting an antibody response) unique to one or the other species. Complex organisms have huge amounts of proteins in common with related species, all of which would induce antibodies capable of detecting all organisms with those proteins. So to produce a specific antibody, one would first have to identify the unique protein and then purify it to the extent that other proteins (common to the two species) are lacking from the preparation. This preparation could then be used to produce a polyclonal antibody.

An alternative, more shotgun approach is to immunize a mouse with a mixture of one species' proteins, and then to prepare monoclonal antibodies, and then to screen these for the unique monoclonal antibody with both species, looking for the unique monoclonal is very expensive, very labour intensive, and luck needed to find unique protein if other proteins are immunodominant.

A further possibility is make a preparation of proteins enriched with the unique protein (i.e. first identify it and then purify it) to screen a recombinant library of proteins, and through panning to obtain evermore enriched preparations of specific antibodies, followed by obtaining a monoclonal recombinant line.

Probably the only remaining alternative is to take the unique sequence (shown by PCR), clone it in an expression system, express the proteins (hoping that a unique piece is competent to express a unique protein epitope), purify this and use it for immunization.

These are all do-able possibilities but as one can see are not trivial and will require that a researcher spend a dedicated period on this piece of work. This is why there are relatively few antisera available to bacteria and fungi (while a lot more antisera to viruses). The actual immunization, bleeding, purification of antibodies, conjugation and development of ELISA do not differ too dramatically from one organism to another and becomes routine very shortly after doing it once or twice. The final aspect is that the antiserum (and ultimate ELISA) would only detect the stages of the fungi in which the unique epitope is expressed but there are relatively major changes in protein expression in different stages.

Future research

Lateral flow devices made by Central Science Laboratories, UK, to produce these is the best institution as they are probably the leaders in this technology and could be approached as soon as such a study is completed. We have obtained PAb for *Phytophthora* and *Pythium* spp. from the same company in Scotland to see if we can shorten the diagnostic procedures in the DC.

4.4 PROJECT: SOILBORNE DISEASES Project Co-ordinator: M.C. Pretorius (CRI)

4.4.1 Project summary

As part of an integrated pest management approach genetic resistance is a reliable replant strategy which is applied as common practice by citrus producers. Twenty years ago 90% of the rootstocks used in South Africa were Rough Lemon, a highly susceptible rootstock to pathogens related to the replant problem, viz. citrus nematodes and *Phytophthora* spp. Today 80% of rootstocks utilized by the industry due to research are Trifoliolate hybrids, of which Troyer, Carrizo citrange and Swingle citrumelo are the most common. Since new rootstocks are frequently introduced into South Africa, it is necessary to screen these rootstocks for their levels of resistance against nematodes. Fourty different rootstocks were screened for resistance against the citrus nematode. This year's data confirms last year's data showing that C35 and Swingle could successfully be utilized as replant rootstocks. C32 citrange can also be recommended to be utilized. According to CRI's

cultivar developer, C32 citrange has excellent production characteristics but due to a shortage of plant material not many commercial trees are available for planting. It is recommended that this rootstock be evaluated on a semi-commercial scale. X639 and Carrizo citrange rootstocks performed well. These rootstocks could be regarded as susceptible. From these results which confirm last year's data, it is clear that Wallace Rough lemon could not be recommended for use on replant soils infested with nematodes. The trial will be terminated (4.4.2).

None of the registered post-plant nematicides have an effect on the citrus nematode, *Tylenchulus semipenetrans*, eggs. The purpose of this trial was to synchronize hatching of nematode eggs and to eradicate the nematode population in the soil by using one or two nematicide treatments. The results obtained after three applications, April 2003, were however disappointing and confusing because no definite correlation was evident between the different treatments regarding time of application, different dosages or the stimulating products on their own. Rugby (3x15g/m²) on its own was the most effective treatment and it can be concluded that it is still the best and most effective control measure to be used by the producers to control citrus nematode populations in citrus. Until such time that more information is available the trial will be terminated (4.4.3).

The work conducted was to find an effective solution to replace the organophosphates with biological control agents. The PL+ trichoderma treatments had a -41 and -17% and -8 an 11% decrease in citrus nematode larvae and female populations in the soil and roots of citrus trees respectively. Although a slight decrease was evident and the results were promising this product should still be evaluated on a commercial scale. The results confirm the fact that a chemical nematicide such as Rugby (3 x 15g/m²) is still the most effective control measure to control the citrus nematode in citrus orchards. Products such as PL+, a biological control product, should be utilized in combination with a nematicide with regular follow-up applications. The trial will be terminated (4.4.4).

The citrus nematode, *Tylenchulus semipenetrans*, is present in most of the citrus replant orchards in South Africa. A few years ago *Hemicycliophora*, the sheath nematode, was detected in root samples in combination with the citrus nematode, in the Gamtoos River Valley. Three species were identified, viz. *H. lalophila*, *H. nortoni* and *H. typical*. However, it was essential to evaluate the effect of this combination of nematodes on popular replant rootstocks currently used by the citrus industry. The following rootstocks were evaluated; Rough lemon, Swingle citrumelo, Carrizo citrange and C35. During the 2003 season the Swingle citrumelo rootstock's nematode female counts were significantly lower than all the other rootstocks. According to the 2004 season's results it is clear that an increase in citrus nematode population numbers in the soil and roots is evident. Even the Swingle rootstock which is regarded as a resistant rootstock was infected. When sampling for nematode analysis, it was difficult to distinguish between roots of the existing trees and roots of the younger trees. This practice caused dramatic stress conditions to the trees and therefore the trees will only be sampled again at the end of 2005 whereafter the trial will be terminated. The Swingle rootstock situation will be monitored closely. The trees are currently still young and therefore no conclusion could be drawn (4.4.5).

The relatively stable behaviour of animal populations in natural environments should serve as a constant reminder that in nature all organisms are subjected to a constant series of checks and balances. In the past applications of the PL+ treatments were done by hand under a micro irrigation system. The use of this biological control product should be evaluated under a drip irrigation system, which could ensure that conditions are more favourable for a Biological control agent to become established in the soil. No suitable site could be found in the Nelspruit region. Trial sites in other citrus growing regions will be visited, sampled and evaluated for utilizing as a suitable site. No results are therefore available (4.4.6).

The phosphonates are currently the most effective product that can be used to control *Phytophthora* spp. in existing infected orchards. However, in the nursery environment, the limited availability of effective fungicides to control root pathogens such as *Phytophthora* and *Pythium* is an enormous concern and unfortunately, the phosphonates did not perform consistently well in the nursery environment therefore other more effective and economically viable compounds had to be evaluated. A standard chemical fungicide (Ridomil), a non toxic product, a possible contact fungicide (Product X), and a biological control product (*Trichoderma*), were evaluated in this trial. The dosages of the non-toxic contact fungicide, supplied by the company were phytotoxic and all the treated trees died. The trial was terminated. Seedlings have been ordered once again from Esselen nursery. The trial will be re-evaluated during 2005 once the seedlings have been transplanted and inoculated with *Phytophthora* (4.4.7).

CRI conducted a demonstration trial for FMC Belgium, to establish the efficacy of Rugby ME (liquid formulation) compared to Rugby G (granular formulation) on a contract basis. A trial was laid out at

Moosrivier Citrus. A final report was sent to FMC for the work conducted during the 2003 and 2004 seasons. FMC Belgium renewed the contract for another year (4.4.8).

CRI evaluated Crop Guard to determine its effect in controlling the citrus nematode, *Tylenchulus semipenetrans*, on a contract basis. Crop Guard is registered as a nematicide on peanuts. Two trials were laid out at Karino (Mpumalanga) and at Citrusdal, Western Cape. This trial is ongoing and Illovo Sugar requested that we keep the information confidential until the final results are available. A progress report was handed to Illovo Sugar for work done during the 2004 season. Illovo renewed their contract for another season (4.4.9).

Applying potassium phosphonate for *Phytophthora* root and collar rot control via foliar sprays can result in phytotoxicity on fruit. In this trial, soil with different clay content was collected in two citrus orchards. Thirteen potassium phosphonate applications via irrigation were done over 3 seasons. Soil was collected from areas with similar soil types that never received soil applications of potassium phosphonate. Although the trial is still ongoing these results could give more clarity on the possible effect of soil borne microbes and/or clay on potassium phosphonate in soils. The analysis was not done by the time of the report and the fate of potassium phosphonate in soil is therefore not known. In California the application of phosphonate to avocado trees via irrigation systems is common practice and no problems regarding accelerated microbial degradation has been experienced after many years' applications (4.4.10).

Accumulation of the phytoalexin scoparone was examined in the roots of citrus rootstocks, to determine whether scoparone, which was shown to be correlated with resistance to *P. citrophthora* collar rot, also plays a role in resistance against *P. nicotianae* root rot. Recent results indicated that the accumulation of the phytoalexin scoparone in the roots as opposed to the stems of citrus rootstocks could not be correlated with tolerance towards *P. nicotianae*. The observed increase in total soluble phenolic concentrations in the roots of citrus rootstocks upon *P. nicotianae* infection, as well as the distinctive accumulation of specific phenolic compounds such as U82 in tolerant rootstocks, however suggests a biochemical mechanism of resistance (4.4.11).

Projekopsomming

Genetiese weerstand, wat die gebruik van onderstamme in herplant situasies insluit, vorm deel van 'n geïntegreerde plaagbeheerprogram wat gevolg word om sitrusaalswurmpopulasies te beheer. Nuwe onderstamme word van tyd tot tyd in die bedryf bekend gestel. Resultate van die 2004 seisoen bevestig verlede jaar se data asook literatuur dat Swingle en C35 wel met groot sukses as herplant onderstamme aangeplant kan word. C32 het vir die tweede jaar ook uitstekend gevaar en daar word aanbeveel dat dié onderstam kommersiëel ge-evalueer word en aan die bedryf beskikbaar gestel word. Die Wallace Rough lemon onderstam het getoon dat dit uiters vatbaar is vir aalwurminfestasie. Die proef word getermineer en geen verdere evaluasie sal gedoen word nie (4.4.2).

Geen geregistreerde aalwurmdoders het 'n effek op die sitrusaalswurm eiers nie. Die kombinasie van eierstimulante en chemiese aalwurmdoders asook die stimulasieprodukte op hul eie is ge-evalueer. In teenstelling met positiewe laboratorium resultate asook aanvanklike proewe was die resultaat op Brits uiters verwarrend. Geen korrelasie kon gevind word waar die eierstimulasieprodukte toegedien is op hul eie of in kombinasie met die chemiese beheer produkte nie. Die Rugby ($3 \times 15 \text{ g/m}^2$) op sy eie was die mees effektiefste behandeling. Die Namacur behandeling het 'n toename in wyfietellings getoon indien vergelyk word met die onbehandelde kontrole en die rede mag toegeskryf word aan versnelde biologiese afbraak van die produk in die grond. Geen addisionele inligting of ondervinding is tans beskikbaar om die onbeantwoorde vrae en benadering t.o.v. aalwurmbeheer te ondersteun nie. Die proef word dus hiermee getermineer (4.4.3).

2004 se PL+ resultaat het 'n 8-11% afname in wyfiepopulasietellings getoon maar nie statisties van die onbehandelde kontrole verskil nie. Die kombinasie van PL+ met 'n geregistreerde aalwurmdoder word aanbeveel. Dit is uiters belangrik dat indien 'n biologiese beheer agent toegedien word, die toestande uiters gunstig behoort te wees aangesien 'n lewende organisme hieself in die grond moet vestig om sodoende 'n positiewe uitwerking te kan hê. Die PL+ toedienings behoort op 'n meer gerêelde basis toegedien te word. Rugby ($3 \times 15 \text{ m}^2$) is steeds die mees effektiefste aalwurmdoder-beheermaatregel wat deur produsente gebruik kan word (4.4.4).

Die sitrusaalswurm is steeds the enkele grootste aalwurm probleem tans in die Suid-Afrikaanse sitrusindustrie. *Hemicyclophora*, die skedeaalswurm, is tans teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in boorde in Mpumalanga se Laeveld. Die skedeaalswurm kom in kombinasie met die sitrusaalswurm op wortels van sitrus voor. Standard na-plant chemiese aalwurmdoders

sal die skedeaalwurm ook beheer. Vier van die gewildste onderstamme word tans in 'n *Hemicycliophora*-geïnfekteerde boord gemonitor, naamlik Growweskiisuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Die Swingle onderstam het in 2003 die laagste aalwurm getalle in vergelyking met die ander onderstamme gehad. Swingle het die afgelope jaar in teenstelling met verlede jaar se data 'n toename in sitrusaalwurm larf- en selfs ook wyfie tellings gehad. Hierdie situasie sal gemonitor word aangesien die neem van wortelmonsters bemoelijk word weens die teenwoordigheid van die wortelstelsel van die bestaande bome. Die bome sal aan die einde van 2005 verwyder word en elke boom se wortels sal individueel gebruik word vir analise wat 'n getrouer weergawe van die situasie sal weergee. Die *Hemicycliophora* tellings is steeds laag maar kan toegeskryf word aan die kombinasie verteenwoordiging van sitrus en skede aalwurms in die grond asook die boompies se ouderdom. Die proef sal getermineer word aan die einde van 2005 (4.4.5).

PL+ se biologiesebeheer produkte se toediening deur 'n drupbesproeiingstelsel word geëvalueer. Gunstige omgewingstoestande kan d.m.v. dié benadering geskep word waar 'n biologiesebeheer agent toegedien word. Geen geskikte drupbesproeiingsperseel metet hoë aalwurmgetalle kon gevind word in boorde wat om Nelspruit geleë is nie. Die soektog sal uitgebrei word na ander sitrusproduserende gebiede (4.4.6).

Fosfonate is tans die mees effektiefste produk beskikbaar vir die beheer van *Phytophthora* spp. op sitrus. Dieselfde resultaat word nie behaal in die kwekery bedryf met die gebruik van fosfonate nie. Die beplande proef het 'n standaard Ridomil swamdoder behandeling asook 'n nie-toksiese kontak swamdoder en 'n biologiese beheer agent (*Trichoderma*) behandeling, teen verskillende dosisse en tye van toediening, wat geëvalueer sou word. Die dosisse van die nie-toksiese produk was fitotoksies en al die bome het gevrek na slegs een toediening. Die proef is getermineer en saailinge is weereens by Esselen kwekery bestel vir reëvaluasie in 2005 (4.4.7).

'n Demonstrasieproef is vir FMC Suid Afrika uitgevoer om die Rugby-korrelformulasie met die vloeibare Rugby-formulasie op 'n kontrakbasis te vergelyk. 'n Verslag is aan FMC België gestuur en die kontrak is vir nog 'n jaar verleng (4.4.8).

CRI is deur Illovo Suiker genader om Crop Guard, 'n nuwe chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te toets. 'n Verslag is aan hulle gestuur en vyf nuwe proewe word gedurende die 2004 en 2005 seisoen op kontrak basis vir hulle uitgevoer (4.4.9).

Blaartoediening van kaliumfosfonaat om *Phytophthora* wortel en kraaggvrot te beheer, veroorsaak soms fitotoksiese skade op vrugte. Kommer het ontstaan grondtoedienings op die langdurig 'n opbou van grondmikro-organismes kan veroorsaak wat versnelde afbraak van kaliumfosfonaat tot gevolg mag hê. Daar bestaan ook 'n persepsie dat kaliumfosfonaat se effektiwiteit in klei-gronde beperk is weens moontlike adsorpsie aan klei-partikels. Verskillende klei-inhoud gronde uit twee sitrusboorde op Letaba Landgoed is versamel. Beide boorde het minstens 13 kaliumfosfonaat toedienings oor die afgelope drie jaar deur die besproeiingssisteem ontvang. Twee grondmonsters is ook uit areas met dieselfde tipe grond, wat nog nooit met kaliumfosfonaat behandel is nie, versamel. Geen resultaat is tans beskikbaar nie. Resultate sal in 2005 beskikbaar wees (4.4.10).

Daar is vasgestel dat die fitoaleksien scoparone wat geassosieer word met weerstandbiedendheid van sitrusonderstamme teen *Phytophthora citrophthora* stam kanker, nie verantwoordelik is vir weerstand teen *P. nicotianae* wortelverrotting nie. Die rol van ander onbekende fenoliese verbindings en meer spesifiek 'n unieke verbinding (U82), wat alleenlik teenwoordig is in weerstandbiedende onderstamme, is ook ondersoek. Die onbekende verbinding U82 is gedeeltelik gekarakteriseer met behulp van dunlaag chromatografie en verskeie spektroskopiese tegnieke word tans gebruik om dit te identifiseer (4.4.11).

4.4.2 **Assessment of citrus rootstocks for citrus nematode resistance** Experiment 281 by M.C. Pretorius (CRI)

Opsomming

Genetiese weerstand, wat die gebruik van onderstamme in herplant situasies insluit, vorm deel van 'n geïntegreerde plaagbeheerprogram wat gevolg word om sitrusaalwurmpopulasies te beheer. Nuwe onderstamme word van tyd tot tyd in die bedryf bekend gestel en daarom is dit essensiële dat hierdie onderstamme geëvalueer word t.o.v. hul weerstand al dan nie teen sitrusaalwurms in herplant situasies. Resultate van die 2004 seisoen bevestig verlede jaar se resultaat asook literatuur dat Swingle en C35 wel met groot sukses as herplant onderstamme aangeplant kan word. C32 het vir die tweede jaar ook uitstekend gevaar en daar word aanbeveel dat dié onderstam kommersiële geëvalueer word en aan die bedryf beskikbaar gestel word. Die Wallace Rough lemon onderstam het getoon dat dit uiters vatbaar is vir

aalwurminfestasie maar volgens literatuur is die onderstam minder vatbaar vir *Phytophthora* infeksie. Die proef word getermineer en geen verdere evaluasie sal gedoen word nie.

Introduction

As part of an integrated pest management approach in controlling the citrus nematode, genetic resistance is a reliable replant strategy which is applied as common practice by citrus producers. Twenty years ago when the term 'replant problem' was a general term, 90% of the rootstocks used in South Africa were Rough Lemon, a highly susceptible rootstock to pathogens related to the replant problem, viz. citrus nematodes and *Phytophthora* spp. Today 80% of rootstocks used are Trifoliate hybrids, of which Troyer, Carrizo citrange and Swingle citrumelo are the most common.

The degree of resistance to citrus nematodes varies from rootstock to rootstock and from hybrid to hybrid. In susceptible rootstocks, the female citrus nematode penetrates the root cortex through the epidermal and hypodermal cells and into the parenchyma cells where they induce nurse cells and form permanent feeding sites.

The nematode populations on the citrange rootstocks do not increase as rapidly as on more susceptible rootstocks, e.g. Rough lemon. With time, however, the populations become as high as on the more susceptible rootstocks. Swingle citrumelo on the other hand used to be nematode resistant in South Africa until a couple of years ago when Miller *et al.* (1997) found the first Swingle orchards in Addo in which the citrus nematode overcame this resistance. This has also been reported in other places in the world, even in open nurseries in Florida. Fortunately the experience in South Africa is that this phenomenon very seldom occurs and in most cases where Swingle is used as a rootstock in replant soils, the trees are nematode-free, despite the soils being infested with nematode eggs after the removal of the previous citrus planting.

Nematode resistance is a dominant character controlled by two genes as resistance is frequently manifested in first generation hybrids. Since new rootstocks are frequently introduced into South Africa, it is necessary to screen these rootstocks for their levels of resistance against the nematode strains found in South Africa. This study was conducted at CRI as a pot experiment.

Materials and methods

The rootstocks screened in this trial are listed in Table 4.4.2.1. The trial was executed in 40 ℓ pots in the open at CRI in Nelspruit. The trees were watered by hand once or twice per week depending on the water requirements. The tree canopies are becoming so large that the root area is permanently shaded, creating more suitable conditions for the nematodes to establish. The trees received the same fertilizer, insect and *Phytophthora* rootrot control programme as the rest of CRI's glasshouse. As inoculation with eggs and juveniles failed to establish nematode populations during the previous years, the trees were re-potted in 2000 with nematode-infested soil collected from Crocodile Valley Citrus Co. The trees were left for eight months to establish and to allow nematode eggs in the soil to hatch and to infect the roots.

Root and soil samples were taken and the second stage juvenile population densities were determined by CRI's Diagnostic Centre according to the method of Whitehead and Hemming (1965), whereas the female population densities were determined according to the method of Van der Vegte (1973). This year trees were sampled by means of a small garden spade to collect as many roots as possible because the trees are much bigger than a year ago. However, this practice could not be repeated as often as we anticipated because it was too dramatic. The result was visible on the tree condition. The tree height and stem diameter were also measured.

Table 4.4.2.1. Rootstocks screened for resistance against the citrus nematode, *Tylenchulus semipenetrans*.

1	F80-8 citrumello	22	Changsha mandarin
2	Pomeroy trifoliate	23	Natsudaidai
3	C-32 citrange	24	Rangpur x Troyer
4	Australian trifoliate	25	Cairn RL
5	Sunki mandarin	26	Cleopatra mandarin
6	Konejime	27	1113 FD X Sunki
7	C. macrophylla	28	C. obovoideae
8	C35 citrange	29	Carrizo citrange
9	Calamandrin	30	Troyer citrange
10	Sun Chu Sha	31	Volkameriana

11	X639 citrange	32	Koethen citrange
12	Yuma citrange	33	Terra Bella citrumelo
13	Milan lemon	34	Roebidoux trifoliolate
14	Orlando tangelo	35	Japanese citron
15	C. amblycarpa	36	Benton citrange
16	Rusk citrange	37	F80-3 citrumelo
17	Jacobsen trifoliolate	38	1112 FD X Sunki
18	Schaub RL	40	Smooth Flat Seville
19	Swingle citrumelo	41	1116 FD X Sunki
20	Rangpur lime	42	Wallace RL
21	Shekwasa mandarin		

Results and discussion

It is clear that only once the canopies are large enough to create a more acceptable micro-climate in the soil do the nematode populations increase. Two to three years ago it appeared as if this trial was a failure and, since then, the shading effect of the trees has allowed the nematode populations to increase.

For the purpose of this discussion the results displayed in Fig. 4.4.2.1 and Table 4.4.2.2 indicate the average female nematode populations on 41 different rootstocks. It is clear from these results that up to Calamandrin these rootstocks could be regarded as tolerant because most of the female nematode populations were at acceptable levels of less than 1500 females per 10 g roots. It is clear that C35 and Swingle could successfully be utilized as replant rootstocks with great success. C32 citrange can also be recommended to be utilized as a replant rootstock. According to CRI's cultivar developer, C32 citrange has excellent production characteristics but due to a shortage of plant material not many commercial trees are available for planting by the citrus industry. It is recommended that this rootstock be evaluated on a semi-commercial scale and eventually be made available as an alternative replant rootstock choice.

During the 2003 season, Australian trifoliolate which was always regarded as a good indicator of tolerance, performed poorly compared to the other trifoliolate rootstocks such as Pomeroy, Jacobsen and Rubidoux trifoliolate. This result was confirmed during this year's trials. Although counts were recorded above the threshold value, they were less than 2000 females per 10 g roots which placed them in an acceptable category of tolerant rootstocks.

On average, X639 and Carrizo citrange rootstocks performed well (although the population counts were more than 1000 females per 10 g roots) compared to the poor performance of Yuma and Benton citrange. These rootstocks could be regarded as susceptible. Carrizo citrange performed better than Benton and Yuma citrange and out-performed Cairn, Wallace Rough lemon and Schaub Rough lemon. As a result, Wallace Rough lemon could be regarded as a highly susceptible rootstock due to its bad performance. A few years ago Wallace Rough lemon rootstock was regarded as a rootstock with good *Phytophthora* tolerance qualities. From these results confirming last year's data, it is clear that Wallace Rough lemon could definitely not be recommended for use on replant soils infested with nematodes.

No significant differences were detected regarding tree-height and stem diameter (Table 4.4.2.3) the past season which did not necessarily correlate with the nematode-susceptibility of the rootstock. The extreme drought and high temperatures experienced during the season could have had an influence on tree growth as a whole. The initial above-ground tree condition could be misleading when compared with the situation in the root system of the trees infested with nematodes. Initially a tree with a high nematode population infestation could still be visually healthy.

Table 4.4.2.2. Listing of 41 rootstocks in order of apparent susceptibility to female nematodes/10g roots and J2 juveniles/250ml soil.

Rootstock	February 2003		December 2003		May 2004	
	J2/250ml soil	♀ / 10g roots	J2/250ml soil	♀ / 10g roots	J2/250ml soil	♀ / 10g roots
Citrus amblycarpa	200 ab	1300 abcde	0 a	666 abcde	200 abcde	600 abcde
C32 citrange	783 bcdef	1508 abcde	116 abc	800 abcdef	216 abcde	800 abcdef
Pomero trifoliolate	200 ab	583 abcd	416 abcdef	500 abc	466 abcdef	800 abcdef
Shekwasha mandarin	250 abc	871 abcde	0 a	3600 hijk	100 abc	4200 jk
C35 citrange	316 abcd	250 a	83 ab	333 ab	66 a	200 ab
Swingle citrumelo	50 a	200 a	33 ab	133 a	50 a	83 a
Australian trifoliolate	333 abcd	1100 abcde	216 abcde	1433 abcdefg	200 abcde	2200 defghi
Sunki mandarin	333 abcd	1141 abcde	500 abcdefg	2333 fghi	480 abcdefg	1400 fghi
Jacobsen trifoliolate	350 abcd	433 ab	0 a	733 abcdef	66 ab	1200 abcdefg
1113 FD x Sunki	650 abcdef	388 ab	300 abcde	933 abcdef	200 abcde	866 abcdef
F80-8 citrumelo	550 abcde	483 ab	100 ab	733 abcdef	100 abc	866 abcdef
Changsha mandarin	283 abcd	500 abc	150 abc	1216 abcdefg	150 abc	933 abcdef
Troyer citrange	950 def	4316 gh	483 abcdefg	1900 bcdefg	600 cdefg	1200 abcdefg
Konjime	250 abc	1433 abcde	283 abcde	2800 ghi	300 abcde	2800 ghi
Smooth Flat Seville	150 ab	2050 abcdef	283 abcde	1666 abcdefg	300 abcde	1666 abcdefg
Carrizo citrange	800 bcdef	975 abcde	950 g	2266 efghi	1000 g	1866 bcdefg
Rangpur x Troyer	1066 ef	5600 h	383 abcde	1366 abcdefg	300 abcde	1200 abcdefg
Calamandrin	16 a	283 a	166 abcd	1300 abcdefg	150 abc	1200 abcdefg
1112 FC x Sunki	66 a	683 abcd	183 abcd	866 abcdef	188 abcd	2266 efghi
Citrus obovoidae	650 abcdef	2405 cdef	233 abcde	1483 abcdefg	200 abcde	2000 cdefgh
F80-3 citrumelo	583 abcdef	741 abcd	250 abcde	2000 cdefgh	250 abcde	1480 abcdefg
Sun Chu Sha	33 a	608 abcd	0 a	366 abc	200 abcde	500 abc
Japanese citron	366 abcd	1471 abcde	366 abcde	1683 abcdefg	1000 g	1366 abcdefg
1116 FC x Sunki	350 abcd	1066 abcde	666 defg	1400 abcdefg	766efg	1400 abcdefg
Rubidoux trifoliolate (?)	416 abcde	416 ab	183 abcd	1100 abcdef	200 abcde	2200defghi
Cairn Rough lemon	900 cdef	2716 efg	616 cdefg	4266 jk	600 cdefg	3600 hijk
Volkameriana	233 abc	1185 abcde	83 ab	3783 ijk	100 abc	4200 jk
X639 citrange	800 bcdef	2758 efg	533 bcdefg	1800 bcdefg	600 cdefg	1800 bcdefg
Koethen citrange	416 abcde	1400 abcde	183 abcd	1866 bcdefg	183 abcd	1800 bcdefg
Wallace Rough lemon	466 abcde	3866 fgh	916 fg	4433 jk	916 fg	5200 k
Benton citrange	650 abcdef	1866 abcde	516 bcdefg	2200 defghi	480 abcdefg	3666 hijk
Citrus macrophylla	316 abcd	1448 abcde	216 abcde	1733 abcdefg	366 abcde	2200 abcdefg
Milan lemon	266 abc	900 abcde	50 ab	1033 abcdef	50 ab	1200 abcdefg
Natsuda dai	1250 f	2241 bcdef	700 efg	4166 jk	583 bcdefg	4266 jk
Schaub Rough lemon	216 ab	720 abcd	0 a	1133 abcdef	200 abcde	4266 jk
Rangpur lime	366 abcd	583 abcd	0 a	266 ab	200 abcde	500 abc
Terra Bella citrumello	266 abc	545 abc	133 abc	1166 abcdefg	83 ab	2800 ghi
Yuma citrange	283 abcd	2491 defg	100 ab	4633 k	100 abc	4100 jk
Orlando tangelo	150 ab	530 abc	283 abcde	1500 abcdefg	200 abcde	1500 abcdefg
Rusk citrange	316 abcd	1866 abcde	333 abcde	600 abcd	333 abcde	2000 cdefgh
Cleopatra mandarin	166	666 abcd	266 abcde	1116 abcdef	200 abcde	2266 efghi

Means in the same column with common letters do not differ significantly at a 5% level according to the Fishers LSD comparison.

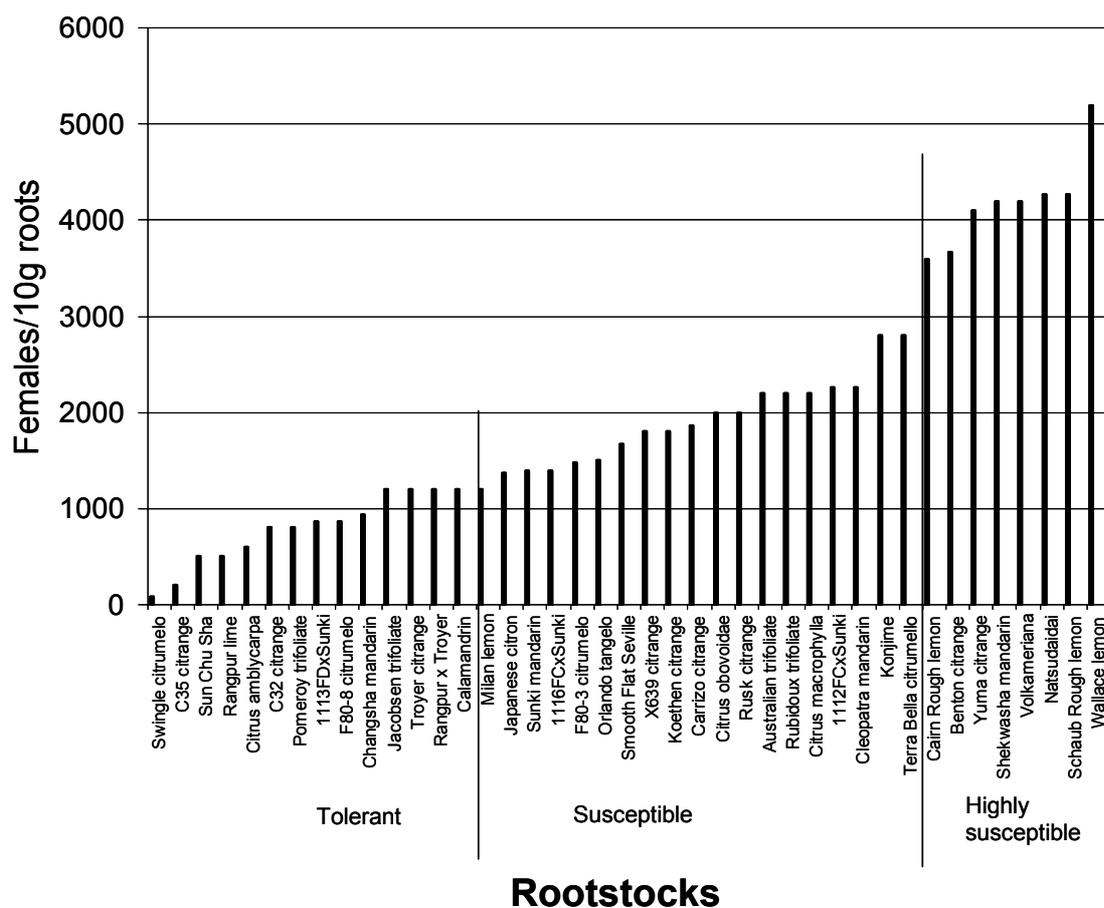


Fig. 4.4.2.1. Average female nematode populations on citrus rootstocks planted in 40 ℓ containers over a period of two years.

Table 4.4.2.3. Differences in stem diameter and tree height due to treatments.

Rootstock	Treatment	December 2003		December 2004	
		Stem diameter	Tree height	Stem diameter	Tree height
F80-8 citrumelo	-N	30.33 a	1.6 a	30.3 a	1.7 a
	+N	27.33 a	1.46 a	28.8 a	1.4 a
Pomeroy trifoliolate	-N	28.66 a	1.63 a	28.66 a	1.4 a
	+N	24.33 a	1.23 a	24.3 a	1.5 a
C-32 citrange	-N	34.83 a	1.4 a	25.01 a	1.3 a
	+N	29.7 b	1.3 a	30.20 b	1.1 a
Australian trifoliolate	-N	30.5 a	1.33 a	31.20 a	1.7 a
	+N	29.06 a	1.26 a	30.10 a	1.2 a
Sunki mandarin	-N	28 a	1.36 a	28.7 a	1.3 a
	+N	27.46 a	1.33 a	28.0 a	1.3 a
Konejime	-N	35.33 a	1.66 b	37.43 a	1.6 a
	+N	33.9 a	1.53 a	35.9 a	1.6 a
Citrus Macrophylla	-N	24.63 a	1.43 a	25.01 a	1.4 a
	+N	20.0 a	1.33 a	21.40 a	1.3 a
C-35 citrange	-N	33.73 a	1.43 a	33.01 a	1.7a
	+N	31 a	1.4 a	31.0 a	1.1 a
Calamandrin	-N	29.06 a	1.5 a	30 a	1.4 a
	+N	25.5 a	1.33 a	25.9 a	1.3 a
Sun Chu Sha	-N	27.96 a	1.46 a	28.10 a	1.4 a
	+N	24.93 a	1.15 a	25.22 a	1.2 a
X639 citrange	-N	31.03 a	1.43 a	31.90 a	1.4 a
	+N	29.6 a	1.23 a	30.66 a	1.9 a

Yuma citrange	-N	33.1 a	1.26 a	34.45 a	1.31 a
	+N	25 a	0.83 a	26 a	1.2 a
Milan lemon	-N	29.5 a	1.53 a	29.56 a	1.55 a
	+N	27.9 a	1.3 a	27.11 a	1.4 a
Orlando tangelo	-N	27.96 a	1.4 a	27.14 a	1.4 a
	+N	27.53 a	1.3a	28.0 a	1.4 a
Citrus amblycarpa	-N	27.83 b	1.43 a	28.0 b	1.52 a
	+N	23.5 a	1.1 a	23.5 a	1.9 a
Rusk citrange	-N	29.03 a	1.4 a	30.42 a	1.6 a
	+N	26.9 a	1.26 a	26.90 a	1.5 a
Jacobsen trifoliolate	-N	27.63 a	1.46 a	27.63 a	1.5 a
	+N	26.53 a	1.36 a	27.10 a	1.3 a
Schaub RL	-N	29.7 a	1.66 a	30.13 a	1.66 a
	+N	29.06 a	1.46 a	29.60 a	1.46 a
Swingle citrumelo	-N	34.83 b	1.46 a	34.83 a	1.51 a
	+N	32.16 a	1.36 a	33.66 a	1.3 a
Rangpur lime	-N	30.73 a	1.73 b	30.77 a	1.77 b
	+N	28.33 a	1.5 a	30.10 a	1.51 a
Shekwasa mandarin	-N	27.16 a	1.43 a	27.16 a	1.43 a
	+N	24.53 a	1.33 a	24.11 a	1.33 a
Changsha mandarin	-N	21.9 a	1.13 a	23.81 a	1.13 a
	+N	20.8 a	1.1 a	20.99 a	1.11 a
Natsudaidai	-N	28.83 a	1.43 a	28.11 a	1.51 a
	+N	26 a	1.36 a	27.04 a	1.41 a
Cairn RL	-N	22.46 a	1.2 a	23.04 a	1.22 a
	+N	19.5 a	1.03 a	21.0 a	1.1 a
Cleopatra mandarin	-N	31 a	1.3 a	31.11 a	1.35 a
	+N	27.33 a	1.2 a	28.34 a	1.2 a
1113FC x Sunki	-N	27.26 a	1.3 a	27.62 a	1.6 a
	+N	22.5 a	1.2 a	22.50 a	1.5 a
Citrus obovoideae	-N	28.73 a	1.26 a	28.73 a	1.30 a
	+N	26.33 a	1.23 a	27.10 a	1.29 a
Carrizo citrange	-N	34.5 a	1.5 a	35.10 a	1.5 a
	+N	34.1 a	1.33 a	34.1 a	1.3 a
Troyer citrange	-N	32.3 a	1.53 a	34.1 a	1.59 a
	+N	27.66 a	1.36 a	28.11 a	1.41 a
Volckameriana	-N	29.47 a	1.51 a	29.90 a	1.6 a
	+N	23.7 a	1.4 a	25.60 a	1.49 a
Koethen citrange	-N	32.63 a	1.63 a	32.63 a	1.63 a
	+N	29 a	1.6 a	29.91 a	1.6 a
Terra Bella citrumelo	-N	33.56 a	1.46 a	34.56 a	1.5 a
	+N	30.36 a	1.23 a	32.10 a	1.56 a
Roebidoux trifoliolate	-N	34.83 a	1.46 b	34.83 a	1.55 a
	+N	33.7 a	1.13 a	30.71 a	1.34 a
Japanese citron	-N	31.63 a	1.33 a	31.83 a	1.32 a
	+N	27.93 a	1.2 a	29.14 a	1.2 a
Benton citrange	-N	29.7 a	1.4 a	30.77 a	1.4 a
	+N	28.96 a	1.05 a	29.84 a	1.1 a
F80-3 citrumelo	-N	27.83 a	1.63 a	28.11 a	1.46 a
	+N	26.13 a	1.36 a	27.63 a	1.4 a
1112FD x Sunki	-N	34.16 a	1.53 a	35.66 a	1.6 a
	+N	32 a	1.5 a	32.11 a	1.58 a
Sampson tangelo	-N	27.5 a	1.4 a	28.50 a	1.58 a
	+N	25.03 a	1.26 a	26.03 a	1.30 a
Smooth Flat Seville	-N	34.33 a	1.56 a	35.66 a	1.50 a
	+N	30.7 a	1.26 a	30.77 a	1.40 a
116FD x Sunki	-N	30.16 a	1.6 a	32.14 a	1.7 a
	+N	29.23 a	1.5 a	29.90 a	1.6 a
Wallace RL	-N	31.96 a	1.7 a	33.13 a	1.9 a
	+N	30.3 a	1.5 a	32.63 a	1.6 a

* -N : Nematode free trees

** +N : Nematode infested trees

Means in the same column with common letters do not differ significantly at a 5% level according to the Fisher LSD comparison.

Conclusion

These data conclude the screening process regarding the effect of nematode populations on 40 different rootstocks. This trial will therefore be terminated.

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4.4.3 Stimulation of egg hatching of *Tylenchulus semipenetrans* eggs Experiment 547 by M.C. Pretorius (CRI)

Opsomming

Geeneen van die geregistreerde aalwurmdoders het 'n effek op die sitrusaalwurm eiers nie. Die kombinasie van eierstimulante en chemiese aalwurmdoders asook die stimulasieprodukte op hul eie is ge-evalueer op 'n proefperseel in Brits. In teenstelling met positiewe laboratorium resultate asook die eerste aanvanklike proewe was die resultaat op Brits uiters verwarrend. Geen korrelasie kon bepaal word waar die eierstimulasieprodukte toegedien is op hul eie of in kombinasie met die chemiese beheer produkte nie. Die Rugby 3 (15 g/m²) was die mees effektiefste behandeling met 'n wyfietelling van 66 wyfies/10 g wortels teenoor die onbehandelde kontrole van 9133 wyfies/ 10 g. Volgens die resultaat is Rugby dus steeds die mees effektiefste aalwurmbheerprodukt wat tans gebruik kan word. Die Namacur behandeling het 'n toename in wyfietellings getoon indien vergelyk word met die onbehandelde kontrole. Die rede mag toegeskryf word aan versnelde biologiese afbraak van die produk in die grond. Geen addisionele inligting of ondervinding is tans wêreldwyd beskikbaar om die onbeantwoorde vrae en moontlike nuwe benadering t.o.v. aalwurmbheer te ondersteun nie. Die proef word dus hiermee getermineer.

Introduction

Although they are aquatic animals, plant parasitic nematodes have evolved protective structures and metabolic adaptations which allow them to survive and flourish in what is often a harsh and competitive soil environment. The body of the nematode is protected by a multi-layered, proteinaceous cuticle, which functions as a flexible skeleton and as a barrier to undesirable elements in the environment. The cuticle is freely permeable to water but differentially permeable to various ions and other chemicals, thus providing nematodes with a selective barrier, which can prevent the entry of some chemicals (Bird, 1971). It is also a relatively resistant structure and is not readily destroyed by chemical or biological agents.

In addition to the structural features which provide protection against antagonism, the physiological capacity of many plant parasitic nematodes to survive adverse conditions (Cooper & Van Gundy, 1971) may give them an advantage over some of their parasites and predators. For example, nematodes are the most successful anhydrobiotic animals (Womersly, 1987) and are less likely to be affected by dry conditions than many of the organisms that prey on them. Also, the behavioural modifications that occur in the anhydrobiotic state (e.g. coiling) possibly reduce the susceptibility of nematodes to parasitism and predation. However, it is important to recognise that the capacity of nematodes to survive adverse conditions does not give them an advantage over all their antagonists.

The high reproductive capacity of most plant parasitic nematodes is one of the features which makes them such significant pests, and it also makes them difficult to control. The life cycle of many of the most important species takes only a few weeks at optimum temperatures, and each female has the capacity to produce hundreds, and in some cases thousands, of progeny resulting in yield losses of thousands of Rands per annum to the growers. On a susceptible crop under ideal conditions for the nematode, populations that are virtually non-detectable at planting can increase to damaging levels in less than three months. This tremendous capacity for multiplication tends to negate the effects of antagonists as high levels of parasitism and predation may do little to diminish final nematode numbers (Stirling, 1990).

None of the registered post plant nematicides have an effect on the eggs of the citrus nematode, *Tylenchulus semipenetrans*. These eggs can survive for up to 9 years in the soil and during favourable conditions the eggs hatch and the life cycle continues. It is therefore essential to follow an integrated nematode control strategy to assist producers in obtaining an economically viable control strategy for effective citrus nematode control. The purpose of this trial was to synchronize hatching of nematode eggs and to eradicate the nematode population in the soil by using one or two nematicide treatments. The initial trial results at Crocodile Valley were very promising and it was clear that the egg hatching stimulants had a positive effect in successfully stimulating the citrus nematode eggs to hatch.

Materials and methods

A trial site with a high nematode population in an eleven-year-old orchard at Bokfontein in the Brits area, was identified. Three single-tree replicates per treatment, were used. A total of 150 trees were monitored. Three liquid formulated nematicides; Rugby EC, Nema-cur EC and Mocap EC were used as the chemical control component. A combination of different treatments and dosages, which included the liquid nematicides in combination with the egg hatching stimulants, the egg hatching stimulants on their own, and a standard chemical nematicide application on its own, were monitored. These consisted of Rugby and Nema-cur only and were compared to an untreated control. Three applications were conducted: November 2002, January 2003 and March 2003. Soil and root samples were taken before the trial was re-applied in September 2002, November 2002, January 2003 and March 2003. These were analysed by the Diagnostic Centre in Nelspruit.

It is known from previous trial results in 2002 that the egg hatching stimulants had a positive effect on the hatching process of the citrus nematode. However, the results obtained during March 2003 after three applications were disappointing and confusing. The trial was not re-applied to save costs but it was re-sampled in March 2004 to determine the long term effect these treatments had, if any, on the hatching process of the citrus nematode.

The results were analysed in three groups to determine the effect of the egg hatching stimulants in combination with three liquid formulations of nematicides. Group A: Rugby EC at different dosages in combination with the egg hatching stimulants. Group B: The aim was to synchronise the egg hatching process and to control the nematode populations with a single or double nematode treatment. The treatments were as follows: Nema-cur EC and Rugby EC at different dosages in combination with the egg hatching stimulants. Group C: Mocap EC and Rugby EC at different dosages in combination with the egg hatching stimulants.

Results and discussion

This year's and the previous year's data were confusing. No definite correlation was evident between the different treatments regarding time of application, different dosages or the stimulating products on their own. The combination of the standard nematicide, Rugby, with the egg stimulating product did not effectively reduce or increase the nematode populations in the soil and roots, as expected, when compared to the Rugby treatment on its own. Rugby at $3 \times 15 \text{ g/m}^2$ on its own was the most effective post-plant chemical control measure to control the citrus nematode, *Tylenchulus semipenetrans*. The results regarding the egg-stimulating treatment on its own and in combination with nematicides were confusing and disappointing.

The nematicides (Nema-cur & Mocap) used in combination with these egg stimulating products did not reduce the nematode populations to acceptable levels of less than $1000 \text{ } \varnothing / 10 \text{ g roots}$ and may therefore be an indication that the stimulating products do have a small effect in stimulating the nematode eggs to hatch. The poor result obtained with the Nema-cur treatment is most likely due to Accelerated Microbial Degradation.

Conclusion

The effect of these possible egg-stimulating products proved to be effective in laboratory trials. It was also clear in the laboratory that these products could be phototoxic to the nematodes when dosages were increased. After these positive results obtained in the laboratory, several field trials were laid out and applications were done in combination with registered nematicides, applied at different rates, and the times of application were also altered. These products were also applied on their own at different dosages. Although a small reaction was detected, no relevant or specific conclusion can be made from the data in the field trial, it is therefore recommended that the trial be terminated. There are too many unanswered questions and no relevant information or experience exists on this approach to enable us to develop a more

effective, environmentally friendly and cost-effective nematode control strategy in citrus. It can be concluded that Rugby 3 (15 g/m²) is still the most effective control measure to control the citrus nematode in citrus.

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4.4.4 Evaluation of biological control agents against *Tylenchulus semipenetrans* Experiment 318 by M.C. Pretorius (CRI)

Opsomming

Mei 2004 se nuwe PL+ behandelingsresultaat het 'n 8-11% afname in wyfiepopulasietellings getoon maar die tellings het nie statisties van die onbehandelde kontrole verskil nie. Alhoewel 'n afname wel voorkom het is die aanleiding van die produk op sy eie nie moontlik nie. Die kombinasie van PL+ met 'n geregistreerde aalwurmdoder kan wel aanbeveel word. Dit is uiters belangrik dat produsente sal besef dat indien 'n biologiese beheer agent toegedien word, die toestande uiters gunstig behoort te wees aangesien 'n lewende organisme in die grond moet vestig om sodoende 'n positiewe uitwerking te kan hê. Die PL+ toedienings behoort op 'n meer gerëelde basis toegedien te word. Dit is baie duidelik uit die resultate dat Rugby (3 x 15/m²) steeds die mees effektiëste aalwurmdoder-beheermaatreeël is wat deur produsente gebruik kan word.

Introduction

Mammalian toxicity of nematicides and the fate of these materials in the environment has rekindled interest in biological control of nematodes. After more than half a century of neglect, biological control is seen as a high priority area for research, and funding has increased worldwide. However, biological control has now reached a critical point in its development. Only a few systems of utilizing antagonists of nematodes for nematode control have been widely adopted with obvious success, and this lack of progress towards the commercialisation of biological control has left many nematologists sceptical of its potential. The difficulties involved in developing a reliable crop protection system are often underestimated.

The relatively stable behaviour of animal populations in natural environments should serve as a constant reminder that in nature all organisms are subjected to a constant series of checks and balances. Populations of individual species do not increase indefinitely but tend to be constrained by the physical environment and by the community of organisms with which they co-exist. Plant parasitic nematodes spend at least some part of their lives in the soil, one of the most complex of environments. Their activities are not only influenced by variation in soil physical factors such as temperature, moisture and aeration, but also by a vast array of living organisms, including other nematodes, bacteria, fungi, algae, protozoans, insects, mites and other soil animals. This biological component of the soil ecosystem is particularly important in limiting, and more or less stabilizing, nematode populations. All organisms are competitors for resources such as space and oxygen, etc. and plant pathogens compete with plant parasitic nematodes for the same food source. It is clear from previous results that these biocontrol products must form part of an integrated control strategy because these products on their own were not effective in controlling the citrus nematode.

The work conducted in this trial was done in collaboration with Biological Control Products (BCP) to initially find an effective solution to replace the organophosphates with biological control agents. A new PL+ Trichoderma combination was evaluated on the previous PL+ treated treatments.

Materials and methods

A twelve-year-old replant orchard with Valencia orange on Rough lemon trees was selected at Bokfontein in the Brits area. The grower experienced a problem with low yields and small fruit size despite the trees being visually healthy. Citrus nematodes were present (>8000 females per 10 g roots) in numbers exceeding the threshold levels recommended for treatment of 1000 females per 10 g roots. Six treatments were replicated seven times in a randomised block design. The treatments were as follows:

Treatment	Applications an dosages
1. Untreated control	-
2. Temik + Rugby	12.5 g/m ² + 20 g/m ²
3. Rugby	3 applications (15 g/m ²)
4. Rugby + PL Plus	20 g/m ² + 2 (PL 4 g + Agr 2 ml + BM 5%)
5. PL Plus (New)	3 applications (PL+ plus Trichoderma)
6. PL Plus (New)	3 applications (PL+ plus Trichoderma)
7. Nema-cur	3 applications (20 g/m ²)

The original trial was retained with a new PL+ formulated *Paecilomyces* + *Trichoderma* combination treatment being applied during the 2003 season to the original PL+ treatments (Treatments 5 & 6). Three applications were done in November 2002, February 2003 and April 2003. Another two applications were done in October 2003 and December 2003 on treatments 5 & 6. Treatment 4 was not re-applied during the 2003 season because of the drought situation in that area which restricted irrigation scheduling, and because water is extremely important when a chemical nematode control strategy is introduced and due to the fact that the female counts were very low. According to the manufacturing company, BCP, this new PL+ tricho product (treatments 5 & 6) would be more effective as the biological control agent on its own in controlling the citrus nematode.

Soil samples were taken prior to each re-application. The samples were analysed by the CRI Diagnostic Centre in Nelspruit. The second stage larvae in the soil were determined according to the method of Whitehead and Hemming (1965) and the female populations in the roots were determined according to the method of Van der Vegte (1973).

Three applications were conducted during the season. Due to the extreme drought situation that was experienced in most of the summer rainfall areas, the trial was only sampled twice during the season. A final sample was taken in January 2005 after good rains fell in the area. The final analysis will be added to the report when the data are analysed.

Table 4.4.4.1. Second stage larvae population counts J2/250 ml soil and female population / 10 g of roots at Brits.

Treatment	Feb 2003	May 2003	Mar 2004
J2 / 250 cc soil			
1. Untreated control	2600 ab (-)	4700 ab* (-)	10314 c (-)
2. Temik (12.5 g/m ²) + 2 x (Rugby 20 g/m ²)	2442.86 ab (-6)*	471.42 a (-90)	900 a (-91)
3. Rugby 3 x (15 g/m ²)	2042.86 ab (-21)	1814.29 ab (-61)	1028 a (-90)
4. Rugby (20 g/m ²) + 2 x (PL Plus)	542.8 a (-79)	1942 ab (-59)	1757 ab (-85)
5. 3 x (PL Plus)	5671.43 b (118)	9985 bc (112)	6728 c (-35)
6. 3 x (PL Plus)	10585.7 c (307)	22042.9 d (369)	6042 bc (-41)
7. Nema-cur 3 x (20 g)	15814.3 d (508)	15042.9 d (220)	8514 c (-17)
♀ / 10 g roots			
1. Untreated control	11871.4 c (-)	6428.57 b (-)	8542 b (-)
2. Temik (12.5 g/m ²) + Rugby 2 x (20 g/m ²)	542.85 a (-95)	400 a (-94)	328 a (-96)
3. Rugby 3 x (15 g/m ²)	457.14 a (-96)	400 a (-94)	514 a (-94)
4. Rugby (20 g/m ²) + 2 x (PL Plus)	542.85 a (-95)	714 a (-89)	1971 a (-77)
5. 3 x (PL Plus)	9200 bc (-23)	7942 b (24)	7842 b (-8)
6. 3 x (PL Plus)	8171.43 b (-31)	12200 c (90)	7600 b (-11)
7. Nema-cur 3 x (20 g)	6800 b (-43)	12657 c (97)	9600 b (12)

*Nematode population results as a percentage increase/decrease compared to the control.

Results and discussion

According to the data in Table 4.4.4.1 the new PL+ Trichoderma treatments (Treatment 5 & 6) had a -41 and -17% and -8 and -11% decrease in citrus nematode larvae and female populations in the soil and roots of the citrus trees respectively accordingly to the 2004 season's data. The decrease was not significantly lower than the untreated control treatment (treatment 1). If compared to the previous year's data (May 2003)

where an increase of nematode larvae and female population counts of >400 and 190% respectively, was evident, the efficacy of the new PL+ formulation were not regarded as promising. Although a slight decrease was evident and if the current drought situation is taken into account, the effect of these treatments cannot look promising. However, the long-term effect of these treatments and the effectivity if utilized in combination with a chemical nematicide should also be monitored. The chemical treatments (2 & 3) indicated significantly lower (96 and 94%) female population counts in the roots compared to all the other treatments. These results confirm that the registered chemical treatments are still the most effective control measures for citrus nematode control.

The combination of Rugby and PL+ (treatment 4) initially also indicated a positive effect in significantly decreasing nematode population numbers over a period of time. This treatment however was not re-applied during the 2003/4 season and as the results indicated a slight increase in nematode numbers was evident. This is a clear indication that regular follow-up applications are necessary when applying a biological control product.

Conclusion

These results confirm the fact that if applied according to recommendations by CRI, i.e. the product being spread evenly when applied and then washed into the soil profile with at least 40 mm of water, a chemical nematicide such as Rugby ($3 \times 15\text{g/m}^2$) is still the most effective control measure to control the citrus nematode in citrus orchards in South Africa. Products such as PL+, a biological control product, should be utilized in combination with a nematicide with regular follow-up applications and a long-term approach in efficacy is necessary. The importance when utilizing a biological control product such as PL+ is the fact that a living organism is applied to the soil and that favourable soil conditions are necessary for it to establish itself which can be created, for instance, with more frequent irrigation cycles, and that a mind shift is necessary to ensure positive, effective results in our country, known for its very hot and arid conditions, compared to the traditional chemical approach known to most producers.

No further studies will be conducted on this trial site and therefore the trial will be terminated.

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4.4.5 Rootstock evaluation against *Hemicycliophora* in the Gamtoos River Valley Experiment 676 by M.C. Pretorius (CRI)

Opsomming

Die sitrusaawurm is steeds the enkele grootste aawurm probleem tans in die Suid-Afrikaanse sitrusindustrie. Aawurms wat die vermoë besit om skade te kan veroorsaak op sitrus is uiters beperk. In Suid-Afrika is *Hemicycliophora*, die skedeaawurm, in verskeie boorde reeds so vroeg as 1963 al geïdentifiseer. Tans is dié aawurm teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in sekere boorde in Mpumalange se Laeveld. Die skedeaawurm kom in kombinasie met die sitrusaawurm op wortels van sitrus voor. Die effek van die skedeaawurm op die sitrusboom word as gevolg van die kombinasie bemoeilik. Drie spesies is geïdentifiseer, nl. *Hemicycliophora halophila*, *H. nortoni* en *H. typical*. Die standard na-plant chemiese aawurmdoders geregistreer op sitrus sal die skedeaawurm ook beheer, soos vasgestel in vorige proewe. Vier van die gewildste onderstamme word tans in 'n *Hemicycliophora*-geïnfekteerde boord gemonitor, naamlik Growweskiisuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Alhoewel die bome nog jonk is, en die situasie oor 'n langer termyn gemonitor behoort te word, het die Swingle onderstam verlede jaar die laagste aawurm getalle in vergelyking met die ander onderstamme gehad. Dit is bekend dat Swingle as 'n weerstandbiedende onderstam suksesvol in herplant situasies gebruik kan word. Swingle het die afgelope jaar in teenstelling met verlede jaar se data 'n toename in sitrusaawurm larf- en selfs ook wyfie tellings gehad. Hierdie situasie sal gemonitor word aangesien die neem van wortel monsters bemoeilik word weens die teenwoordigheid van die wortelstelsel van die bestaande bome in die proefperseel en die resultaat kan beïnvloed. Die bome sal aan die einde van 2005 verwyder word en elke boom se wortels sal individueel gebruik word vir analise wat 'n getrouer weergawe van die situasie sal weergee. Die *Hemicycliophora* tellings is steeds laag maar kan toegeskryf word aan die kombinasie verteenwoordiging van sitrus en skede aawurms in die grond asook die boompies se ouderdom. Die proef sal getermineer word aan die einde van 2005.

Introduction

Nematodes other than *T. semipenetrans* currently known to be capable of damaging citrus tend to be very limited in distribution. A number of species of *Hemicycliophora* have been identified from the citrus rhizosphere. *Hemicycliophora arenaria* is a species native to plants in the desert valleys of southern California that causes damage in citrus nurseries (McElroy *et al.*, 1966). The nematode was closely studied (Van Gundy, 1959) and quarantined to prevent its spread to other areas of that state. It appears to have a wide host range (ten of nineteen hosts tested) although the rutaceous host status is variable. *Citrus limon*, *C. aurantifolia*, *C. reticulata* and *C. sinensis* are resistant (Van Gundy & Rackham, 1961). The nematode feeds in large numbers at root tips whose roots typically develop round galls arising from hyperplasia. Seedling growth in pot studies was reduced by 35%. *Hemicycliophora nudata* causes similar symptoms on citrus in Australia (Colbran, 1963). *H. arenaria* can be eradicated from root systems with hot water dips (10 min at 46°C), preplant soil fumigation with methyl bromide is very effective and a number of rootstocks resistant to the nematode are available (Van Gundy & McElroy, 1969). Three spp. that were detected from samples in the Gamtoos River Valley were identified by Dr. Ester v/d Berg, *viz.* *Hemicycliophora halophila*, *H. nortoni* and *H. typica*.

It is clear from previous trial results that post-plant chemical nematicides did control both the citrus nematode and *Hemicycliophora*. It is, however, essential to determine the effect of *Hemicycliophora* on rootstocks currently used by the citrus industry.

Materials and methods

Four popular rootstocks currently used by citrus producers were obtained from Paksaam nursery, *viz.* Rough lemon, Swingle citrumelo, Carrizo citrange and C35. An orchard infected with *Hemicycliophora* was identified and one of each rootstock was randomly planted next to a tree in a wagon wheel formation with ten replicates. A drip irrigation system was installed in the orchard. The producer assisted in ensuring that aboveground insects were controlled by means of stem applications every second month. These trees were fertilized by means of a liquid fertilizer mixture through the drip irrigation system.

The following parameters will be evaluated for the duration of this trial:

1. Soil analysis after one year, 18 months and thereafter once per year to determine the nematode population status.
2. Visual rating.
3. Stem diameter.
4. Tree height.

The trees were sampled and evaluated in February 2003, ten months later in December 2003 and September 2004.

Results and discussion

It is clear from the results in Table 4.4.5.1 that the citrus nematode female counts in the Swingle rootstock treatment for the previous year are significantly lower than all the other rootstocks. It is known that the Swingle rootstock is more tolerant to nematodes and therefore an excellent replant rootstock although not very popular. Nematode larvae will attack the root system of Swingle but will never complete its life cycle due to the characteristic nature of the rootstock. Because no host is therefore available, the citrus nematode populations in these situations will decline to very low or undetectable levels. The December 2003 results indicated that the Rough lemon and Carrizo Citrange rootstocks had the highest citrus nematode female counts.

Table 4.4.5.1. *Tylenchulus semipenetrans* and female population counts and *Hemicycliophora* juvenile and adult population counts on 4 different rootstocks.

Treatments	<i>Tylenchulus semipenetrans</i>						<i>Hemicycliophora</i>					
	J2/250ml soil			♀ / 20 g roots			J2/250ml soil			♀ / 20 g roots		
	Feb 2003	Dec 2003	Sept 2004	Feb 2003	Dec 2003	Sept 2004	Feb 2003	Dec 2003	Sept 2004	Feb 2003	Dec 2003	Sept 2004
Swingle citrumelo	990a	1270a	5180ab	320a	240a	2700a	196.66a	174.5b	240a	360ab	330 a	140a
Carrizo Citrange	3130	4020b	7790b	2020ab	2580b	3420a	60a	44a	300a	90a	250 a	200a
Rough lemon	3330a	2580ab	6200ab	1940ab	2820b	3960a	193a	27.5a	60a	810b	230 a	140a
C35 citrange	1790a	1610a	3790a	2660b	1460b	3740a	126.5a	98.5ab	140a	340ab	230 a	180a

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

It is clear from the results obtained during 2004 that citrus nematode populations are on the increase. The *Hemicycliophora* counts are still very low and no significant differences occurred. The reason for the delay in an increase in the *Hemicycliophora* populations can be ascribed to the presence of both the citrus and Sheath nematodes in this orchard and due to the fact that the trees are still too young and do not have well-established root systems where infection can take place. It is also very difficult to distinguish between roots of the existing older trees in the trial site and those of the younger trees when taking samples for analysis to determine nematode populations. Rather than take roots from older trees, samples were taken very close to the stems of young trees. For this reason it was not possible to sample the trees more than once a year because of the age of these trees and damage caused to their root systems. The increase in the Swingle rootstock treatment could possibly be due to the high incidence of citrus nematode population pressure in the soil. However, the trial will be sampled in September 2005, one year after the previous samples were taken. This situation will be monitored closely because of the characteristic nature of Swingle where nematode female infection should not be possible. The trees will be removed and a more definite result will be possible, as the roots from each tree will be analysed. The final evaluation regarding the other parameters will then be done. The trees were under stress due to the competition effect of the older trees and due to the removal of half of the young trees' root systems for analysis.

Conclusion

The trees have been monitored but are too young therefore no significant conclusions can be drawn. The trial will be monitored and terminated at the end of 2005.

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4.4.6 Biological control of the citrus nematode under a drip irrigation system

Experiment 760 by M.C. Pretorius (CRI)

Opsomming

Om die effektiwiteit van PL+ se biologiese beheer produkte wat gewoonlik per hand toegedien is te verhoog is die toediening van die produk deur 'n drupbesproeiingstelsel voorgestel. Gunstige omgewingstoestande kan d.m.v. dié benadering geskep word waar 'n biologiese beheer agent toegedien word. Die produk sal oor 'n tydperk toegedien word en die aalwurmpopulasies in die grond en wortels sal gemonitor word. Geen

geskikte drupbesproeiingsperseel het hoë aalwurmgetalte kon gevind wor din boorde wat om Nelspruit geleë is nie. Die soektog sal uitgebrei word na ander sitrusproduserende gebiede.

Introduction

The relatively stable behaviour of animal populations in natural environments should serve as a constant reminder that in nature all organisms are subjected to a constant series of checks and balances. Plant parasitic nematodes spend at least some part of their lives in the soil, one of the most complex of environments. Their activities are not only influenced by a variation in soil physical factors such as temperature, bacteria, fungi, algae, protozoans, insects, mites and other soil animals. This biological component of soil ecosystem is particularly important in limiting or stabilizing nematode populations. In the past applications of the PL+ treatments were done by hand under a micro irrigation system. The use of this biological control product should be evaluated under a drip irrigation system, which could ensure that conditions are more favourable for a Biological control agent to become established in the soil. The efficacy of the product will be determined over a period of time.

Materials and methods

Once a suitable drip irrigation orchard with nematode numbers exceeding the threshold value of 1000 females/10 g roots has been found, a semi-commercial trial will be laid out. Six treatments with 20 trees per row will be treated at different intervals. Soil and root samples will be taken every second month before the next application. The standard chemical, Rugby ME, application will be done as registered.

Results and discussion

Although nematode numbers were tested at several locations, no suitable site could be found in the Nelspruit region. Trial sites in other citrus growing regions will be visited, sampled and evaluated for their suitability.

Conclusion

No results were obtained.

4.4.7 To evaluate enhancing products for the control of *Phytophthora* root rot diseases in citrus nurseries

Experiment 761 by M.C. Pretorius (CRI)

Opsomming

Die fosfonate is tans die mees effektiefste produk beskikbaar vir die beheer van *Phytophthora* spp. op sitrus. Dieselfde resultaat kan ongelukkig nie van getuig word in die kwekery bedryf met die gebruik van fosfonate nie. Die beplande proef het 'n standaard Ridomil swamdoder behandeling asook 'n nie-toksiese kontak swamdoder en 'n biologiese beheer agent (*Trichoderma*) behandelings, teen verskillende dosisse en tye van toediening, wat ge-evalueer sou word. Die dosisse van die nie-toksiese produk was fitotoksies en al die bome het gevrek na slegs een toediening. Die proef is getermineer en saailinge is weereens by Esselen kwekery bestel vir re-evaluasie in 2005.

Introduction

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is an aggressive root rot and fruit rot pathogen in citrus. The pathogen has a wide range of host species and can be isolated from soil in most of the older citrus orchards in southern Africa as well as in young nursery trees.

Most of the research on the chemical control of fungal root pathogens of citrus concerns *P. nicotianae* var. *parasitica* and *P. citrophthora*. Godfrey (1953) recommended that young citrus trees be painted with a fungicidal trunk paint to protect them against *Phytophthora*. Sleeth (1966) reported the effectiveness of paints containing 1-5% copper. Timmer (1977) found that copper ammonium carbonate, cupric hydroxide and captafol were all active for at least 33 weeks when applied at a concentration of 60 mg/m². Captab and pyroxychlor were less effective and retained activity for only 17 weeks. The latter compound limited the expansion of root rot lesions but was not translocated from the treated area. Excision of effected tissue and painting with a copper fungicide slightly improved tree recovery (Timmer, 1977).

During the seventies a new class of systemic fungicides, the acylalanines, proved to be effective in the control of diseases caused by *Phytophthora* spp and certain other oomycetes under field and greenhouse

conditions (Young, Seifried & Biehn, 1977; Darvas, Kotzé & Toerien, 1978). Metalaxyl controlled fruit, stem and/or root infections of citrus by *P. nicotianae* var. *parasitica* and *P. citrophthora* (Timmer, 1979; Farih, Menge, Tsao & Ohr, 1981) and drastically reduced or even eliminated *P. nicotianae* var. *parasitica* populations in treated soils (Farih et al, 1981). Low concentrations of the compound were highly inhibitory to mycelial growth as well as to the formation of sporangia, chlamydo spores and oospores (Farih et al, 1981).

Soon after the launching of the acylalanine fungicides, resistant strains were observed among the populations of various oomycete species in field situations (Clerjeau, Piganeau, Bompeix & Malfatti, 1984). After five years of application to avocados in South Africa, metalaxyl has shown a loss of effectiveness (Darvas & Becker, 1984). This probably was due to soil bacteria metabolising the compound (McKenzie & Margot, 1982), and not to the induction of resistant strains of *P. cinnamomi*. However, Pegg (1983) claims that strains tolerant to metalaxyl pre-exist in field populations of other *Phytophthora* spp. and that the selection of resistant strains results in the loss of disease control. Zentmeyer & Ohr (1978) reported the control of *Phytophthora* root rot by another systemic fungicide, fosetyl aluminium (aluminium tris-o-ethyl phosphonate). The effectiveness of this compound in the control of gummosis and root rot of citrus caused by *P. nicotianae* var. *parasitica* and *P. citrophthora* was proved by Farih et al (1981). The formation of sporangia, chlamydo spores and oospores was highly sensitive to fosetyl-Al, but zoospore and chlamydo spore germination, as well as germ tube growth were insensitive to low concentrations of the fungicide (Farih, Tsao & Menge, 1981). Fosetyl-Al can thus be regarded as an anti-sporulant.

Since fosetyl-Al has a low activity against mycelial growth *in vitro*, it was proposed that the compound might act indirectly by triggering a host resistance response (Zentmyer & Ohr, 1978). In the plant, fosetyl-Al is degraded to H₃PO₃. The latter compound has a similar, though generally higher efficiency in reducing stem infection of *Persea indica* seedlings by *P. citricola* Sawada (Fenn & Coffey, 1984).

The phosphonates are currently the most effective product that could be used to control *Phytophthora* spp. in existing infected orchards. However, in the nursery environment, the limited availability of effective fungicides to control root pathogens such as *Phytophthora* and *Pythium* is an enormous concern to the nursery industry. Unfortunately, the phosphonates did not perform consistently well in the nursery environment and therefore other more effective and economically viable compounds should be evaluated.

Materials and methods

Rough lemon citrus seedlings from Esselen Nursery, Malelane, were used in the glasshouse at CRI. Ten trees per treatment were used. Trees were replanted in 5 litre plastic bags in a potting soil mixture. The trees were left for a month to settle in the bags and adapt to the glasshouse environment. Excluding the untreated control, the remaining trees were infected with *Phytophthora*-infested irrigation water. The treatments and time of application are shown in Table 4.4.7.1. A standard chemical fungicide (Ridomil), a non toxic product, a product that would act as a contact fungicide (Product X) at different rates and a biological control product (*Trichoderma*), were evaluated in this trial.

Table 4.4.7.1. Treatments and time of application of trees manually infected with *Phytophthora*-infested irrigation water, excluding the untreated control.

PHYTOPHTHORA TRIAL		
Treatment	Dosage	Treatment schedule
1. Untreated control -	No <i>Phytophthora</i>	-
2. Treated control +	With <i>Phytophthora</i>	Inoculate trees for 3-4 wks
3. Product x	1 % product	Inoculate trees for 3-4 weeks apply product every 2 weeks
4. Product x	2.5 % product	Inoculate trees for 3-4 weeks apply product every 2 weeks
5. Product x	5 % product	Inoculate trees for 3-4 weeks apply product every 2 weeks
6. Product x	1 % product	Treat and inoculate (repeat these two steps for 3-4 weeks) then treat every 2 weeks
7. Product x	2.5 % product	Treat and inoculate (repeat these two steps for 3-4 weeks) then treat every 2 weeks
8. Product x	5 % product	Treat and inoculate (repeat these two steps for 3-4 weeks) then treat every 2 weeks
9. Trichoderma 1	2.1 ml/bag	Inoculate trees for 3-4 weeks apply product every 2 weeks
10. Trichoderma 2	2.1 ml/bag	Treat and inoculate (repeat these two steps for 3-4 weeks) then treat every 2 weeks
11. Ridomil	2 g/bag	Inoculate trees for 3-4 weeks apply product

Trees were sampled after 4, 8, 14 and 20 weeks. The final parameters to be evaluated are tree height; stem diameter; wet-or-dry root mass and a visual tree health rating.

Results and discussion

The recommended dosages for the non-toxic contact fungicide were phytotoxic and all the treated trees died. The trial has terminated. Seedlings have been ordered once again from Esselen nursery. The trial will be re-evaluated during 2005 once the seedlings have been transplanted and inoculated with *Phytophthora*.

4.4.8 Evaluation of cadusafos on citrus trees in nematode infested soils

Experiment 684 by M.C. Pretorius (CRI)

Opsomming

'n Demonstrasieproef is vir FMC Suid Afrika uitgevoer om die Rugby-korrelformulasie met die vloeibare Rugby-formulasie op 'n kontrakbasis te vergelyk. 'n Verslag is aan FMC België gestuur en die kontrak is vir nog 'n jaar verleng.

Summary

FMC Southern Africa approached CRI to conduct a demonstration trial to establish the efficacy of Rugby ME (liquid formulation) compared to Rugby G (granular formulation) on a contract basis. A new trial was laid out at Moosrivier Citrus. The trial site was selected on 12-year-old Valencia trees with ± 8000 ♀/10 g roots. The trial was applied and sampled three times during the season. A final report was sent to FMC for the work conducted during the 2003 and 2004 seasons. FMC Belgium renewed the contract for another year.

4.4.9 Evaluation of Crop Guard against the citrus nematode, *Tylenchulus semipenetrans*

Experiment 675 by M.C. Pretorius (CRI)

Opsomming

CRI is deur Illovo Suiker genader om Crop Guard, 'n nuwe chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te toets. 'n Verslag is aan hulle gestuur en vyf nuwe proewe word gedurende die 2004 en 2005 seisoen op kontrak basis vir hulle uitgevoer.

Summary

Illovo Sugar approached CRI to evaluate Crop Guard, a non-organophosphate nematicide on a contract basis, and to determine its effect in controlling the citrus nematode, *Tylenchulus semipenetrans*. Crop Guard is registered as a nematicide on peanuts. Two trials were laid out at Karino (Mpumalanga) and at Citrusdal, Western Cape. This trial is ongoing and Illovo Sugar requested that we keep the information confidential until the final results are available. A progress report was handed to Illovo Sugar for work done during the 2004 season.

4.4.10 *Phytophthora* root rot control - Determining the long term effect of phosphorous acid equivalent products in soil applied systems

Experiment QMS 2005/11 by J.J. Serfontein, S. Serfontein, & S.H. Swart (QMS)

Opsomming

Blaartoediening van kaliumfosfonaat om *Phytophthora* wortel en kraaggvrot te beheer, veroorsaak soms fitotoksiese skade op vrugte. Die ander algemene wyse van toediening, nl. stamverf, is duur weens die hoë arbeidsinset. Produsente is bekommerd dat die alternatiewe, nl. grondtoedienings op die langduur 'n opbou van grondmikro-organismes kan veroorsaak wat versnelde afbraak van kaliumfosfonaat tot gevolg mag hê. Daar bestaan ook 'n persepsie dat kaliumfosfonaat se effektiwiteit in klei-gronde beperk is weens moontlike adsorpsie aan klei-partikels.

In die proef is grond met verskillende klei-inhoud uit twee sitrusboorde op Letaba Landgoed tydens mid somer (Januarie) tydens die die hoogste verwagte grond mikrobe aktiwiteit versamel. Beide boorde het minstens 13 kaliumfosfonaat toedienings oor die afgelope drie jaar deur die besproeiingssisteem ontvang. Twee grondmonsters is ook uit areas met dieselfde diepe grond wat nog nooit met kaliumfosfonaat behandel

is nie, versamel. Grond is by steriele water gevoeg (100 g/100 ml) wat 40 µg/l kaliumfosfonaat (Phytext) bevat, geskudinkubeer waarna steriele minimale medium wat 40 µg/l kaliumfosfonaat bevat, met die grondwater geïnkuleer is. Die media is weer geskudinkubeer waarna dit gevries is om in te dien vir kaliumfosfonaat konsentrasie bepaling. 'n Ongeïnkuleerde minimale medium bevattende kaliumfosfonaat het as kontrole gedien. Grond met 'n hoë klei-inhoud en 'n sanderige grond is ge-outoklaveer om mikrobe aktiwiteit te inaktiveer. Die grond is daarna by steriele gedistilleerde water bevattende 40 µg/l kaliumfosfonaat gevoeg, geskudinkubeer, die bostand verwyder en gevries vir kaliumfosfonaat bepaling.

Hierdie resultate behoort meer duidelikheid te gee wat die effek van grondgedraagde mikrobe en/of klei op kaliumfosfonaat in grond het.

Introduction

The use of phosphonates to control *Phytophthora* root and collar rot is common practice in South Africa. The two most common methods of phosphonate application on citrus are foliar sprays and painting of trunks. Foliar sprays sometimes result in phytotoxicity on fruit while the intensive use of labour makes stem application costly. Soil application of phosphonates via irrigation is becoming a popular method of application. Producers fear, however, that the long term use of this method may result in the building up of soil micro-organisms that are able to biodegrade potassium phosphonate or related products. There is also a perception that potassium phosphonate is not very effective if applied in clay soils due to possible adsorption to clay particles. The aim of this study was to determine the fate of potassium phosphonate in soils with a history of application of the product as well as in soils with high clay content without taking the plants into consideration.

Material and methods

Soil with different clay content was collected from two orchards on Letaba Estates with a history of phosphonate application via irrigation. The soil, ~2 kg, was collected with a clean spade into plastic bags. The spade was cleaned with 70% ethanol between samples to prevent possible cross contamination by soil microbes. Soil of similar type as the two mentioned samples was collected from areas where no potassium phosphonate has been applied via the irrigation system. Soil was stored in the cold room (~11°C) until further processing. Sterile soft water (300 ml quantities) containing 40 µl/l was prepared in 500 ml Erlenmeyer flasks and inoculated with 30 g quantities of the different soil samples respectively. A flask to which no soil was added, served as control. These flasks (one control and four with soil added) were shake-incubated for ~72 h at room temperature to promote the growth of possible phosphonate degrading bacteria. Flasks containing 300 ml of a minimal medium (Parekh, *et al.* 1994, Roberts, *et al.*, 1991) amended with 40 µl/l potassium phosphonate were inoculated with 300 µl of the supernatant of each of the soil cultures. These cultures were shake incubated for 72 h at room temperature after which the medium was frozen in Ziplock bags to be submitted for chemical analysis (phosphonate presence).

In a second experiment soil with a high clay content, and sand was added to different beakers, wetted to about field capacity and autoclaved at 121° for 15 min in order to eliminate possible interfering microbes. Soil (200 ml) was added to 400 ml sterile soft water containing 40 µl/l potassium phosphonate in 1000 ml Schott bottles and shaken vigorously by hand for two minutes and then shake incubated for 20 min. The bottles were left on the table for the soil to settle (~20 to 30 min) after which the supernatant (~200 ml) was poured off into Ziplock bags and frozen for chemical analysis.

Results and discussion

The phosphonate content analysis was not done by the time of the report and the fate of potassium phosphonate in soil is therefore not known. In California the application of phosphonate to avocado trees via irrigation systems is common practice and according to Prof Menge (*personal communication*) no problems regarding enhanced microbial degradation have been experienced after many years of applications. In his view, it is highly unlikely that the chemical will be degraded in soil at a rate that will interfere with its efficacy. In the absence of roots, due to advanced root rot, application via irrigation will be ineffective as the roots will not be able to take up the chemical.

This experiment will, hopefully, give more clarity on the fate of phosphonate products in soil in order to pursue this method of phosphonate application as a possible replacement for foliar and stem paint applications.

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4.4.11 Resistance of citrus rootstocks to root pathogens

Experiment by Prof N. Labuschagne (UP)

Opsomming

Dit is vasgestel in die huidige studie dat die fitoaleksien scoparone wat geassosieer word met weerstandbiedendheid van sitrus onderstamme teen *Phytophthora citrophthora* stam kanker, nie verantwoordelik is vir weerstand teen *P. nicotianae* wortelverrotting nie. 'n Verhoging in die konsentrasie van totale oplosbare fenoliese verbindings en die akkumulering van spesifieke fenoliese verbindings in die wortels van sitrus onderstamme, dui egter daarop dat die meganisme van weerstand teen *P. nicotianae* wel biochemies van aard is. Die rol van ander onbekende fenoliese verbindings en meer spesifiek 'n unieke verbinding (U82), wat alleenlik teenwoordig is in weerstandbiedende onderstamme, is ondersoek in die huidige studie. Onbekende verbinding U82 is gedeeltelik gekarakteriseer met behulp van dunlaag chromatografie en verskeie spektroskopiese tegnieke word tans gebruik om dit te identifiseer.

Introduction

The viability of the citrus industry is to a great extent dependent on the rootstocks in use (Castle, 1987). The systemic fungicides metalaxyl and fosetyl-Al have been effectively used in citrus to control *Phytophthora* diseases, but the excessive dependence on the use of metalaxyl has resulted in the development of resistant *Phytophthora* isolates (Timmer *et al.*, 1998). Rootstocks therefore must have a high degree of resistance or tolerance to root and collar rots. Although the use of resistant rootstocks is a good approach for the control of *Phytophthora* diseases, some resistant rootstocks might still be susceptible to other diseases or they might not be suited for specific horticultural conditions. In the search for alternative rootstocks, it is therefore necessary to develop screening techniques that are rapid and reliable. To identify possible markers for resistance, the objective of the present study was to determine the biochemical mechanisms involved that govern resistance of citrus rootstocks against *P. nicotianae* root rot.

In our previous research it was shown that the determination of total soluble phenolics from citrus roots correlated well with tolerance or susceptibility towards *P. nicotianae* and it can therefore be used as a good pre-screening method for citrus rootstock resistance against *P. nicotianae* root rot. Treatment with the systemic fungicide fosetyl-Al further elevated total phenolic levels in the roots of citrus rootstocks. This indicates that elevation of total phenolics play a role in the mode of action of this fungicide, therefore suggesting an indirect mode of action.

Accumulation of the phytoalexin scoparone was examined in the roots of citrus rootstocks, to determine whether scoparone, which was shown to be correlated with resistance to *P. citrophthora* collar rot (Afek & Szejnberg, 1988; 1989; 1993; 1995), also plays a role in resistance against *P. nicotianae* root rot. Using thin layer chromatography (TLC), scoparone was found in low concentrations within the bark and very little or no scoparone at all could be detected within the roots of any of the citrus rootstocks tested. No correlation between the accumulation of scoparone and citrus root rot resistance could therefore be demonstrated. These results correlate with our previous studies (Aucamp *et al.*, 2000) where very little or no scoparone was detected in citrus roots when infected by *P. nicotianae*, confirming that scoparone which dictates resistance to *P. citrophthora* stem canker is not *per se* involved in the mechanism of resistance in the roots.

TLC-analysis of citrus root extracts revealed a number of other unidentified fluorescent compounds. A distinct yellow fluorescent compound, provisionally labelled U82, was detected in tolerant Swingle, Sour orange, Troyer and Macrophylla rootstocks. This compound was absent in susceptible Rough lemon and Volckameriana rootstocks. Such a unique compound that is only associated with tolerant rootstocks could potentially be used in developing a high throughput screening technique for citrus rootstock resistance. In the current study U82 and other phenolic compounds were characterized using TLC and HPLC and their antifungal activity determined using a bioassay with *Cladosporium* as an indicator fungus. Various spectroscopy techniques such as infrared and nuclear magnetic resonance spectroscopy are currently being used to finalise the identification unknown compound U82.

Materials and methods

Phytophthora susceptible Rough lemon and tolerant Swingle rootstocks were compared for phenolic production in their roots, in response to *P. nicotianae* infection. For HPLC, the liquid chromatograph was equipped with a MALsil C18 micron reverse-phase analytical column. For qualitative analysis, a gradient elution schedule consisted of an initial one minute run of 10% acetonitrile in ultra distilled water followed by a linear gradient to 50% acetonitrile over 20 minutes at a flow rate of 2 ml per minute. Samples were injected and compounds photometrically detected by a System Spectra 6000 LP UV diode array detector. For TLC, a volume of 5 μ l root extract was loaded onto pre-coated Silica Gel 60 plates without fluorescent indicator, developed with toluene:ethyl acetate (1:1) and analysed under UV light (365 nm). A two-dimensional TLC procedure was established to separate products even further. Samples were firstly separated on TLC plates using 12% acetic acid, after which it was run in a second direction perpendicular to the first separation with a toluene:ethyl acetate (1:1) mixture. Antifungal activity was determined using a bioassay with *Cladosporium* (Homans & Fuchs, 1978). The TLC chromatograms were sprayed with a conidial suspension of *C. cladosporioides* and incubated in a moist atmosphere for 2-3 days at 25°C. Inhibition zones indicate the presence of fungitoxic compounds.

Uninfected roots of citrus rootstocks were subjected to TLC analysis, in order to quantify U82 by measuring the length of the spot on the TLC chromatograms. To purify and determine the structure of unknown phenolic compound U82, crude phenolic root extracts were firstly separated and concentrated from other compounds using preparative TLC, after which HPLC analysis was used to determine its purity (Mousa et al., 1997; De Ascensao & Dubery, 2003). Preparative TLC entailed loading the maximum of crude phenolic extract on a preparative TLC plate, followed by development with toluene: ethyl acetate (1:1) and examination under UV light (360 nm). Partially separated, yellow fluorescent U82 was scratched off and re-extracted from the silica plates with 80% methanol for 1 hour, loaded onto preparative TLC plates again and then development with 12% acetic acid. This preparative TLC purification method with different TLC solvents proved to be quite time consuming and it did not provide adequate separation and purification of unknown compound U82 from the rest of the root phenolics.

A method was developed to replace the preparative TLC purification procedure, in order to increase the yield and purity of U82. This involved separating U82 on a solid phase extraction (SPE) column, where the crude phenolic root extracts were loaded onto a solid sorbent (Strata-X, Normal phase, SPE column, Phenomenex), followed by selective elution. Methanol (70%) was used as solvent for the elution. Fractions (1.5 ml) were collected, loaded on TLC plates and developed to confirm separation. Fractions that contained only compound U82 were combined and evaporated under a stream of nitrogen at room temperature. TLC analysis revealed a single yellow fluorescent spot, confirming that it is a pure compound of substantial volume. Nuclear magnetic resonance spectroscopy was subsequently used to determine the carbon-hydrogen framework of U82, ultraviolet spectroscopy to determine the nature of the pi electron system or the wavelength of maximum absorption, mass spectroscopy to determine the molecular weight and formula and infrared spectroscopy to establish what functional groups are present on the compound (Picerno et al, 2003).

The activity of key defence enzymes involved in the production of phenolic compounds was studied to determine their role in citrus rootstock resistance against *P. nicotianae* root rot. Induction of defence responses is observed in the early stages of attempted infection by a pathogen. Defence gene products may include peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL). PAL catalyses the conversion of L-phenylalanine to trans-cinnamic acid, which is a key starting point in the production of secondary compounds in plants. Roots of uninfected and *P. nicotianae* infected rootstocks were subjected to enzyme analysis 21 days following inoculation by the pathogen. Rootstocks used were Swingle, Troyer, Sour orange, Macrophylla, Carrizo, C35, Volckameriana and Rough lemon. During a modified procedure of Cahill and McComb (1992) for PAL extraction and activity determination, citrus roots were removed, freeze-dried for 48 hours, after which 50 mg of material was ground in liquid nitrogen together with 1 ml Borate-Na buffer (0.05M, pH 8.8) plus 1% of Polyvinylpyrrolidone (PVP). It was shaken for 20 min at 4°C and centrifuged for 15 min at 40 000g. Extraction was repeated 4 times. Enzyme specific substrate (450 μ l L-phenylalanine) was added to 450 μ l enzyme extract and the production of cinnamic acid measured by reading absorbance at 290 nm.

Results and discussion

Phenolic compounds from citrus root extracts were characterised and screened for antifungal activity against *P. nicotianae*, in order to identify possible markers for resistance. HPLC analysis of crude phenolic extracts showed complex patterns of constitutively present as well as induced phenolic compounds (Figure 4.4.11.1).

Although differences could be seen between susceptible and tolerant rootstocks, the phenolic mixture was too complex for accurate evaluations.

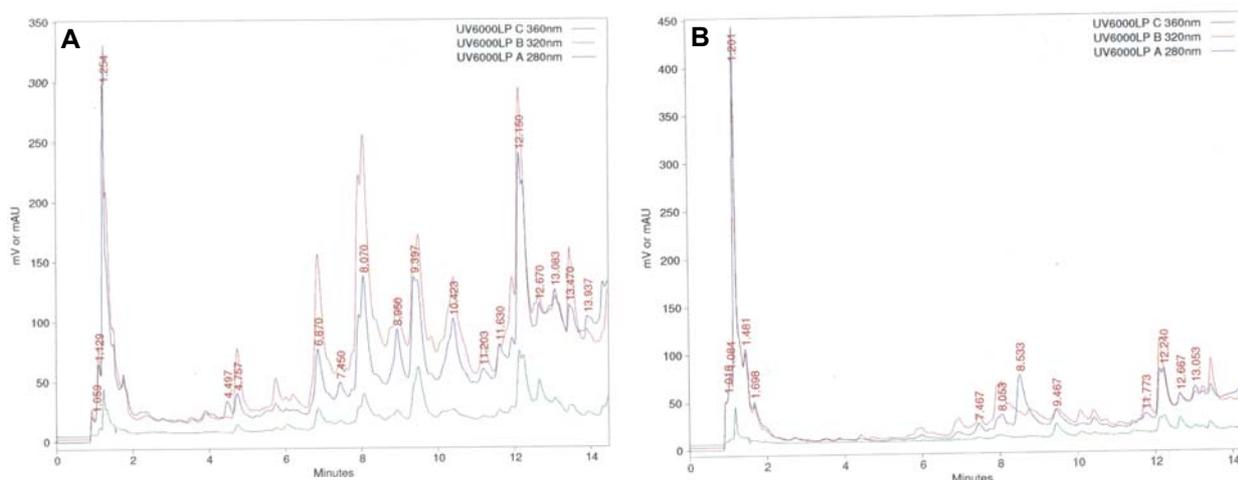


Figure 4.4.11.1. HPLC analysis of crude phenolic root extracts from Rough lemon (A) and Swingle (B) rootstocks, 21 days following inoculation by *P. nicotianae*.

TLC analysis revealed quite a number of unidentified fluorescent compounds (Figure 4.4.11.2). The R_F value of scoparone has experimentally been determined as 0.34. Some of the R_F – values of the unknown phenolic compounds corresponds with that found in the literature. It is highly likely that the unknown compounds are structurally related to scoparone, for example umbelliferone and scopoletin (Figure 4.4.11.3) and that they probably play a role in host defence.

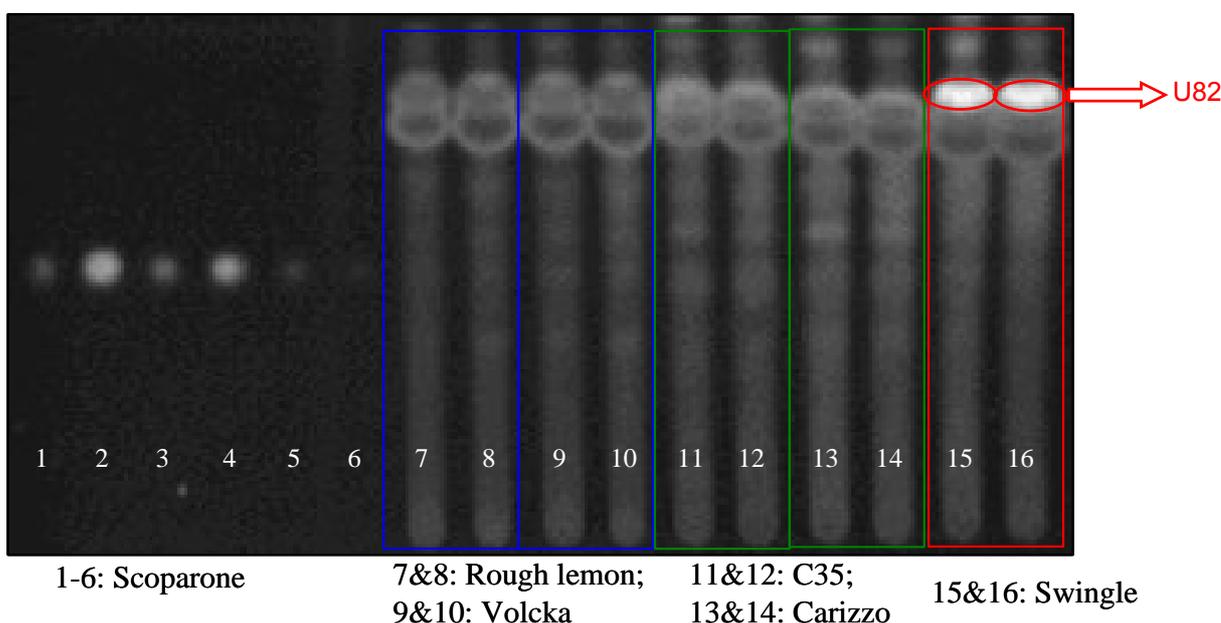


Figure 4.4.11.2. TLC analysis of crude phenolic extracts from the roots of different citrus rootstocks, following inoculation by *P. nicotianae*. Chromatograms were developed with Toluene: Ethyl acetate (1:1) and fluorescence observed under UV light (360 nm). Lane 1 - 6, scoparone standards 10-200 $\mu\text{g}/\text{ml}$ and Lane 7 - 16, induced citrus root extracts. Arrow indicates unknown yellow fluorescent compound (U82) with R_f value of 0.82.

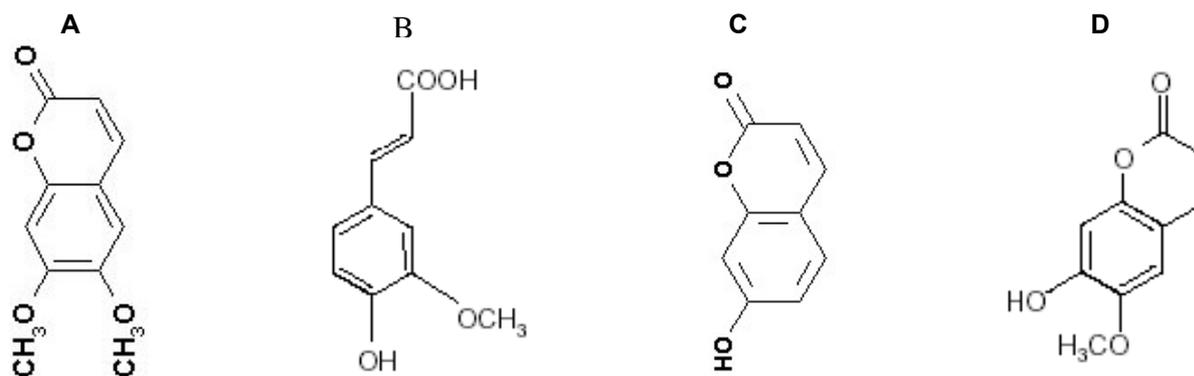


Figure 4.4.11.3. Structures of the phytoalexin scoparone (A), which is associated with resistance of citrus rootstocks against the collar rot fungus *Phytophthora citrophthora* and structurally related ferulic acid (B), umbelliferone (C) and scopoletin (D), that could possibly be produced in the roots upon *P. nicotianae* infection.

These compounds were further separated using a two-dimensional TLC method. A distinct yellow fluorescent compound (U82) was detected in tolerant Swingle that was absent in susceptible Rough lemon rootstocks (Figure 4.4.11.2). Tolerant Sour orange and Swingle rootstocks produced considerable amounts of U82 followed by moderate concentrations in Troyer, Macrophylla, Carrizo and C35, whereas it was absent in susceptible Volckameriana and Rough lemon rootstocks (Table 4.4.11.1.) Yellow fluorescence under UV light (360 nm) is characteristic of flavanoid type compounds. This compound was produced constitutively but increased in concentration upon infection by *P. nicotianae*. Several compounds, including the yellow fluorescing compound, displayed antifungal activity during the *Cladosporium* bioassay (Figure 4.4.11.4).

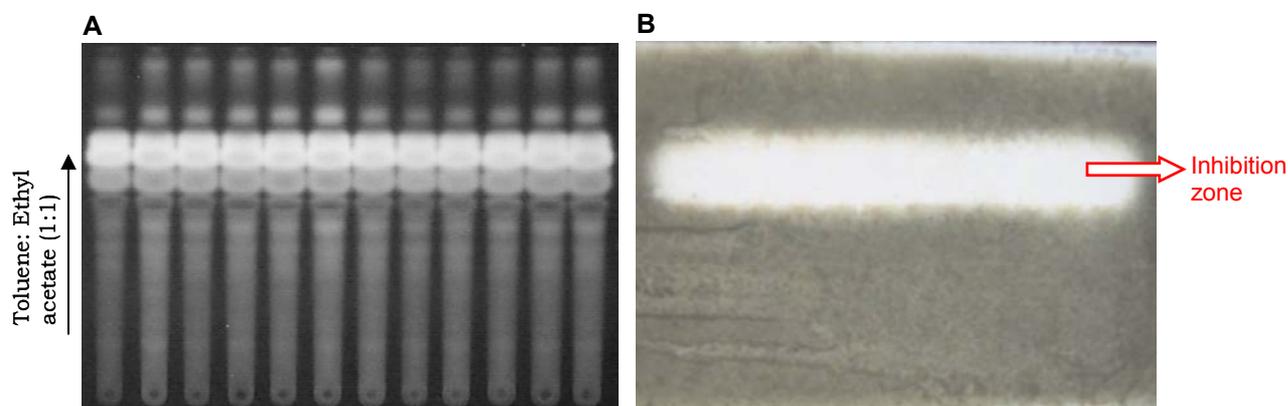


Figure 4.4.11.4. TLC analysis of crude phenolic extracts from the roots of Swingle rootstock following inoculation by *P. nicotianae* (A) and bioassay with *Cladosporium cladosporioides* (B).

Table 4.4.11.1. TLC separation and subsequent quantification of unknown phenolic compound U82, occurring in uninduced citrus rootstocks.

Rootstock	U82 spot length* (mm)	Duncan grouping
Sour orange	4.3	a
Swingle	4.1	a
Troyer	1.42	b
Macrophylla	1.28	bc
Carrizo	1	cd
C35	0.72	d
Volckameriana	0	e
Rough lemon	0	e

* Length of U82 spot on TLC chromatograms was measured. Values are the means of combined averages of four replications. Values followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

Unknown compound U82 was separated and purified from other citrus root phenolics by means of preparative TLC (Figure 4.4.11.5). This procedure did work to some extent, but it was rather tedious and yielded low quantities of U82, which was not completely pure. A solid phase extraction (SPE) method was implemented to resolve this problem. Strata-X SPE columns (Phenomenex) was used to bind citrus root phenolics that allowed for selective subsequent elution. The multimode retention mechanisms of SPE allow for selective retention and elution, which gives consistently improved recoveries for substituted aromatic analytes, in comparison to traditional reverse phase sorbents that retain analytes primarily by hydrophobic interaction. Strata-X are also deconditioning resistant and will not lose selectivity after the sorbent bed runs dry in comparison to traditional columns. The deconditioning resistant ability results in consistent sample processing, preventing variable recoveries as a result of the sorbent becoming deconditioned. Strata-X SPE columns produced considerable pure amounts of the compound U82 and therefore has the advantage of being a high throughput method and allowing large volume cleanup or purification.

During the structure determination and identification of U82, ultraviolet spectroscopy revealed a maximum absorbance at 280 nm (Figure 4.4.11.6), which is characteristic of some flavanoid type of compounds. Nuclear magnetic resonance (NMR) revealed a complex hydrogen composition or framework, indicating that U82 is a reasonably large compound compared to that of other phenolic compounds or phytoalexins. Although the SPE extraction method produced pure and considerable amounts of U82, it proved to be not enough for a proper NMR analysis of the carbon-framework. Current efforts are now focussed on obtaining enough pure compound for adequate analysis. Infrared spectroscopy in addition needs a crystalline compound to establish what functional groups are present therefore more of compound U82 is needed for crystallization. Since mass spectroscopy is destructive, it will be the final procedure in the structure determination of U82 to determine its molecular weight. Such a unique compound that is constitutively produced and does not need induction and that is only associated with tolerant rootstocks could potentially be used in developing a high throughput screening technique for citrus rootstock resistance.

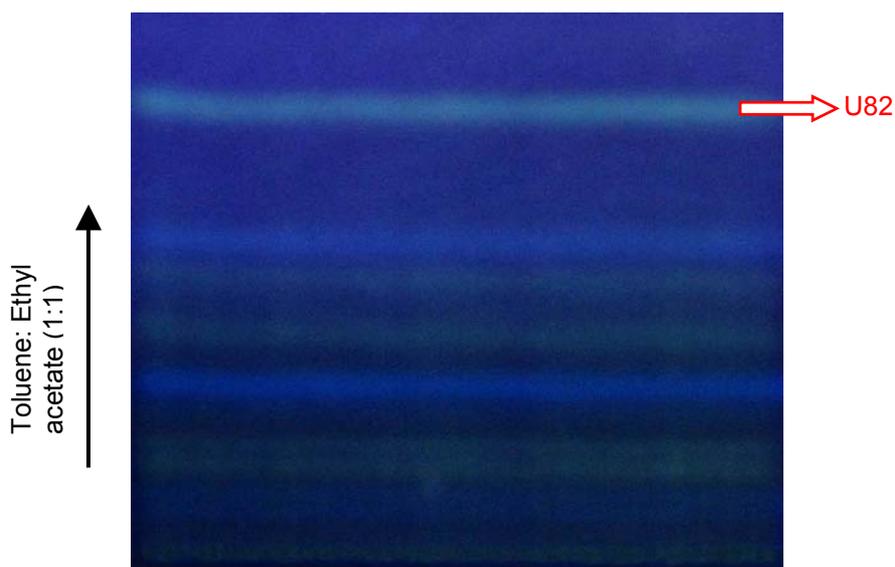


Figure 4.4.11.5. Preparative TLC analysis of crude phenolic extracts from the roots of Swingle rootstock following inoculation by *P. nicotianae*. Arrow indicates unknown yellow fluorescent compound (U82) with Rf value of 0.82.

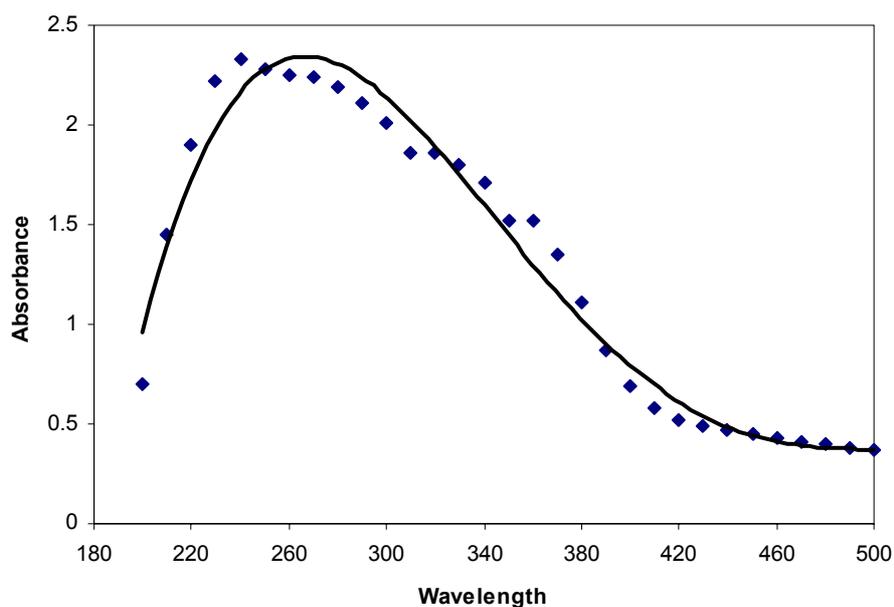


Figure 4.4.11.6. UV spectrum (200 – 500 nm) of unknown yellow fluorescing compound U82, with maximum absorbance at 280 nm.

Phenylalanine ammonia-lyase (PAL) was investigated as key enzyme in the production pathway of resistance related phenolic compounds. During the enzyme extraction procedure, phenolic compounds were extracted with the enzyme that interfered with the absorbance readings in the PAL activity assay. Powdered citrus root samples were consequently extracted 5 times with cold acetone for 30 minutes and centrifuged at 12 000 g for 5 minutes, to get rid of all possible phenolic compounds. The resulting acetone powder extracts were then used for further enzyme extraction. Following removal of the phenolic compounds, PAL activity was observed in most of the root extracts of the rootstocks used. Unfortunately PAL activity was very low and no significant differences could be observed between rootstocks or roots inoculated with *P. nicotianae* in comparison to the uninfected control. Further work is therefore necessary to optimise PAL extraction and activity determination from citrus roots.

Conclusion

Graham (1995) demonstrated that tolerance to *P. nicotianae* root rot might be due to the greater ability of tolerant citrus rootstocks to regenerate roots under certain environmental conditions. There were, however, rootstocks such as Trifoliate orange that were tolerant, but it did not have a strong root regeneration capability, therefore suggesting a biochemical mechanism of resistance. In citrus, the production of induced antifungal phenolic compounds has been demonstrated in fruits (Rodov *et al.*, 1994), peels (Dubery *et al.*, 1999), leaves (Manthey *et al.*, 2000) and in citrus roots (Sulistyowati *et al.*, 1990). Afek *et al.* (1986; 1988; 1989; 1995) implicated the phytoalexin scoparone in the resistance mechanism active within the stem bark of citrus rootstocks against the collar rot fungus *P. citrophthora*. In our studies, accumulation of the phytoalexin scoparone in the roots as opposed to the stems of citrus rootstocks could not be correlated with tolerance towards *P. nicotianae*. The observed increase in total soluble phenolic concentrations in the roots of citrus rootstocks upon *P. nicotianae* infection, as well as the distinctive accumulation of specific phenolic compounds such as U82 in tolerant rootstocks suggests a biochemical mechanism of resistance.

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4.5 PROJECT CITRUS BLACK SPOT

Project Co-ordinator: J.M. Kotzé

4.5.1 Project summary

Three groups are responsible for CBS research. The University of Pretoria provides the vital basic techniques and information to solve CBS, while the CRI and QMS are responsible for the field experiments and spray programmes.

It was a great step forward when Black Spot could be detected with DNA technology on export fruit. It is an accredited ISO facility at UP. The challenge to detect the Black Spot pathogen in dead leaves and soil was won by Lise Korsten and Linda Meyer. She succeeded in eliminating the interfering factors in soil and dead leaves. The technique is now used to satisfy the export needs to USA (4.5.2).

The new Inoculum Monitor was developed, tested, calibrated, and is now used extensively to detect potential ascospore outbreaks and to apply sprays accurately and more cost effectively. This technology exists only in South Africa (4.5.4 & 4.5.10).

It was established that the leaves of Valencia and lemon trees stay susceptible to Black Spot infection for up to 10 months. The work is being repeated and found to be true so far (4.5.3).

The focus now is to eliminate the infection in leaves before they drop, to break the life cycle (4.5.5 & 4.5.6). This approach fits perfectly with the project where dead leaves are removed and destroyed to prevent primary inoculum. Biological agents and cultural practices are employed successfully to break the life cycle. We are moving towards practical application. This approach has resulted in a situation where Black Spot incidence has become so low, that spraying is becoming almost unnecessary or the spray programme can be simplified (4.5.5).

By using the new Inoculum Monitor in three different areas, viz. Brits (Mooinooi), Burgersfort and Letsitele, it becomes evident that the first available inoculum for infection after fruitset in October varies according to the climate. In the cooler areas (winters) the first ascospore releases are up to three weeks later than the hotter areas. This work is done through teamwork and co-operation between UP and QMS. So far, it has become clear that the main cause of CBS epidemics is the leaves which drop between July and December. Furthermore, if the orchards can be kept free of fallen leaves between October and December, there will be no epidemic. The exception is when the climate is very conducive to infection in old Valencia orchards after 15th February. A suitable eradication treatment for this eventuality is being investigated. Indeed the onset of resistance of citrus fruit to infection is not fully understood throughout the citrus industries. Inoculation studies are underway to clear up the problem (03/68n). The dry seasons of the past two years did not contribute to obtain the required results. It was found that under certain conditions ascospores can be produced on dead leaves in the tree (4.5.11) but this source of inoculum seems unimportant.

Chemical control strategies are intensively investigated. The results so far, show that mancozeb can be replaced if overseas buyers refuse to accept fruit which is sprayed with this chemical group. Indications are that benomyl may still play an important role and that the strobilurins will be with us for some time. By re-arranging the position of chemicals in a programme, better control was achieved (4.5.7, 4.5.8, 4.5.9 & 4.5.13)

The choice of different copper fungicides is getting bigger every season. Experiments showed that copper stippling can be overcome, but not without sacrificing external fruit quality (4.5.9).

An interesting development is that the addition of Sporekill to mancozeb and copper seem to improve the overall control. However, this information is based on one season's experiments and there is still a lot more experimentation needed before a recommendation can be made.

One nursery (Du Roi) is being used to establish a protocol for monitoring CBS (4.5.12). The work is done in co-operation with UP.

The brief summary is made possible by the excellent research at the University of Pretoria, lead by Prof. Lise Korsten, assisted by Dr. Linda Meyer, Ms. Mariette Truter, Rene Jacobs and various students; also by Dr. Fanus Swart and his team. Dr. Tian Schutte and his assistants, as usual, are responsible for most of the chemical programmes and testing of new chemicals.

To all the co-operators and participants in the CBS programme, my sincere gratitude.

Projekopsomming

By Swartvleknavoring word drie groepe navorsers betrek. Die Universiteit van Pretoria ontwikkel nuwe tegnieke en stel inligting beskikbaar wat die Swartvlek probleem kan oplos, terwyl die CRI en QMS hoofsaaklik betrokke is by veldproewe, opnames, waarnemings en die ontwikkeling van bestrydings programme. Dit was 'n baie groot stap vorentoe, toe Prof. Korsten en Dr. Linda Meyer nou ook die Swartvlek swam kan opspoor in dooie blare en grond. Hierdie inligting word nou ingespan om uitvoere na VSA en ander lande te bevorder. Baie bruikbare inligting kom hier na vore (4.5.2).

Die nuwe Inokulum Monitor was ontwikkel, getoets, gekalibreer en word intensief gebruik om potensiële askospooruitbrake te bepaal en om spuitprogramme meer koste effektief aan te wend. Hierdie tegnologie is nuut in die sitruswêreld (4.5.4 & 4.5.10).

Dit is vasgestel dat die blare van Valencia en Suurlemoenbome vatbaar bly vir infeksie tot 10 maande. Die werk word herhaal en dit word weer so gevind (4.5.3). Dit plaas die fokus nou op die uitwissing van besmette blare, voordat die blare val om die swam se lewensiklus so te breek. Die benadering pas perfek in by die projek waar dooie blare verwyder en vernietig word (4.5.5 & 4.5.6). Biologiese agente en verbouingspraktyke word ingespan om die lewensiklus suksesvol te breek en ons beweeg na praktiese aanwending van hierdie bevindings. Die situasie word bereik waar chemiese bespuitings drasties verminder kan word (4.5.5 & 4.5.6).

Deur gebruik te maak van die nuwe Inokulum Monitor in drie verskillende areas, naamlik, Brits, Mooinoi, Burgersfort en Letsitele, het dit duidelik geword dat die begin van die infeksieperiode in die verskillende gebiede nie dieselfde is nie. In die "koeler" gebiede is die eerste spoorvystellings heelwat later. Beter programmering van spuitskedules kan met behulp van hierdie instrument nou gedoen word, met gevolglike kostebesparings. Dit is ook nou duidelik dat die hoofbron van besmetting kom van blare wat val tussen Julie en Desember. Dit toon ook dat as dooie blare uitgeskakel word tussen Oktober en Desember, geen bespuitings nodig sal wees nie. Die uitsondering is wanneer sterk infeksie periodes voorkom in ou boorde na middle-Februarie. Geskikte programme word spesifiek vir hierdie situasies ondersoek. Inokulasie studies is ook tans aan die gang om presies te bepaal wanneer sitrusvrugte bestand raak teen infeksie (QMS 03/68n). Die droë weer gedurende die afgelope twee seisoene het ons in die wiele gery. Onder sekere toestande kan askospore wel op (aan) bome geproduseer word (4.5.11) maar hierdie bron van inokulum is nie baie belangrik nie.

Chemiese beheer word intensief nagevors. Mankoseb is besig om uitgedruk te word deur die markte. So ver is daar verskeie koperbevattende middels wat net so goed werk as Mankoseb. Die stippelvorming van die koperverbindings word deeglik aangespreek en sal in die toekoms 'n groter aandeel in die spuitprogram hê. Aanduidings is dat 'n nuwe rol vir Benomil gevind word (4.5.7, 4.5.8, 4.5.9 & 4.5.13).

'n Interessante ontwikkeling is dat 'n produk soos Sporekil onverwags belowende resultate gegee het wanneer dit in programme gebruik word. Die werk is egter onvolledig en tans kan geen aanbevelings in die verband gedoen word nie (4.5.9).

Een Kwekery (Du Roi) word tans gemoniteer om 'n protokol saam te stel om swartvlek se teenwoordigheid in kwekerye vastestel en te beheer (4.5.12).

Hierdie opsomming lig net uit enkele van baie goeie vordering wat met die Swartvlek probleem gemaak word. Dit is ook die resultaat van uitstaande navorsing by die Universiteit van Pretoria onderleiding van Prof. Lize Korsten, bygestaan deur Dr. Linda Meyer, Rene Jacobs, Mariette Truter en verskeie studente. Dan is Dr. Fanus Swart van QMS en sy span ywerig besig met meer prakties-gerigte ondersoeke. Dr. Tian Schutte van CRI lewer 'n waardevolle bydrae deur die evaluering van nuwe middels en ontwikkeling van spuitprogramme.

Aan alle medewerkers en die bekwame leiding van Dr. Tim Grout, baie dankie.

4.5.2 Detection of *Guignardia* from soil ecosystems Experiment PPL 4 by L Meyer, R Jacobs and L Korsten (UP)

Opsomming

'n Protokol vir die isolasie van DNA van die *Guignardia* sitrus patogeen en endofiet uit verskillende grondtipes is ontwikkel. DNA van *Guignardia citricarpa*, wat geïsoleer is uit grond versamel in 'n natuurlike geïnfekteerde boord, is suksesvol vermeerder met PKR. Die ekstraksieproses behels 'n kombinasie van die

SoilMaster en FastDNA™ Spin tegnieke en monsters word vinniger en meer effektief voorberei met die FastPrep instrument. Die PKRs is verder verfyn om inhibisie te verlig. Die toevoeging van Bees serum albumien (BSA = 300 ng/μl) tot die PCR mengsel vorm nou deel van die standaard protokol vir amplifikasie van grond en plant DNA. Negatiewe resultate kan verder m.b.v. 'n eenvoudige inhibisie toets bevestig word voor finale terugvoering. Die protokol vir DNA ekstraksie en PCR vermeerdering van die patoëen in grond word aangepas en getoets vir die opsporing van die patoëen in simptoomblose groen blare en verbetering van patoëen opsporing in blaar molm.

Introduction

The objective of this study was to compare the most common elements of DNA extraction and purification protocols from soil and to use the information obtained to develop a comprehensive method for obtaining *Guignardia citricarpa* (GC) DNA from different soil samples. As *Guignardia* is difficult and even impossible to isolate directly from soil, molecular methods are expected to give a more realistic view of the presence of the pathogen. DNA-based methods do not depend on the culturability of microorganisms and therefore they offer an attractive alternative for the study of complex fungal community structures.

Materials and methods

Soil samples

Techniques were evaluated using (i) naturally infested soil collected from 5 different locations with a history of CBS, (ii) 4 different soils amended with 3 different concentrations of GC pycnidiospores (spores/μl), and (iii) 4 different soils amended with 3 different concentrations of GC mycelium (mg/μl).

DNA extraction

1. DNA extraction using the SoilMaster DNA extraction kit (Epicentre) according to the manufacturer's protocol.
2. Rapid purification of DNA from soil using the FastDNA™ Spin Kit for soil (BIO101) and the FastPrep Instrument (Bio101) according to the manufacturer's protocol.
3. A combination of the SoilMaster and FastDNA Spin techniques.

Optimisation and troubleshooting

1. Removing humic acids in the DNA extract and alleviating inhibition of PCR reaction using dilutions, benzyl chloride, bovine serum albumin and PureTaq PCR beads.
2. Developing a protocol to rule out inhibition with negative PCR results.
3. Detection of very low pathogen numbers using nested PCR amplification reactions.

Results and discussion

FastPrep Instrument

Preliminary tests with a bead beater (FastPrep Instrument from Bio101) using fungal cultures, leaf litter and soil proved successful. Bead beating is based on the physical disruption of cells by glass or ceramic beads under rapid agitation and the protection of the DNA by a stabilizing lysis buffer. Cells are lysed with minimal shearing of the nucleic acids. The procedure eliminates the major concerns in isolation of nucleic acids from cells that are difficult to lyse without enzymes, manual grinding, or homogenizing. It is these laborious and time consuming lysing steps which allow nucleases to act and can make nucleic acid isolation a chore.

DNA extraction

1. Soilmaster™ DNA extraction kit: The SoilMaster™ DNA extraction kit provides all of the reagents necessary to recover PCR-ready DNA from a variety of environmental samples. The kit utilizes a hot detergent lysis process combined with a chromatography step, which removes enzymatic inhibitors known to co-extract with DNA from soil and sediment samples. This is the most recent development/kit on the market, which concentrates on removal of humic acids. Total alleviation of inhibition was obtained with this kit. The only negative being the insufficient lyses of the fungal material using a mortar and pestle together with liquid nitrogen.
2. BIO101 FastDNA™ SPIN Kit for soil (BIO 101): The FastDNA™ SPIN Kit for soil is designed to extract PCR-ready genomic DNA from soil samples in less than 30 minutes. The kit enables the extraction of genomic DNA from all bacteria, fungi, plants and animals in a soil community. Good DNA at high concentrations was obtained (Fig. 4.5.2.1), but inhibition in some of the samples was still evident.

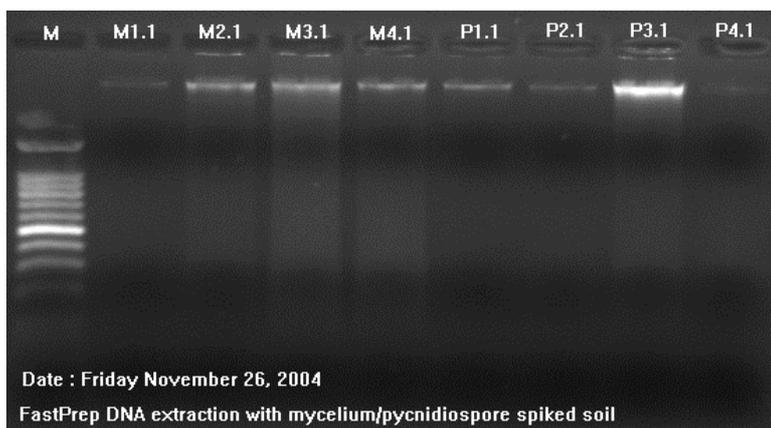


Figure 4.5.2.1. DNA isolated from artificially infested soil using the combined SoilMaster and FastDNA Spin kits.

3. Combined technique: The FastPrep Instrument and Matrix E tubes were used in combination with the SoilMaster kit and successful DNA extraction was obtained. PCR amplification had a 100% success-rate in all samples tested so far (Fig. 4.5.2.2). More sample (up to 300 mg of soil) can be used with this altered SoilMaster technique. Fine-tuning is still necessary.

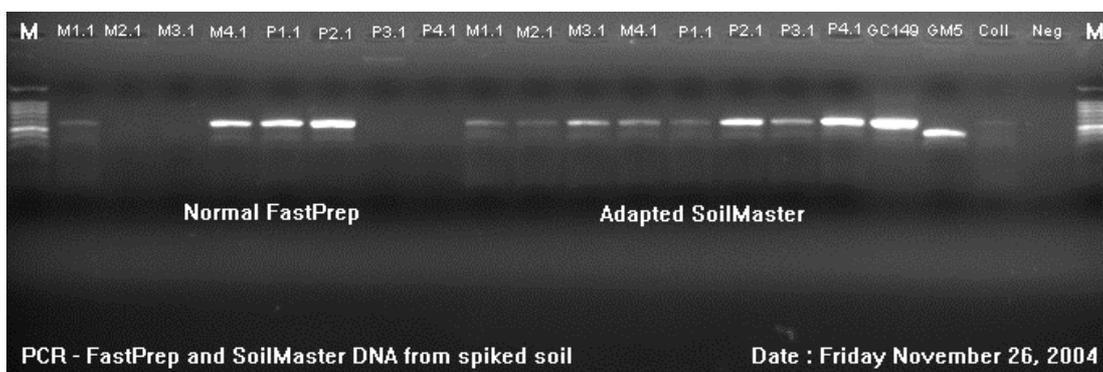


Figure 4.5.2.2. PCR products of DNA isolated using the standard FastPrep protocol and the adapted SoilMaster kit.

Optimisation and troubleshooting

1. Addition of benzyl chloride (BC) to the DNA extraction steps often helps to eliminate humic acids in the DNA extract and therefore limits any inhibition of the PCR reaction. In our study, the addition of benzyl chloride during DNA extraction did not alleviate inhibition, and seeing that it is a highly toxic substance, experimentation with BC was discontinued.
2. DNA polymerases, have been found to be inhibited by as little as 1 μ l of undiluted humic acid-like extract, regardless of the amount of DNA present. In many such cases dilution rather than further purification of soil DNA extracts will achieve a successful PCR reaction. Dilution exploits the sensitivity of PCR by reducing the concentration of inhibitors relative to target DNA. We did not get consistent results with diluted DNA, and although it can be used in problematic cases it will probably not form part of the final protocol.
3. Bovine serum albumin (BSA) has had widespread use for relieving interference in PCR. The optimum concentration of BSA for relief of inhibition from humic acids in our case was determined to be 300 ng/ μ l of the PCR reaction mix. The addition of 300 ng/ μ l BSA to the PCR mix is now standard protocol when the DNA to be amplified originates from soil or plant material.
4. The use of nested PCR improves the sensitivity of the assay by allowing the detection of minute amounts of target DNA, even from soil with a high organic matter content. Nested PCR was performed with all samples extracted from naturally infested soil. DNA was subjected to PCR amplifications with universal primers ITS1 and ITS4 as well as ITS1 and ITS2. The resulting PCR

products were amplified in a second PCR with primer pairs ITS4, CITRIC1 and CAMEL2 under the same conditions. This was repeated several times. The PCR products from GC and GM isolates could not be distinguished on agarose gels and therefore the testing with nested PCRs was discontinued.

5. The use of PureTaq PCR beads resulted in successful amplification even with inhibitors present, but is only used in special circumstances due to cost implications.
6. Inhibition test: The test is very trustworthy and was used successfully to display and confirm inhibition of PCR reactions with DNA originating from soil extracted using the standard FastDNA extraction method.

Conclusion

The SoilMaster DNA kit is being used in conjunction with the FastDNA spin kit and FastPrep Instrument to extract DNA of the *Guignardia* citrus pathogen and endophyte from soil. The sensitivity of the procedure still needs to be defined. The survival of the pathogen in soil under different environmental conditions, the presence/absence of the host, various cultivation practices etc., can now be easily studied. Preliminary tests suggest that mycelium and even thin-walled pycnidiospores can survive in soil for an undetermined period of time. No further work will be done on this subject due to the termination of this part of the project.

PCR reactions with the designed primers for GC and GM are being done with the addition of 300 ng/μl BSA to the reaction mix to alleviate possible inhibitory substances, such as humic and phenolic acids when working with soil and plant material. Negative results can now be screened for inhibition before final confirmation.

Future research

The protocol for DNA extraction and PCR amplification of the pathogen in soil are being adjusted and tested for pathogen detection in green symptom-less leaves and improvement of leaf litter pathogen detection.

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4.5.3 Inoculation of leaves with *Guignardia citricarpa* pycnidiospores

Experiment PPL 6 by M Truter, B Greyvenstein and L Korsten (UP)

Opsomming

Blaar inokulasies met pycnidiospore van *Guignardia citricarpa* is weer uitgevoer op nuwe swart vlek vrye bome. Bome is ontblaar, nuwe blare is gemerk en maadeliks geïnokuleer. Herisolasies is uitgevoer 'n maand na elke inokulasie. Teen einde Desember 2004 is blare tot die ouderdom van 5 maande suksesvol geïnokuleer en *G. citricarpa* geherisoleer. Die eksperiment sal voortgaan en blare tot die ouderdom van 14 maande sal geïnokuleer word. Die beskermende waarde van nuwe chemiese of ander middels kan vinnig en meer akkuraat geëvalueer word deur die inokulasie tegniek.

Introduction

It has been observed that fruit become resistant to pathogen infection between four and five months after flowering (Kotzé, 2000). To date, no data are available regarding the age of leaves at which they become resistant to new ascospore infections. This paper reports on the period of leaf susceptibility to *G. citricarpa* pycnidiospore infections in Valencia leaves.

Materials and methods

Plant materials

Two-year-old Valencia orange and Eureka lemon trees from Western Cape Province were obtained in April 2004. The trees were transplanted to 4 ℓ plastic bags and maintained in a greenhouse with temperatures ranging between 12 to 30°C. The trees were manually defoliated and petioles of new flushes (less than 14 days old) were labelled with short plastic straws. Three replicate trees were used per inoculation.

Fungal isolate

A *G. citricarpa* isolate (GC252), originally obtained from naturally infected fruit, were maintained in the Plant Pathology Laboratories fungal culture collection at the University of Pretoria. The isolate was sub-cultured onto half-strength potato dextrose agar (PDA) (Difco) and incubated at 20°C (\pm 2°C) under continuous fluorescent light for 18 to 21 days to stimulate sporulation. Pycnidiospores were harvested and a spore suspension of 10³ spores ml⁻¹ was prepared in sterile water. Leaf inoculation was done within 4 h after preparation of the spore suspension.

Leaf inoculation

The entire tree was covered with a plastic bag and inoculated by spraying the leaves abaxially and adaxially with the pycnidiospore suspension, until run-off. After 48 h, the bags were removed and 5 marked leaves per tree were collected after a month for re-isolations. New trees were inoculated each month. The control trees were treated the same, but were inoculated with only sterile water.

Re-isolation

Collected leaves were surface disinfected for 1 min with 1% sodium hypochlorite, rinsed with sterile water, blotted dry and sections (10 mm²) of the leaves aseptically plated to half-strength PDA. The plates were incubated for two weeks at 25°C and developing colonies noted. The retrieved colonies were identified and for confirmation the polymerase chain reaction (PCR), according to the test method PPL009 (Meyer *et al.*, 2001) was performed on selected re-isolated cultures in the ISO 17025 accredited Plant Pathology Laboratory, University of Pretoria. Five replicate leaves per tree were used.

Results and discussion

Valencia orange and Eureka lemon leaves up to the age of 5 months were successfully infected with pycnidiospores and *G. citricarpa* could be re-isolated from the leaves after an infection period of one month. The identity of selected isolates obtained from the leaf segments was confirmed with PCR as *G. citricarpa*. Since citrus leaves could remain on the tree for up to three years (Kotzé, 2000), more research is required to determine for how long the pathogen can be re-isolated.

Conclusion

Valencia orange and Eureka lemon leaves can be infected by pycnidiospores of *G. citricarpa* up to 5 months.

Future research

The experiment will continue during 2005 without funding and leaves up to the age of 14 months will be inoculated.

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4.5.4 Seasonal availability of ascospore inoculum of *Guignardia citricarpa* Experiment PPL 12 by M Truter, B Greyvenstein and L Korsten (UP)

Opsomming

Die beskikbaarheid en rywording van *Guignardia citricarpa* askospore is ondersoek in 'n Valencia boord in Burgersfort en Valencia en Eureka boorde in Brits. Natuurlike blaar monsters is maandeliks versamel en die beskikbare askospore op die materiaal gevang op 'n Vaseline bedekte mikroskoopplaatjie d.m.v. 'n Kotzé Inokulum Monitor. Natuurlike geïnfecteerde volwasse groen blare is maandeliks gepluk en in die boorde

geplaas in roosters om die rywording van askospore te ondersoek. Natuurlike Valencia blaarafval het gemiddeld 0 tot 657 askospore opgelewer per 20 blare, en Eureka blaarafval gemiddeld 0 tot 3511. Geen askospore is gevang van die geplukte groen Eureka en Valencia blare na 6 maande nie. Die ondersoek sal voortduur tot einde 2005.

Introduction

Citrus Black Spot (CBS), caused by *Guignardia citricarpa* Kiely, affects the production of citrus in subtropical regions with a summer rainfall climate (Kotzé, 2000). Infection occurs through ascospores released from pseudothecia on dead infected leaves on the orchard floor. Thus far, information on ascospore release in South Africa was based on data obtained with a Burkhard volumetric sampler (Campbell & Madden, 1990) and from 1997 onwards with a Quest spore trap, which is based on the Burkhard volumetric sampler and locally manufactured by Quest Developments. The Quest spore trap is used in the field and can determine the inoculum present in the air for a couple of hours or up to eight days, but results are not always in time to implement control measures. To establish the potential inoculum at a specific time in a particular locality, a different type of sampler was required. A new apparatus was therefore designed and manufactured by Quest Developments in collaboration with Prof J.M. Kotzé to determine the inoculum present in samples of leaf material at any given time (Truter *et al.*, 2004). Information obtained using the new Kotzé Inoculum Monitor (KIM) can be used to estimate the potential inoculum load available in orchards to cause new infections by *G. citricarpa* or other plant pathogens that are mainly disseminated through airborne spores.

Materials and methods

The availability and maturation of ascospores on citrus leaf litter was assessed from 2 Valencia orchards in Burgersfort, Mpumalanga and from a Eureka and a Valencia orchard in Brits, Mpumalanga during 2004 with the aid of the KIM. The availability of ascospores was determined by collecting natural leaf litter at a monthly interval and processing it with the KIM. The leaf litter was submersed in water at 40°C for 5 min, placed on paper towels for 5 min to remove excess water, and placed in plastic grids (10 mm mesh size) in the KIM. A standard microscope slide, coated with a thin layer of Vaseline, was used to collect spores. After a two-hour operation, the slide was stained with lactofuchin and ascospores resembling *Guignardia* were counted in four transverse rows in the centre of the slide.

The maturation pattern of leaves and developing *Guignardia* fruiting structures was assessed in 1 Valencia orchard in Burgersfort and a Eureka and a Valencia orchard in Brits by picking mature green leaves, placing them in grids (10 mm mesh size) and leaving these underneath trees in the orchard for up to 6 months. Three grids were collected each month and processed with the KIM as described above.

Results and discussion

Natural Valencia and Eureka leaf litter (average of 20 leaves) contained 0 to 657 and 0 to 3511 ascospores, respectively, upon collection as determined with the KIM (Fig. 4.5.4.1). Large variation in available ascospores occurred between different sampling dates and between farms sampled on the same date. No ascospores could be captured from the green Eureka and Valencia leaves that were detached and placed in grids on the orchard for 1 to 6 months.

Future research

The process of inoculum maturity of *G. citricarpa* on leaf litter will be monitored over at least two growing seasons ending with the 2005/2006 season.

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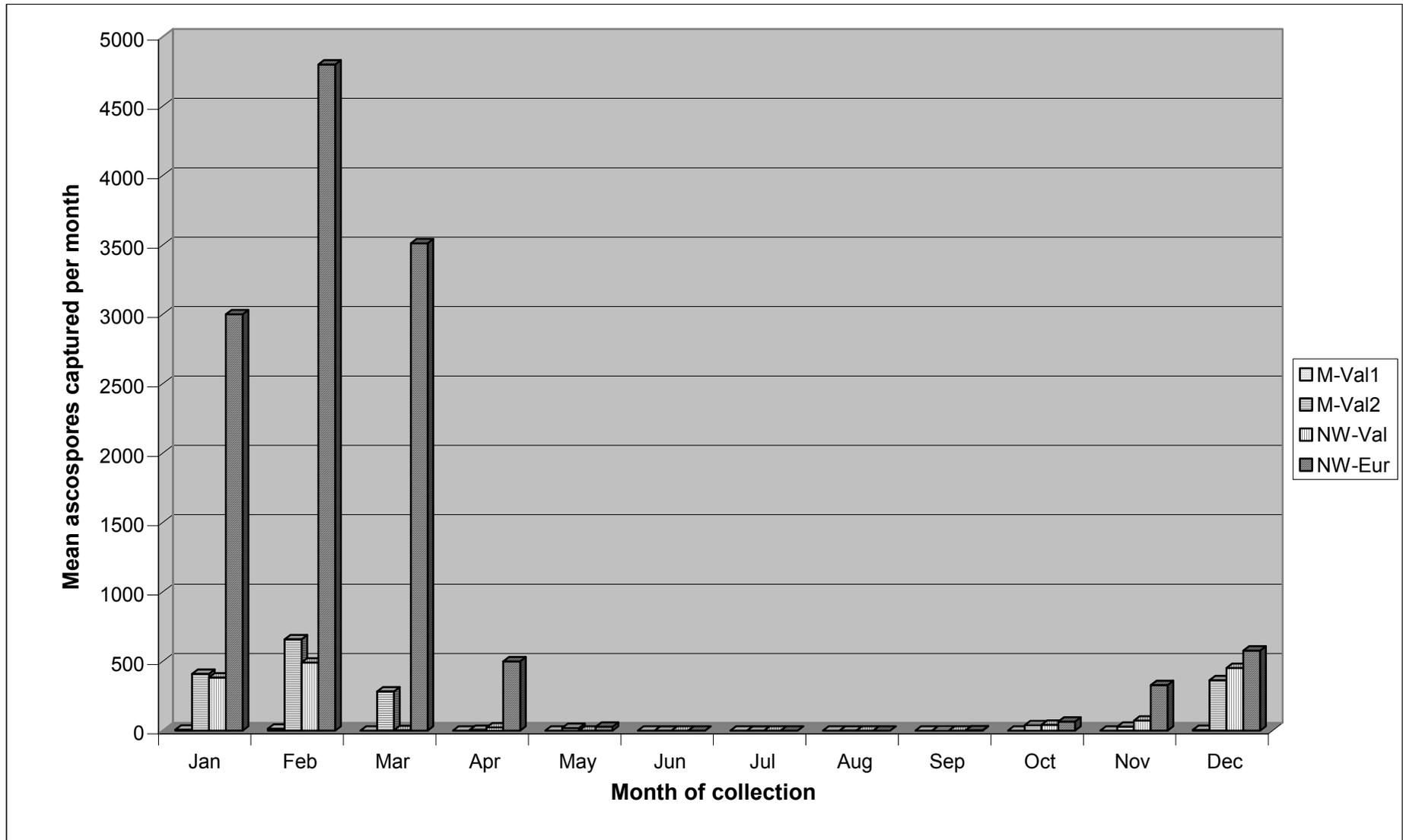


Figure 4.5.4.1. *Guignardia* ascospores captured on Vaseline coated microscope slides with the Kotzé Inoculum Monitor from citrus leaf litter collected during 2004 from the orchard floor. M-Val1 = Valencia orange orchard in Mpumalanga; M-Val2 = Valencia orange orchard in Mpumalanga; NW-Val = Valencia orange orchard in North-West; NW-Eur = Eureka lemon orchard in North-West.

4.5.5 Breaking the life cycle of *Guignardia citricarpa*: Removal or confinement of inoculum Experiment PPL 13-A by M Truter, B Greyvenstein and L Korsten (UP)

Opsomming

Verwydering of vasvangung van die oorwinterde inokulum van *Guignardia citricarpa* was ondersoek in 'n 36-jarige Valencia boord naby Burgersfort. Al die blare op die boordvloer was met die hand verwyder en verbrand middel Oktober 2003. Agt aangrensende rye van 20 bome elk was gebruik vir die behandelings en het tydens die proef tydperk geen chemiese bespuiting vir swart vlek ontvang nie. Die boordvloer oppervlak van vier van die agt rye was bedek met 'n laag koringstrooi. By die ander vier rye was die blare weer verwyder van die boordvloer 'n maand later. Die omliggende boorde was met drie maal met chemiese middels gespuit vanaf Oktober 2003 tot Januarie 2004. Evaluering van die boorde tydens die opeenvolgende seisoen het 'n sitrus swart vlek insidensie aangedui van 1.84% in die strooi bedekte rye en 0.77% in die onbedekte rye. Feitlik geen sitrus swart vlek was sigbaar op die chemiese behandelde vrugte nie.

Introduction

The most important inoculum source of citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is airborne ascospores (Kotzé, 1981). Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40 to 180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during spells of rain (Kotzé, 1963) or irrigation (Smith, 1996). Exclusion of the pathogen is the ultimate aim in any disease control programme. The most critical period for infection occurs at fruit set and can persist for ca. four months. Therefore, infected leaf litter must be removed before and during this critical infection period, to reduce the available CBS inoculum. In South Africa the critical infection period is between October and January. Reduction of available inoculum in this period can be achieved by entire removal, inactivation or immobilisation of overwintering inoculum residing in infected leaf litter on the orchard floor. This report describes an experiment aimed at elucidating the efficacy of removal of leaf litter and mulching of the soil under infected trees for the control of CBS.

Materials and methods

The experimental site comprised an orchard near Burgersfort with 36-year-old Valencia orange on Rough Lemon rootstock. All the leaves on the orchard floor were manually removed and burned in October 2003. A total of eight adjacent rows of 20 trees each were used and received no chemical spray for CBS. In early November, the surface under the trees in four of the eight rows, was mulched with a layer of wheat straw, whereas leaves were removed again from the unmulched area in the other four rows. In the adjacent orchards, leaf litter was not removed. Trees were evaluated in August 2004. Forty-eight evenly distributed fruit on each tree were assessed for CBS according to a rating scale: 0 = clean; 1 = 1-5 spots per fruit; 2 = 6-50 spots per fruit; 3 = fruit extensively infected. A severity index was calculated for each tree by means of the following formula:

$$\text{CBS-index} = 100 \times \frac{(0n_0 + 0.25n_1 + 0.5n_2 + 0.75n_3)}{n_{\text{total}}}$$

Where n represents the total number of infected fruit falling into each of the categories.

Data were analysed using the statistical program GenStat (2002). Analysis of variance was used to test for differences between values and means were separated according to Fisher's protected *t*-test least significant difference.

Results and discussion

Due to the low disease incidence, no significant differences were detected between treatments with a CBS incidence of 1.84 % in the mulched rows and 0.77 % in the unmulched rows (Table 4.5.5.1). The mulched trees had a higher CBS incidence compared to the unmulched ones in terms of total infected fruit per treatment (results not shown). No significant differences were evident between fruit borne within the canopy and on the outside (results not shown). Differences were evident between fruit of different aspects of the tree, with CBS occurring only on the northern and eastern side of the tree (results not shown). No CBS was present on chemically sprayed fruit in the adjacent orchards at the time of assessment.

Table 4.5.5.1. Incidence and severity of citrus black spot in a citrus orchard where leaf litter was either removed or removed together with mulching with wheat straw

Parameter	Mulched*	Unmulched*	Chemical sprayed*
Infected trees (%)	2.63	0.92	0.28
Infected fruit (%)	0.06	0.03	0.01
CBS-index	1.84	0.77	0.59

* Values do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \geq 0.05$).

Conclusion

No significant differences were observed between treatments due to low disease incidence. The available newly produced ascospores were reduced in the orchard by continuous leaf litter removal and almost no ascospores could be detected from the newly formed leaf litter with a Kotzé Inoculum Monitor (Section 4.5.4, Experiment PPL 12).

Future research

The treatments were repeated for the last time in the same orchard during the 2004/2005 season, with first leaf litter removal in October 2004. The fruit will be evaluated in September / August 2005.

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4.5.6 Biological control of *Guignardia citricarpa*

Experiment PPL 13-B by M Truter, B Greyvenstein and L Korsten (UP)

Opsomming

Blare van jong Valencia lemoen bome (ongeveer 2 jaar oud) was beskerm teen *Guignardia citricarpa* infeksies deur 'n voorkomende spuit behandeling met sitrus afval kompos tee, Trykocide (*Trichoderma harzianum*) en Avogreen (*Bacillus subtilis*) na 8, 2 en 4 dae, onderskeidelik. Trykocide was die enigste behandeling wat beskerming teen infeksie gegee het na spuiting vir meer as 1 periode wat getoets is. Meer navorsing is nodig voordat enige konklusies afgelei kan word van die data.

Introduction

Citrus black spot (CBS) is an economically important disease that can effectively be controlled by the timely application of appropriate control measures (Kellerman, 1976). Since the nature of the infection is latent, it allows a curative approach (eradicate infection and prevent symptom development) towards disease control (Kellerman & Kotzé, 1977), although a preventative approach (applying control agents before infection to protect fruit) has been reported to be more reliable (Kellerman, 1976). The most actively researched control measures alternative to chemicals is currently in the field of biological control. The objective of this study was to evaluate biocontrol agents for its ability to reduce inoculum in infected leaves.

Materials and methods

Plant materials

Two-year old Valencia orange trees from Western Cape Province were obtained in April 2004. The trees were transplanted to 4 l plastic bags and maintained in a greenhouse with temperatures ranging between 12 to 30°C. Three replicate trees were used per treatment.

Fungal isolate

A *G. citricarpa* isolate (GC252), originally obtained from naturally infected fruit, were maintained in the Plant Pathology Laboratories fungal culture collection at the University of Pretoria. The isolate was sub-cultured

onto half-strength potato dextrose agar (PDA) (Difco) and incubated at 20°C (\pm 2°C) under continuous fluorescent light for 18 to 21 days to stimulate sporulation. Pycnidiospores were harvested and a spore suspension of 10^3 spores ml⁻¹ was prepared in sterile water. Leaf inoculation was done within 4 h after preparation of the spore suspension.

Biocontrol treatments

Compost tea was prepared each from composted kraal manure and citrus waste compost by adding 2 l water to 1 l compost and aerating the mixture for 3 weeks. The filtered compost tea was stored at 4°C for up to 5 days before being used.

Trees were sprayed until run off with the compost tea extracts or commercial available biocontrol agents, Trykocide (*Trichoderma harzianum*) at 10 ml/l and Avogreen (*Bacillus subtilis*) at 10 ml/l at 1, 2, 4 and 8 days before inoculation with *G. citricarpa*. The control trees received only sterile water.

Leaf inoculation

The entire tree was covered with a plastic bag and inoculated by spraying the leaves abaxially and adaxially with the pycnidiospore suspension, until run-off. After 48 h, the bags were removed and 5 marked leaves per tree were collected after a month for re-isolations. The control trees were treated the same, but were inoculated with only sterile water.

Re-isolation

Collected leaves were surface disinfected for 1 min with 1% sodium hypochlorite, rinsed with sterile water, blotted dry and sections (10 mm²) of the leaves aseptically plated to half-strength PDA. The plates were incubated for two weeks at 25°C and developing colonies noted. The retrieved colonies were identified and for confirmation the polymerase chain reaction (PCR), according to the test method PPL009 (Meyer *et al.*, 2001) was performed on selected re-isolated cultures in the ISO 17025 accredited Plant Pathology Laboratory, University of Pretoria. Five replicate leaves per tree were used.

Results and discussion

Colonisation of citrus leaves by *G. citricarpa* was prevented with a preventative spray of 8, 2 and 4 days for citrus waste compost tea, Trykocide and Avogreen, respectively. The colonisation of the biocontrol agents on the citrus leaves needs to be studied to determine the optimum period required for establishment.

Table 4.5.6.1. Retrieval of *Guignardia citricarpa* from artificially inoculated trees previously sprayed with biocontrol agents ^a

Biocontrol agent	Days after biocontrol treatment			
	1	2	4	8
Composted kraal manure tea	+ ^b	+	+	+
Citrus waste compost tea	+	+	+	-
Trykocide	+	-	-	-
Avogreen	+	+	-	+

^a Mean of 5 leaves per tree, with 3 trees per treatment.

^b + = *G. citricarpa* was successfully isolated; - = *G. citricarpa* was not isolated.

Conclusion

More research is required before any conclusions can be drawn from this data.

Future research

None.

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4.5.7 Positioning of a single benomyl application in a strobilurin spray programme Experiment 707 by G.C. Schutte (CRI)

Opsomming

Die strobilurin, trifloxystrobin (Flint) in 'n tenkmengsel met koperhidroksied (Copstar) teen 'n verlaagde dosis van 300 ml/h ℓ water wat afgewissel word met die benzimidazole, carbendazim of benomil, toon groot potensiaal vir die beheer van swartvlek en dit in boorde waar die benzimidazool weerstandbiedende populasie 87% beslaan. Dit wil voorkom asof die spuitvolgorde van trifloxystrobin+koperhidroksied+olie/carbendazim of benomyl+mancozeb+olie (A+B:C+D) instelle van carbendazim+mancozeb+olie /trifloxystrobin+koperhidroksied+olie (C+D:A+B) die ideale spuitvolgorde kan wees, maar die proewe moet herhaal word om dit te bevestig.

Introduction

Fungal resistance development to strobilurins is an ever existing possibility. Resistance to fungicides such as benzimidazoles, dicarboximides and triazoles has been reported. Therefore, anti-resistance strategies using fungicides with different modes of action have to be investigated without losing the edge on effective control with less spray rounds. It was reported by a consultant that a single Benlate application gave good CBS control at Lisbon Estates where resistance towards benzimidazoles was reported in the 1980s. Since then, no benzimidazoles have been sprayed on that Estate. However, with new ownership of the Estate, CBS control was intended although no CBS applications were performed during the summer of 2001 and 2002. A consultant was approached and asked what control options could be considered for CBS at the end of February 2002. A single benomyl application at a rate of 75 g/h ℓ water was advised and applied. With this application, good CBS control was achieved and this after 21 years! Therefore we need to investigate how a single curative benomyl application will perform in a benzimidazole resistant environment and the placement thereof in a strobilurin spray programme also consisting of two applications in November and January according to the label, but where a tank mixture with mancozeb is replaced with a copper fungicide.

Materials and methods

Two orchards were selected. One was at Crocodile Valley Citrus Co. and one at Friedenheim Estates, both in the Nelspruit region. A randomized block design with 5 and 3 single-tree plots per treatment was used respectively. Trees in both groves were selected for uniformity in canopy density and tree size. Guard trees were located between plots within rows. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500- 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Certain treatments commenced in mid-October 2004 as previously recommended, depending on the climatological information required for infection during the critical infection period. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. The fungicides tested included mancozeb (Sancozeb, 80% WP), benomyl (Benlate, 50% WP), trifloxystrobin (Flint, 50% WG), carbendazim (Bavistin 50% SC), copper hydroxide (Copstar 12% SC) and the mineral spray oil, Citrex. At fruit maturity in July or August, CBS severity was rated on 100 fruits per tree according to a 3-point index : 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test ($P=0.05$). They will be sprayed as either A+B : C+D in November and January or as C+D : A+B also in November and January where A = Benlate, B = mancozeb, C = Flint and D = copper.

Fruit infested with CBS were sampled throughout the orchard at Friedenheim and about 150 isolates were made from the fruit and plated onto PDA and incubated at 25°C under artificial light for 14 days. Thereafter an aqueous stock suspension of benomyl was made up in sterile distilled water at a rate of 5 ppm. This was added to PDA after it was autoclaved at 120°C for 15 minutes and cooled to approximately 45°C. The amended agar (20 ml) was thoroughly mixed and poured into 80 mm diameter petri dishes.

Fungal plugs (5 mm in diameter) of 14-day old *P. citricarpa* cultures were aseptically removed. Five plugs were placed onto each petri dish and were then incubated at 25°C. Aqueous stock suspensions of maneb (Trimangol (SC), 435/4,7 g/l, Saadchem) and mancozeb (Sancozeb (WP), 800 g/kg, Sanachem) were made up separately at rates consisting of 100 g or ml/h ℓ water (1/2x); 200 g or ml/h ℓ water (1x or registered rate) and 400 g or ml/h ℓ water (2x) in sterile distilled water. These concentrations were added to PDA after it was autoclaved at 120°C for 15 minutes and cooled to approximately 45°C. The amended agar (20 ml) was mixed thoroughly and poured into 80 mm diameter petri dishes.

Fungal plugs (5 mm in diameter) of 14-day old *P. citricarpa* cultures were aseptically removed and five plugs were placed onto each petri dish, and were then incubated at 25°C under artificial light. Five replicates were used per isolate per concentration. Radial growth was recorded after 21 days and growth was recorded after 21 days. Fungal plugs were also taken from the same isolates and plated onto oatmeal-agar to distinguish between pathogenic and non-pathogenic strains as described by Baayen *et al.*, (2002).

Results and discussion

According to Table 4.5.7.1 the trial site at Friedenheim Estates showed that the alternation of the tank mix combination with either trifloxystrobin+copper hydroxide+oil/ benomyl +mancozeb+oil (A+B:C+D) or benomyl + mancozeb+oil/ trifloxystrobin + copper hydroxide + oil carbendazim + mancozeb + oil (C+D:A+B) sprayed in mid-November and mid-January, only resulted in 86% clean exportable fruit that was significantly different ($P < 0.05$) from the standard mancozeb treatment. The mancozeb treatment resulted in 10% more clean exportable fruit. However, where benomyl was replaced with its breakdown product, carbendazim, also in tank mixtures with mancozeb and oil, these treatments sprayed as either trifloxystrobin+copper hydroxide+oil/carbendazim+mancozeb+oil (A+B:C+D) or carbendazim+mancozeb+oil/trifloxystrobin+copper hydroxide+oil (C+D:A+B) resulted in more clean exportable fruit for export of 99.33%, that is 3% better than the standard mancozeb treatment. All these treatments were significantly different from the control that had only 19.33% clean exportable fruit.

The trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P>0.05$) between any of the treatments. However, they were all significantly different from the control that resulted in 39.60% clean exportable fruit. In both trials, the trifloxystrobin+copper hydroxide+oil/ carbendazim+mancozeb+oil (A+B:C+D) sequence at Friedenheim Estates and the trifloxystrobin+copper hydroxide+oil/ benomyl +mancozeb+oil (A+B:C+D) sequence at Crocodile Valley Citrus Co., was the only spray programme that resulted in fruit with no lesions in the category with four and more CBS lesions. The latter spray programme sprayed at Friedenheim Estates had 7% fruit with four and more CBS lesions. This can be attributed to the poor spraying and coverage of one particular tree. It was also impossible to exclude this tree from the statistical analysis because there were only 3 replicates.

From the initial 150 CBS isolates made from the fruit, only 49 grew and 87% of these isolates were resistant to CBS. Furthermore, 85% of the same isolate represented *G. citricarpa* and the rest were *G. mangiferae*.

Conclusion

It appears that the residual action of the strobilurin, trifloxystrobin, is long enough to protect fruit for 8 weeks if mixed with copper hydroxide and spray oil. Moreover, according to the restrictions on the use of plant protection products on export citrus, the other two registered strobilurins, azoxystrobin (Ortiva) and pyraclostrobin (Cabrio), both have a pre-harvest interval restriction of 77 and 82 days respectively, while trifloxystrobin is not permitted to be sprayed later than mid-January, illustrating that these fungicides do have a long residual action. Benomyl on the other hand, although it was sprayed where the benzimidazole population was 87%, still maintained clean fruit and could still provide a curative action perhaps in a synergistic way with the strobilurins. The addition of copper hydroxide to the tank mixture with trifloxystrobin and spray oil at a reduced rate of 300 ml/hl water showed no phytotoxic symptoms and can play a role in the extended protection period required and should rather be used instead of mancozeb. Therefore, the alteration of strobilurins with benzimidazoles is not only effective against CBS, but can also result in less residues on fruit, especially with regards to mancozeb, and the latter can be successfully replaced with copper fungicides such as copper hydroxide. Although not conclusive, it seems as if the trifloxystrobin+copper hydroxide+oil/ benomyl + mancozeb+oil (A+B:C+D) sequence of spraying can be recommended in future for the control of CBS. Although only one field trial with the other benzimidazole, carbendazim, was conducted with excellent results, it should be repeated during the coming season.

Future research

The experiment will be repeated and *G. citricarpa* and *G. mangiferae* populations will be monitored closely to determine if there has been a population shift in the orchards.

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Table 4.5.7.1. Evaluation of spray programmes consisting of tank mixtures of Flint, Copstar and spray oil and Benlate/Bavistin, Dithane and spray oil during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co., S.A. and Friedenheim Estates during 2003 and 2004.

Treatment (a+b/c+d)	Rate / 100ℓ water	Friedenheim Estates, Nelspruit ^y			Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^w	% fruit with 1 – 3 CBS lesions ^w	% fruit with four and more CBS lesions ^w	%Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x
Carbendazim+mancozeb+oil/ Trifloxystrobin+copper hydroxide+oil	55 ml + 150 g + 0.25%/ 10 g + 300 ml+ 0.25%	99.33 a	0.33 a	0.34 a	ND	ND	ND
Trifloxystrobin+copper hydroxide+oil/ Carbendazim+mancozeb+oil	10g + 300 ml + 0.25%/ 55 ml + 150 g + 0.25%	99.33 a	0.67 ab	0.00 a	ND	ND	ND
Mancozeb	200 g	96.33 a	0.66 ab	3.00 a	95.20 a	1.60 a	3.20 a
Trifloxystrobin+copper hydroxide+oil/ Benomyl+mancozeb+oil	10 g + 300 ml + 0.25%/ 50 g + 150 g + 0.25%	86.00 b	7.00 bc	7.00 a	100.00 a	0.00 a	0.00 a
Benomyl+mancozeb+oil/ Trifloxystrobin+copper hydroxide+oil	50 g + 150 g + 0.25%/ 10 g + 300 ml+ 0.25%	86.00 b	8.00 c	6.00 a	95.00 a	2.20 a	2.80 a
Control		19.33c	20.33 d	60.33 b	39.60 b	14.60 b	54.20 b

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004 for mancozeb alone and 13 November 2003 and 8 January 2004 for the other treatments sprayed in a tank mixture

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004 for mancozeb alone and 11 November 2003 and 6 January 2004 for the other treatments sprayed in a tank mixture

ND = Not determined

4.5.8 *In vitro* and *in vivo* evaluation of maneb (Trimangol and Manager) and mancozeb (Sancozeb) against pathogenic *Guignardia citricarpa* isolates, the cause of citrus black spot on Valencias Experiment 744 by G.C. Schutte (CRI)

Opsomming

Maneb is sedert 1999 weer geherregistreer vir die beheer van sitruswartvlek (SSV) en verskeie navrae is ontvang van kwekers of die nuwe produkte geskik is vir die beheer van die siekte aldan nie. Twee produkte is bekom vir die evaluasie, naamlik Trimangol en Manager. Slegs Trimangol is vir die *in vitro* proef gebruik en slegs Trimangol is ook in al die veldproewe gebruik terwyl Manager in net een veldproef ingesluit is. Mancozeb (Sancozeb) is as geregistreerde standaard ingesluit. Resultate van die *in vitro* proewe toon dat daar statistiese verskille tussen al die mancozeb en maneb konsentrasies is wat met die vergiftigde agar tegniek getoets is en dat mancozeb beter werking toon teen SSV, maar dat die vloeibare voedingsmedium tegniek nie soortgelyke resultate opgelewer het nie, omrede die tegniek te robuust is en die swam nie genoegsaam kon ontwikkel in die toetsperiode nie. Veldproefresultate toon weer dat daar verskille van 0, 4 en 10% was tussen Trimangol en Sancozeb in die drie veldproewe wat uitgevoer is en 9% tussen Manager en Sancozeb wat slegs in een veldproef beproef is.

Introduction

Maneb is back on the market for the control of citrus black spot (CBS). How and where the newly formulated fungicides were tested, is not known. Reports on its efficacy are not promising and that is why it has to be re-evaluated. Furthermore, why maneb's active ingredient was lowered to 435 g/l and the manufacturer still expects to achieve the same level of control as mancozeb (registered at 800 g/l), is not known, and can and should be explained by the manufacturer. According to consultants, maneb, which was originally registered as 800 g/l in 1972 (Bot, J., Genis, De L., & Hollings, N., 1972) was reported to be phytotoxic to citrus fruit and could be the reason why the formulation was lowered to 435 g/l. Maneb was only re-registered in 1999 while mancozeb's rate has never changed and is still regarded as the best dithiocarbamate on the market. Zineb (640 g/l), another dithiocarbamate, was also registered for use against CBS in 1972, but more spray applications (6-7) at 19-21 day intervals are needed, in comparison with mancozeb and maneb's spray applications at 24 day intervals. This was not favoured by the growers and is no longer considered as an option for CBS control by citrus growers. Isolates, that were obtained from Hennie Korf (BASF) isolated from Valencia oranges from Bosveld Citrus and Vorster in the Letsitele area during the 2001/2002 season, were used in this study. Both isolates were sent to and tested by the University of Pretoria for their pathogenicity according to the method described by Baayen *et al.* (2002) and Meyer *et al.* (2001). Both isolates belonging to the pathogenic strain *viz. Phyllosticta citricarpa* tested positive. From these isolates, sub-cultures were made, placed onto potato dextrose agar (PDA) and incubated at 25°C under artificial light. From these, isolates were used to determine the potential difference of fungicidal activity of maneb and mancozeb against pathogenic *P. citricarpa* using the poisoned agar and nutrient broth techniques.

Materials and methods

In vitro studies

a) *Poisoned agar technique*

Aqueous stock suspensions of maneb (Trimangol (SC), 435/4,7 g/l, Saadchem) and mancozeb (Sancozeb (WP), 800 g/kg, Sanachem) were made up separately at rates consisting of 100 g or ml/hl water (1/2x); 200 g or ml/hl water (1x or registered rate) and 400 g or ml/hl water (2x) in sterile distilled water. These concentrations were added to PDA after it was autoclaved at 120°C for 15 minutes and cooled to approximately 45°C. The amended agar (20 ml) was mixed thoroughly and poured into 80 mm diameter petri dishes.

Fungal plugs (5 mm in diameter) of 14 day old *P. citricarpa* cultures were aseptically removed. Five plugs were placed onto each petri dish and were then incubated at 25°C under artificial light. Five replicates were used per isolate per concentration. Radial growth was recorded after 21 days as the mean value of two diameter points subtracting the diameter of the fungal plug. Results were statistically analysed according to Fisher's LSD test ($P = 0.05$) and the percentage mycelial inhibition was then calculated accordingly.

b) Nutrient broth technique

Sixteen grams of Nutrient broth (Biolab diagnostics (Pty) Ltd, Midrand) were dissolved in 1 litre of distilled water, mixed and 100 ml poured into 250 ml Erlenmeyer flasks and autoclaved at 120°C for 15 minutes and left to cool afterwards. Fungal plugs (5 mm in diameter) that were 14 days old taken from *P. citricarpa* cultures, were aseptically removed and placed into each nutrient broth solution. Three replicates were used per isolate per concentration. All the flasks were placed onto a GFL 3016 shaking apparatus at a rotation speed of 1 rotation/second and artificial light was also used above the shaking apparatus. After 30 days, the mycelial growth was separately removed, weighed (recorded as wet weight) and placed in an oven for 14 days to dry out (35°C) then weighed again (recorded as dry weight). Results were statistically analysed according to Fisher's LSD test ($P = 0.05$) and the percentage mycelial inhibition was then calculated accordingly.

In vivo studies

Three orchards were selected to do the evaluations. One, at Croc Valley Citrus Co., and one at Friedenheim Estates in the Nelspruit region and one at Bayer CropScience's Experimental Farm in Hectorspruit. A randomized block design with 3 or 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Certain treatments commenced in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees in all the groves were selected for uniformity in canopy density and tree size. In the orchard at Flippie Walters, guard trees were located between plots within rows. Spray programs, dates and rates of application are listed in Table 4.5.8.1. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. At fruit maturity in July or August, CBS severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test ($P=0.05$).

Results and discussion

In vitro studies

a) Poisoned agar technique

In vitro results from the poisoned agar technique, showed that mancozeb tested at rates of 100, 200 and 400 g/hl water gave significantly better control at all the rates tested than maneb also tested at the same rates. Where the mancozeb rate was lowered to 100 g/hl water, only 83% mycelial inhibition was achieved in comparison with the 100% where the registered rate of 200 g/hl water was used as well as the 400 g/hl water rate. This shows again that growers should not lower the mancozeb rate to 100 g/hl water or less as it will not give the desired fungicidal effect against the disease. On the other hand, maneb evaluated at the same rates as mancozeb using 200 ml/hl water as the registered rate with 100 ml/hl water (1/2x) and 400 g/hl water (2x), performed inadequately even at 2x. All the mancozeb rates gave significantly ($P < 0.05$) better control than maneb tested at the same rates as mancozeb – even at the 1/2x rate (Figures 4.5.8.1, 2 and 3b).

b) Nutrient broth technique

Results from the nutrient broth technique showed that there was a significant difference ($P < 0.05$) between the 1/2x and 2x maneb and mancozeb rates, but not at the 1x (registered) rates (Fig. 4.5.8.3a) where the wet weight was determined. Although mancozeb was shown to inhibit *P. citricarpa* after the mycelial balls were dried out at 35°C for 14 days (dry weight), there were no significant differences between any of the rates of any of the two fungicides tested (Fig. 4.5.8.3b). This technique can, however, not be considered as ideal to evaluate fungicidal effects on *P. citricarpa*, as this slow growing fungus is sensitive to any physical changes (such as shaking) and also requires light to grow under.

In vivo studies

Results from Table 4.5.8.1 show that there were no significant differences ($P > 0.05$) between the standard registered Sancozeb (mancozeb) treatment and all the Trimangol (maneb) treatment with regards to all three criteria and in all three different trial sites used for evaluation. However, all these treatments were significantly different from the controls. There were no significant differences between the control and the

mancozeb and maneb treatments (with regards to the amount of clean fruit yielded) the differences of between 4 and 10% can be contributed towards the total yield. The same results were obtained with the Manager treatment in the Crocodile Valley trial site where no significant difference between Manager on the one hand and Trimangol and Sancozeb on the other hand was obtained, but a difference of 9% between the above mentioned treatments is also not acceptable in a zero tolerance market.

Conclusion

Maneb is, according to the *in vitro* study, not an ideal fungicide for the control of citrus black spot. On the other hand, Trimangol resulted in less CBS control of between 4 and 10% if compared to the registered Sancozeb treatment, while a difference of 9% was obtained between the Manager and Sancozeb treatments and is not acceptable in a zero tolerance overseas market. Although both are registered for the control of CBS, growers can choose which product to use.

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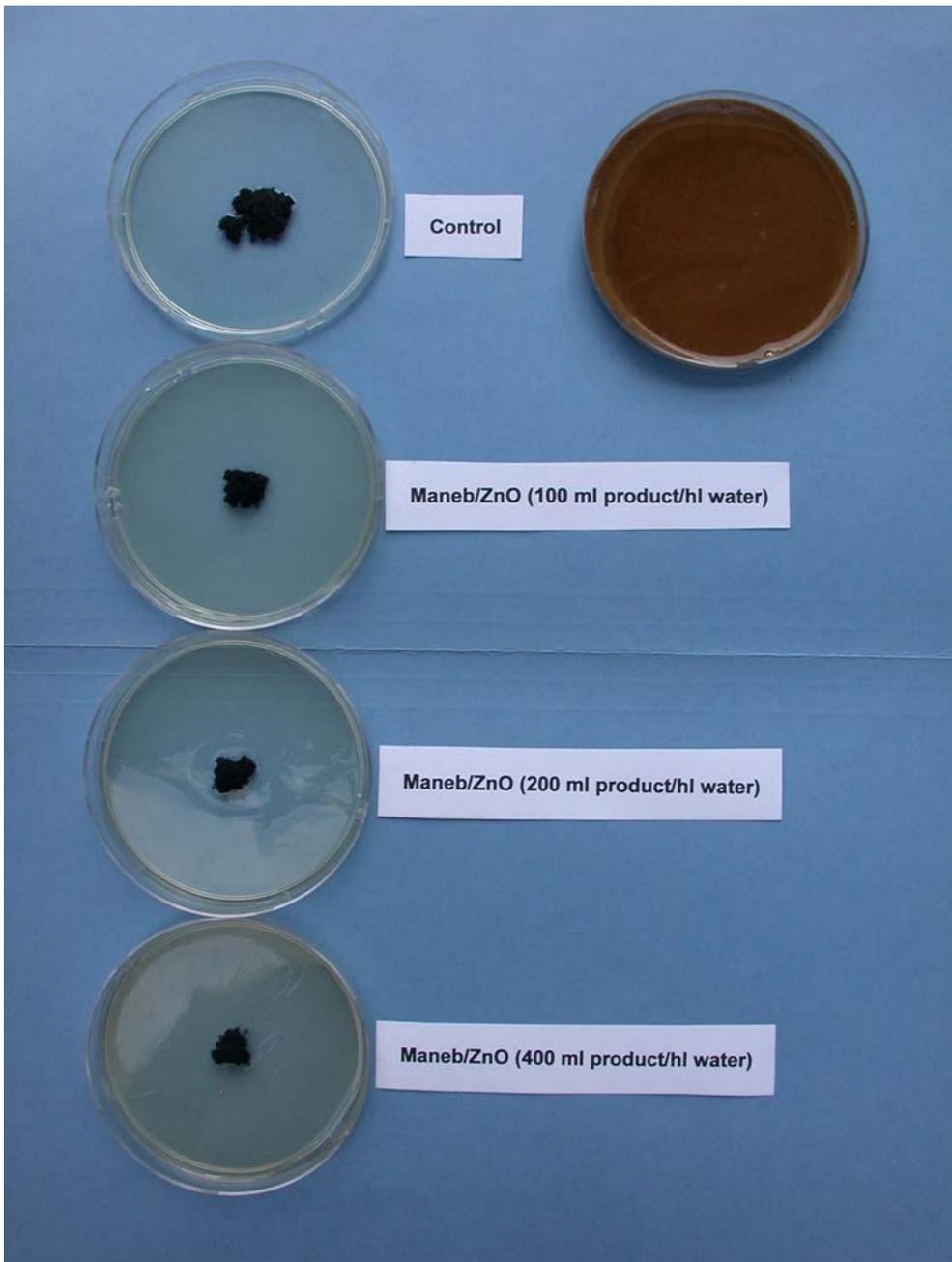


Fig. 4.5.8.1. *In vitro* evaluation of maneb at rates of 100 ml/hl water (1/2x), 200 ml/hl water (1x – registered rate) and 400 ml/hl water (2x) against pathogenic isolates of *Phyllosticta citricarpa*.

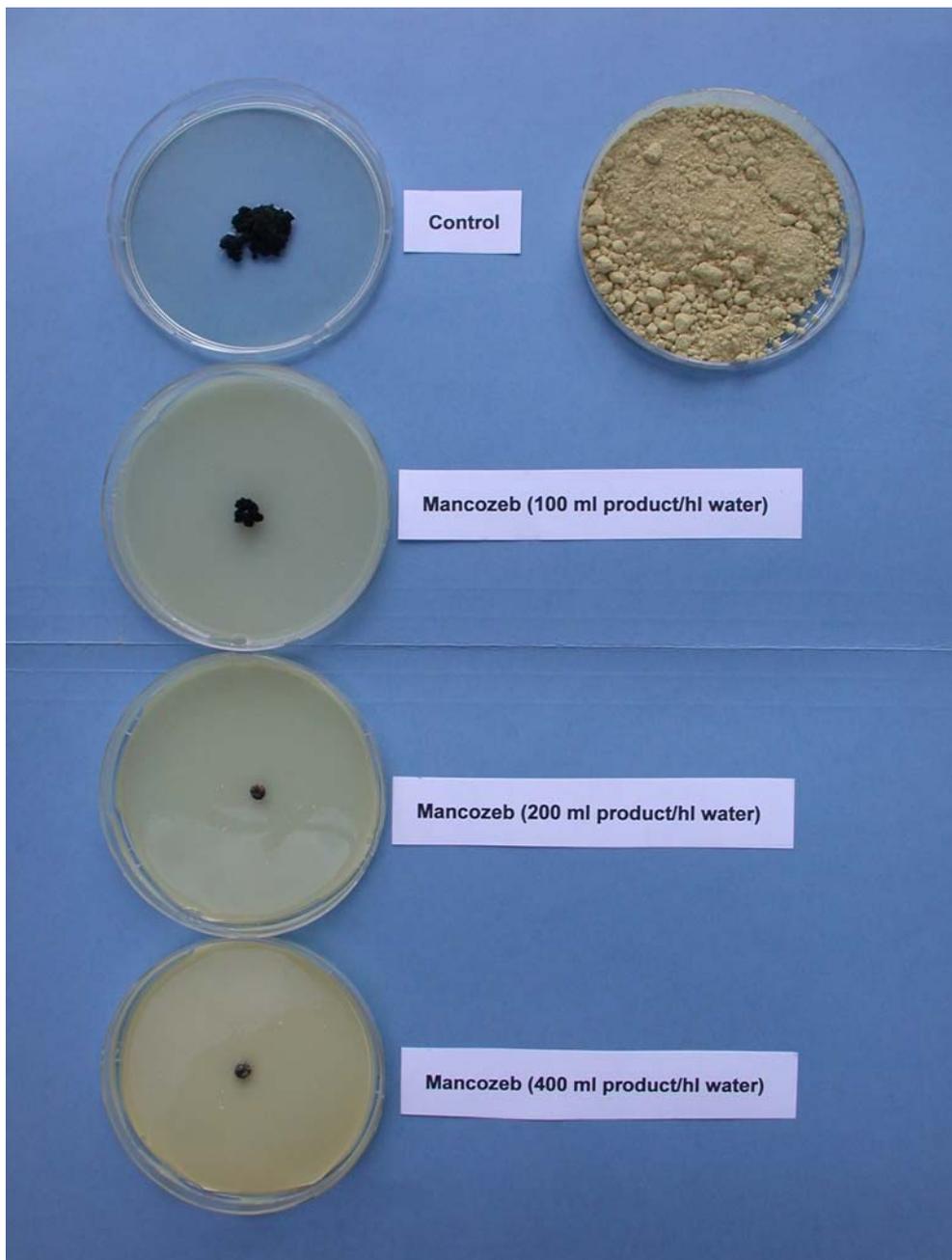


Fig. 4.5.8.2. *In vitro* evaluation of mancozeb at rates of 100 ml/hl water (1/2x), 200 ml/hl water (1x – registered rate) and 400 ml/hl water (2x) against pathogenic isolates of *Phyllosticta citricarpa*.

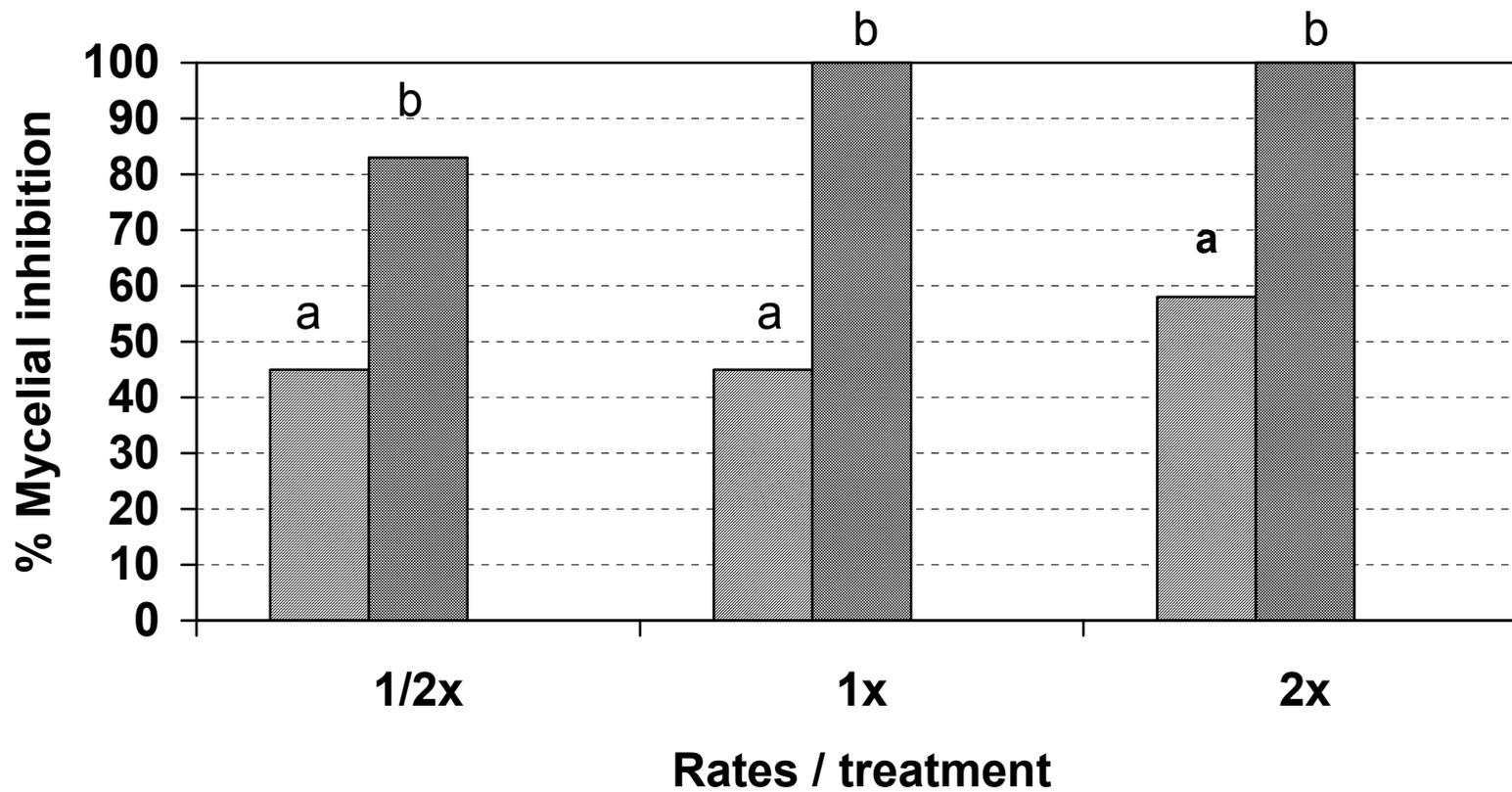


Fig. 4.5.8.3a. Susceptibility of mycelium of *Phyllosticta citricarpa* to maneb (▨) and mancozeb (▩) amended agar tested at rates of 1/2x (100 g or mL/hL water), 1x (200 g or mL/hL water) and 2x (400 g or mL/hL water). Bars with the same letters in each figure are not significantly different ($P>0.05$) according to Fisher's LSD test.

Table 4.5.8.1. Efficacy of Sancozeb (mancozeb) and Trimangol/Manager (maneb) for the control of a benzimidazole-resistant citrus black spot (CBS) strain sprayed during the susceptible period from October 2003 to January 2004 at Friedenheim Estates, Nelspruit, Hectorspruit and Crocodile Valley Citrus Co., Nelspruit.

Treatment	Rate / 100ℓ water	Friedenheim Estates, Nelspruit ^y			Hectorspruit ^z			Crocodile Valley Citrus Co., Nelspruit ^y		
		% Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x	% Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x	% Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x
Sancozeb	200 g	96.33 a	0.66 a	3.00 a	83.67 a	1.00a	15.33 a	95.20 a	1.60 a	3.20 a
Trimangol	200 ml	92.00 a	4.33 a	3.67 a	73.00 a	4.00 a	23.00 a	95.00 a	2.80 ab	2.20 a
Manager	200 ml	ND	ND	ND	ND	ND	ND	86.40 a	8.00 b	5.60 a
Control		19.33 b	20.33 b	60.33 b	5.33 b	7.67 a	87.00 b	39.60 b	14.60 c	54.20 b

^xMeans in a column (based on three (Flippie Walters & Hectorspruit) or five (Crocodile Valley Citrus Co.) replicates) followed by the same letter are not significantly different (P = 0.05) according to Fishers LSD test.

^y Spray dates: 15 October 2003, 12 November 2003, 10 December 2003 and 7 January 2004.

^z Spray dates: 14 October 2003, 11 November 2003, 9 December 2003 and 6 January 2004.

ND = not determined

4.5.9 Evaluation of spray programmes using copper fungicides alternated with mancozeb as well as combinations of copper fungicides with other fungicides for the control of citrus black spot in South Africa

Experiment 748 by G.C. Schutte (CRI)

Opsomming

Tenkmengsels van $\frac{3}{4}$ x Copstar en $\frac{3}{4}$ x Copflo Super met $\frac{1}{2}$ x mancozeb konsentrasies het belowende resultate getoon en kan sodanig aanbeveel word vir die beheer van swartvlek. So ook het afwisselende Copstar en Copflo Super behandelings met trifloxystrobin (in geregistreerde tenkmengsel met mancozeb en olie) ook goeie beheer van swartvlek opgelewer (tussen 95-100% skoon uitvoerbare vrugte) en dien ook as 'n goeie strategie om weerstand teen die strobilurines hok te slaan en ook om koperstippelvorming te beperk. So ook het die afwisselende volgorde van A:B:A:B waar A = koper en B = mancozeb, die beste resultate opgelewer ten opsigte van swartvlekbeheer en beperking van koperstippelvorming. Alhoewel die A:B:B:A volgorde ook goeie swartvlekbeheer gegee het, was daar 'n toename van 10% in koperstippelvorming weens die laaste bespuiting in Januarie. Sporekill teen 'n dosis van so hoog as 300 ml/h ℓ water in 'n tenkmengsel met 'n $\frac{1}{2}$ x mancozeb konsentrasie het nie net goeie swartvlekbeheer gegee nie, maar was ook nie fitotoksies nie. Netso het Sporekill dosisse van 100 en 150 ml/h ℓ water ook in 'n tenkmengsel met 'n $\frac{1}{2}$ x mancozeb konsentrasie uitstekende beheer van swartvlek gegee en moet laer Sporekill konsentrasies ook oorweeg word vir verdere evaluering en ook met ander swamdoders. Furfural is nie effektief teen swartvlek nie.

Introduction

Control of citrus black spot (CBS) disease, caused by *Guignardia citricarpa* Kiely, is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in South Africa (Kotzé, 1963; McOnie, 1964; Kellerman & Kotzé, 1977, Schutte, 1995). When properly applied, fungicides containing copper afford excellent and inexpensive control and are used widely to control citrus diseases like CBS, scab, melanose, greasy spot, Phytophthora brown rot, Botrytis blight, Alternaria brown spot, mal secco, blast, and canker (Brodrick, 1970). Copper fungicides do not only have a broad spectrum of activity, but are also cheap. Registered spray programmes for the control of CBS are expensive. For instance, strobilurin spray programmes cost growers about R14/tree/season consisting of 4 applications if sprayed according to the label. On the other hand, 4 mancozeb sprays cost the growers between R6-8/tree/season. Moreover, mancozeb is not permitted on fruit destined for the USA. In countries like Canada, a 28 day pre-harvest interval is required and only packhouses with wet lines, i.e. where fruit is washed or rinsed, can be used. In all other countries a 21 day pre-harvest interval is permitted. However, mancozeb is more expensive than any of the currently registered copper fungicides, costing about R20-24/kg in comparison with R14-16/kg for copper fungicides. Furthermore, copper fungicides are registered at 35-day intervals in comparison with the 24-25-day intervals for mancozeb. Preliminary trials during the 2001/2002 season showed that copper oxychloride and cuprous oxide showed effectivity against CBS if sprayed as 2 applications according to the old strobilurin label (November and January applications). No stippling was detected either. New copper formulations include copper ammonium carbonate, copper hydroxide (Champion, 770 WG), as well as five other newly formulated coppers with secret ingredients. The formation of copper stippling will also be determined. The aim of these studies is: to look for tank mixtures of current registered fungicides at lower than registered rates; to use different systemic fungicides with different contact fungicides only as two applications; to alter the sequence of copper and mancozeb spray programmes with the aim to limit stippling due to frequent copper applications and to evaluate new copper formulations and other compounds such as furfural.

Materials and methods

Two experiments were conducted in commercial Valencia orange (*Citrus sinensis* (L.) Osbeck) groves on Rough lemon rootstock (*C. jambhiri* Lush.) at Crocodile Valley Citrus Co. and Friedenheim Estates near Nelspruit during 2003 and 2004. The trees were 37 and 58 years old respectively, 3 to 4 m high. The rows ran directly north to south. Each treatment was replicated five and three times respectively in a randomized block design. The fungicides were applied with a trailer-mounted high-volume, high-pressure (2,500 to 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree, but all trees were sprayed to the point of run-off, each receiving approximate 35 ℓ of spray mix per tree per application. All treatments commenced in mid-October before the onset of the first summer rain (Fig. 4.5.9.1.1, Tables 4.5.9.1.1 and 4.5.9.1.2). At fruit maturity during June, CBS severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analyzed by ANOVA, using Fisher's LSD test ($P = 0.05$). Spray dates and rates of applications are listed in Tables 4.5.9.1 to 4.5.9.8.

a) Tank mixtures of Copstar and Copflo Super with mancozeb

Two field trials were conducted. One was at Crocodile Valley Citrus Co. and the other at Friedenheim Estates near Nelspruit during 2003 and 2004. The fungicides tested included Copstar (copper hydroxide, 120 SC), Copflo Super, and the standard treatment, Sancozeb (mancozeb, 800 WP). The aim of this study is to see if the rates of the copper fungicides, Copstar and Copflo Super, can be reduced from 350 to 260 ml and from 250 ml to 180 ml respectively in tank mixtures with mancozeb also at half the registered rate of 100 g/hl water (Table 4.5.9.1.).

b) Alternating copper fungicides such as Copflo Super and Copstar with the registered tank mixture consisting of trifloxystrobin, mancozeb and spray oil as well as comparative evaluations with the registered copper hydroxide and mancozeb treatments

Three field trials were conducted during 2003 and 2004. One was at Crocodile Valley Citrus Co., the other at Friedenheim Estates near Nelspruit and the third at Bayer Cropscience's Experimental farm at Hectorspruit (Table 4.5.9.2). The fungicides tested included Copstar (copper hydroxide; 120 SC), Champion (copper hydroxide; 770 WG), Flint (trifloxystrobin, 500 WG) and the standard treatment, Sancozeb (mancozeb, 800 WP). The aim was to see if the placement of two liquid copper formulations (Copflo Super and Copstar) can result in an increase of clean exportable fruit and where the alternating placement of these applications will not result in stippling due to copper.

c) Alternating different copper hydroxide formulations with mancozeb to determine efficacy against CBS and to limit the possibility of copper stippling

Two field trials were conducted during 2003 and 2004. One was at Crocodile Valley Citrus Co. and the other at Friedenheim Estates near Nelspruit. The fungicides tested included Copstar (copper hydroxide; 120 SC), Champion (copper hydroxide; 770 WG) and the standard treatment, Sancozeb (mancozeb, 800 WP) (Tables 4.5.9.3 & 4.5.9.4).

d) Evaluation of Copper Count N, Cupro Plex and MegaCop for the control of CBS

Only one field trial was conducted at Crocodile Valley Citrus Co., near Nelspruit during 2003 and 2004. Copper Count N, Cupro Plex and MegaCop were sprayed at rates of 500 ml and 1000 ml/hl water (Table 4.5.9.6).

e) Evaluation of Sporekill in tank mixtures with mancozeb for the control of CBS

Two field trials were conducted. One was at Crocodile Valley Citrus Co. and the other at Friedenheim Estates near Nelspruit during 2003 and 2004. The sanitizing agent, didecyl dimethylammonium chloride (Sporekill) was used in tank mixtures with reduced rates of mancozeb (100 g/hl water) (Table 4.5.9.7).

f) Evaluation of furfural for the control of CBS

Only one field trial was conducted at Crocodile Valley Citrus Co. near Nelspruit during 2003 and 2004. Furfural was obtained from Illovo Sugar and was tested at rates of 1, 2.5 and 5% (Table 4.5.9.8).

Results and discussion

a) Tank mixtures of Copstar and Copflo Super with mancozeb

Significant differences ($P < 0.05$) in the percentage clean exportable fruit were achieved between all fungicide treatments and the control in both trial sites. Of these, Copflo Super sprayed at a lower rate of 180 ml/hl water instead of the recommended rate of 250 ml/hl water (if sprayed alone), in a tank mixture with mancozeb (100 g/hl water) resulted in more clean exportable fruit (97% and 98%) without CBS lesions (Table 4.5.9.1). The same tendency was also achieved with the other criteria used for evaluation. Copstar on the other hand, also sprayed at a lower rate of 260 ml/hl water with mancozeb instead of the recommended rate of 350 ml/hl water if sprayed alone (registered rate) respectively, did not perform that well in the trial site at Friedenheim Estates, and resulted in only 85.67% clean exportable fruit that was also significantly different from the other treatments. However, the same treatment resulted in the highest percentage of clean exportable fruit (98.67%) at the trial site at Crocodile Valley Citrus Co. and the same was also achieved with regards to the other criteria used for evaluation. If the 9% fruit with 1-3 CBS lesions are to be added to the 85.67% clean exportable fruit as in previous years, and were permitted for export, it would add up to 94.67%

fruit for export. The outstanding 5.33% fruit had four and more CBS lesions and was not significantly different from the other fungicide treatments. Therefore the poor result from this trial site regarding the tank mixture of Copstar in a tank mixture with mancozeb can only be ascribed to the poor coverage obtained with the handguns used for application. Mancozeb on the other hand, if used at a rate of 100 g/hℓ water in combination with other fungicides, can be successfully used for CBS control and will also result in lower residue levels on the fruit (Table 4.5.9.1).

b) Alternating copper fungicides such as Copflo Super and Copstar with the registered tank mixture consisting of trifloxystrobin, mancozeb and spray oil as well as comparative evaluations with the registered copper hydroxide and mancozeb treatments

Significant differences ($P < 0.05$) in the percentage clean exportable fruit were achieved between all fungicide treatments and the control in all trial sites. Of these, Copflo Super (sprayed at the recommended rate of 250 ml/hℓ) or Copstar (sprayed at the recommended rate of 350 ml/hℓ) water alternated with trifloxystrobin in a tank mixture with mancozeb (150 g/hℓ water) and spray oil and again alternated Copflo Super or Copstar sprayed at the recommended rate of 250 ml/hℓ water alternated with trifloxystrobin in a tank mixture with mancozeb (150 g/hℓ water) and spray oil for a second time, resulted in more than 95% clean exportable fruit in all three test sites (Table 4.5.9.2). At Crocodile Valley Citrus Co., these two spray programmes resulted in 100% clean exportable fruit. Where mancozeb (200 g/hℓ water) was included instead of the copper formulations, it resulted in only 80% clean exportable fruit that is 19% worse than the abovementioned treatments. This spray programme, although only evaluated at the Crocodile Valley Citrus Co., also resulted in 15% less clean exportable fruit than the registered standard of mancozeb sprayed four times at 200 g/hℓ water. These treatments were however, not significantly different from each other. At the Hectorspruit trial site, the registered standard mancozeb treatment sprayed four times at 200 g/hℓ water, also resulted in 12% less clean exportable fruit and was significantly different from the other spray programmes consisting of Copflo Super (sprayed at the recommended rate of 250 ml/hℓ) or Copstar (sprayed at the recommended rate of 350 ml/hℓ) water alternated with trifloxystrobin in a tank mixture with mancozeb (150 g/hℓ water) and spray oil (Table 4.5.9.2).

c) Alternating different copper hydroxide formulations with mancozeb to determine efficacy against CBS and to limit the possibility of copper stippling

Due to a lack of trees at the trial site at Friedenheim Estates to conduct a proper duplicate spray programme to the one at Crocodile Valley Citrus Co., only a few combinations of copper hydroxide and mancozeb were selected for evaluation. Results from the trial site at Friedenheim Estates showed that there were no significant differences ($P > 0.05$) between the alternating copper hydroxide formulations with mancozeb treatments (except for Copstar spray alone as four applications under the criterion, clean exportable fruit) with regards to all the criteria used for evaluation (Table 4.5.9.4). However, all the fungicide treatments were significantly different from the control where the inoculum pressure was extremely high. Due to the cool microclimate in this orchard, no stippling could be detected and therefore all the treatments, irrespective of the alternating sequence followed, were suitable for CBS control (Table 4.5.9.4).

Results from the trial site at Crocodile Valley Citrus Co. (Table 4.5.9.3) showed that there were no significant differences ($P > 0.05$) between the alternating copper hydroxide formulations with mancozeb treatments with regards to all the criteria used for evaluation. All the fungicide treatments were significantly different from the control where the inoculum pressure was relatively high.

Effect of copper-containing spray programmes on copper stipple formation

It is interesting to note that spray programmes where copper (A) and mancozeb (B) were alternated, i.e. alternating A:B:A:B, resulted in less copper stippling than those spray programmes where either copper or mancozeb was sprayed in succession, viz. A:B:B:A, when applied during the susceptible period from October to January if sprayed at monthly intervals (Table 4.5.9.5). The copper hydroxide WG and SC formulations (Champion) sprayed in the A:B:A:B sequence, resulted in 95.0 and 93.3% fruit without stippling. Although the A:B:B:A sequence resulted in 9% less clean fruit from stippling with regards to the previous sequence, these treatments were not significantly different ($P > 0.05$) from each other. As expected and shown in previous trials during the 2002/2003 season, sequences of A:A:A:A or B:A:A:B where two or more copper fungicides were sprayed in succession, more stippling occurred and resulted in between 58 to 78% fruit without stippling. The same was observed with the criterion, mild stippling, where the same sequences resulted in 15 to 40% fruit with more mild stipple formation than the A:B:A:B and A:B:B:A sequences. From these results it is clear that 10% more fruit had stippling if the A:B:B:A sequence was applied with the last application in January in comparison with the last copper application in December (Table 4.5.9.5).

As expected and proven before, the copper hydroxide WG (Champion) formulation, if sprayed in succession with another copper treatment, resulted in more fruit with mild stippling and severe stippling. This is because the metallic copper content (if sprayed at the registered rate of 200 g/hℓ water) is 77 g in the WG (Champion) formulation and 42 g in the SC (Copstar) formulation (if sprayed at the registered rate of 350 ml/hℓ water). There were, however, no significant differences between any of the treatments in the criterion: severe stippling.

It is clear from this trial site that temperature plays a definite role in stipple formation as there was a remarkable difference in the microclimates at the two trial sites. As shown in previous tables, the application dates only differed by one day and are about 2-3 km apart and situated in the same region. Both sites are also subjected to the same climatic conditions. However, the trial site at Friedenheim Estates had healthier trees, better irrigation and the tree density was also higher than the trial site at Crocodile Valley Citrus Co. Applications of copper in January, but not December, in the alternating sequence, resulted in about 10% more fruit with stippling. The pH of the different applications was not measured.

d) Evaluation of Copper Count N, Cupro Plex and Mega Cop for the control of CBS

Results from Table 4.5.9.6 show that there were no significant differences ($P > 0.05$) between the standard registered mancozeb treatment and all the copper fungicide treatments at Crocodile Valley with regards to the criterion clean fruit (or clean exportable fruit) used for evaluation. Concomitantly, all the fungicide treatments were significantly different ($P < 0.05$) from the control that yielded only 39.6% clean fruit, showing that the inoculum pressure was exceptionally high the past season. Although not significantly different from each other, results showed that there was a difference of 5% between the Mega Cop rates of 500 ml and 1 ℓ/hℓ water. The same with Cupro Plex and Copper Count N where there was a difference of 15% for both treatments with regards to the criterion, clean fruit. With regards to the criterion 1-3 lesions, there was a significant difference between Mega Cop sprayed at a rate of 1 ℓ/hℓ water and Cupro Plex and Copper Count N, both sprayed at a rate of 500 ml/hℓ water including the control. On the other hand, there were significant differences between all the treatments and the control with regards to the criterion, 4 or more lesions. This was although the Cupro Plex and Copper Count N, both sprayed at a rate of 500 ml/hℓ water, had 10 and 14.4% fruit with 4 or more lesions.

e) Evaluation of Sporekill in tank mixtures with mancozeb for the control of CBS

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.5.9.7) show that there were no significant differences ($P < 0.05$) between the standard registered mancozeb treatment and all tank mixtures of reduced mancozeb with Sporekill at all three different rates tested. There were no dosage responses as the highest rate of Sporekill (300 ml/hℓ water) with mancozeb was only 0.2% less effective than the lower rate of Sporekill (150 ml/hℓ water). The same scenario was achieved with Sporekill evaluated at a rate of 100 ml/hℓ water that was also 0.2% less effective. These differences are so small and are also not significantly different from each other ($P > 0.05$). The same results were observed with the other criteria. All the treatments were significantly different from the control.

Results from the field trial conducted at Friedenheim Estates (Table 4.5.9.7) show that there were no significant differences ($P > 0.05$) between the standard registered mancozeb treatment and all tank mixtures of reduced mancozeb with Sporekill at all three different rates tested. There was a difference of 3% between the tank mixtures of reduced mancozeb with Sporekill at the highest rate tested (300 ml/100 ℓ water) and the standard mancozeb treatment. In this experiment there was, however, a dosage response between all the rates of Sporekill evaluated. The same results were observed with the criterion, fruit with 1 to 3 CBS lesions where all the treatments were significantly different from the control. With regards to the criterion fruit with 4 and more CBS lesions, a significant difference was achieved between the Sporekill tank mixtures (100 ml) on mancozeb (100 g) and the standard registered mancozeb treatment. The latter treatment had 2.6% less fruit with CBS than the lowest rate Sporekill plus mancozeb. The higher dosage Sporekill mixtures and the registered mancozeb had 60% less fruit with CBS than the control (Table 4.5.9.7).

f) Evaluation of furfural for the control of CBS

Results from Table 4.5.9.8 show that there were significant differences ($P < 0.05$) between the standard registered mancozeb treatment and all of the furfural treatments with regards to all three criterion used for evaluation. However, the only furfural treatment that was not significantly different from the mancozeb treatment was the furfural sprayed at 1 ℓ/hℓ water for the criterion, 4 or more CBS lesions. This criterion had 18.4% more CBS infected fruit with regards to this furfural treatment versus the mancozeb treatment, which is not acceptable in a zero tolerance overseas market. With regards to the criterion no lesions (or percentage

clean fruit free from CBS), there was a difference of 31 – 40% between the standard mancozeb treatment and the furfural treatments.

Conclusion

The first ascospore release occurred on the 12 November 2003 and is exactly as recorded before in previous studies (Kellerman & Kotzé, 1977) (Fig. 4.5.9.1.1) Results of tank mixtures of Copstar and Copflo Super with mancozeb were promising and the results were discussed with the registrar with regards to registration of spray programmes consisting of different fungicides. This spray programme is highly recommended for CBS control.

Alternating copper fungicides such as Copflo Super and Copstar with the registered trifloxystrobin treatment as well as comparative evaluations with the registered copper hydroxide and mancozeb treatments gave between 95 –100% clean exportable fruit and is an excellent strategy not only to limit the possibility of CBS resistance development to the strobilurins, but also to limit the possibility of copper stippling. This spray programme is highly recommended for CBS control.

Alternation of different copper hydroxide formulations with mancozeb in the A:B:A:B sequence is the only recommended spray programme if copper is to be sprayed more than once per annum. This is because 10% more fruit had stippling if the A:B:B:A (A = copper) sequence was followed with the last application in January in comparison with the last copper application in December of the A:B:A:B sequence.

Sporekill in tank mixtures with reduced mancozeb rates of 100 g/h ℓ water sprayed at 28 day intervals, resulted in the same amount of clean exportable fruit as the standard mancozeb treatment sprayed at 200 g/h ℓ water. There is a synergistic effect between these two fungicides and the fact that mancozeb can be sprayed at such a low rate and at a 28 day interval as well is an achievement. Moreover, the highest rate of Sporekill tested (300 ml/h ℓ water), did not give any phytotoxicity. It is recommended that Sporekill should be evaluated at rates of lower than 100 ml/h ℓ water and in combination with other contact fungicides such as copper. It will be a breakthrough if the rates of the different copper formulations can be reduced which will hopefully result in less stippling. The same scenario is applicable to mancozeb, where less fungicide on the citrus fruit will result in less residue on the fruit. Concomitantly, Sporekill incorporates a spreader/sticker which is not required in tank mixtures and can conversely save the citrus grower spray costs (Fig. 4.5.9.1.2 & 3).

Furfural is not recommended for use against CBS and is also an irritating compound on the skin and difficult to work with, especially at the rates prescribed to us.

Future research

More field trials of Sporekill in combination with different fungicidal groups are planned as well Sporekill at lower rates.

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Table 4.5.9.1. Evaluation of spray programmes consisting of tank mixtures of Copstar and CopFlo Super with mancozeb during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co., S.A. and Friedenheim Estates during 2003 and 2004¹

Treatment (number of applications)	Rate / 100ℓ water	Friedenheim Estates, Nelspruit ^y			Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^w	% fruit with 1 – 3 CBS lesions ^w	% fruit with four and more CBS lesions ^w	%Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x
CopfloSuper + mancozeb (x4)	180 ml + 100 g	97.00 a	2.33 ab	0.67 a	98.00 a	1.20 a	0.80 a
Copstar + mancozeb (x4)	260 ml + 100 g	85.67 b	9.00 b	5.33 a	98.67 a	1.00 a	0.33 a
Mancozeb (x4)	200 g	96.33 a	0.66 a	3.00 a	95.20 a	1.60 a	3.20 a
Control		19.33 c	20.33 c	60.34 b	39.66 b	14.60 b	45.74 b

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004

ND = Not determined

Table 4.5.9.2. Efficacy of alternating copper fungicides such as Copflo Super, Copstar with the registered trifloxystrobin treatment as well as comparative evaluations with the registered copper hydroxide and mancozeb treatments for the control of a benzimidazole resistant citrus black spot (CBS) strain sprayed during the susceptible period from October 2003 to January 2004.

Treatment sequence	Rate / 100l water	Friedenheim Estates, Nelspruit ^x			Crocodile Valley Citrus Co., Nelspruit ^y			Hectorspruit ^z		
		% Clean exportable fruit ^u	% fruit with 1 – 3 CBS lesions ^u	% fruit with four and more CBS lesions ^u	% Clean exportable fruit ^v	% fruit with 1 – 3 CBS lesions ^v	% fruit with four and more CBS lesions ^v	% Clean exportable fruit ^w	% fruit with 1 – 3 CBS lesions ^w	% fruit with four and more CBS lesions ^w
CF/trifloxystrobin+mancozeb+oil/CF/trifloxystrobin+mancozeb+oil	250 ml/ 10 g + 150 g+0.25%/ 250 ml/ 10 g + 150 g + 0.25%	100 a	0 a	0 a	99.40 a	0 a	0.60 a	94.0 a	0.66 a	5.34 a
CS/trifloxystrobin+mancozeb+oil/CS/trifloxystrobin+mancozeb+oil	350 ml/ 10 g + 150 g+0.25%/ 350 ml/ 10 g + 150 g + 0.25%	100 a	0 a	0 a	99.40 a	0.40 a	0.20 a	95.50 a	0 a	4.50 a
Copper hydroxide (Champion) (x4)	200 g	99.67 a	0.33 a	0 a	98.00 a	0.80 a	1.20 b	ND	ND	ND
Mancozeb (x4)	200 g	96.33 ab	0.67 a	3.00 a	95.20 a	1.60 a	3.20 c	83.67 b	1.00a	15.33 b
Copper hydroxide (Copstar) (x4)	350 ml	91.00 b	7.67 b	1.33 a	97.20 a	2.00 a	0.80 a	ND	ND	ND
Mz/trifloxystrobin+mancozeb+oil/Mz/trifloxystrobin+mancozeb+oil	200 g/ 10 g + 150 g+0.25%/ 200 g/ 10 g + 150 g + 0.25%	ND	ND	ND	80.60 a	10.20 b	9.20 d	ND	ND	ND
Control	-	19.33 d	20.33 c	60.34 b	39.66 c	14.60 b	45.74 e	5.33 c	7.67 b	87.00 c

^u Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^v 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.

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^x Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004

^y Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004

^z Spray dates were 13 October 2003, 10 November 2003, 8 December 2003, 5 January 2004

Mz = Mancozeb CS = Copstar CF = Copflo Super ND = Not determined

Table 4.5.9.3. Efficacy of alternating spray programmes mancozeb and copper fungicides such as Copstar or Champion (WP) for the control of a benzimidazole resistant citrus black spot (CBS) strain sprayed during the susceptible period from October 2003 to January 2004 at Crocodile Valley Citrus Co., Nelspruit.

Treatment sequence	Rate / 100ℓ water	Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^y	% fruit with 1 – 3 CBS lesions ^y	% fruit with four and more CBS lesions ^y
Ch/Mz/Mz/Ch	200 g (x4)	98.80 a	0.60 a	0.60 a
Champion (x4)	200 g / 200 g / 200 g / 200 g	98.00 a	0.80 a	0.20 a
CS/Mz/CS/Mz	200 g / 350 ml / 350 ml / 200 g	97.80 a	1.20 a	1.00 a
Mz/CS/CS/Mz	200 g / 350 ml / 350 ml / 200 g	96.80 a	2.00 a	1.20 a
Mz/Ch/Ch/Mz	200 g / 200 g / 200 g / 200 g	96.20 a	1.20 a	2.60 a
Ch/Mz/Ch/Mz	200 g / 200 g / 200 g / 200 g	95.60 a	0.20 a	4.20 a
Mancozeb (x4)	200 g	95.20 a	1.60 a	3.20 a
CS/Mz/Mz/CS	350 ml / 200 g / 200 g / 350 ml	95.0 a	2.00 a	3.00 a
Copstar (x4)	350 ml	93.40 a	2.20 a	4.40 a
Control	-	39.66 b	14.60 b	45.74 b

^y 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.

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004

Mz = Mancozeb

CS = Copstar

Ch = Champion

Table 4.5.9.4. Efficacy of alternating spray programmes mancozeb and copper fungicides such as Copstar or Champion (WP) for the control of a benzimidazole resistant citrus black spot (CBS) strain sprayed during the susceptible period from October 2003 to January 2004 at Friedenheim Estates, Nelspruit.

Treatment sequence	Rate / 100ℓ water	Friedenheim Estates, Nelspruit ^z		
		% Clean exportable fruit ^y	% fruit with 1 – 3 CBS lesions ^y	% fruit with four and more CBS lesions ^y
Champion (x4)	200 g (x4)	99.66 a	0.34 a	0.34 a
Mz/Ch/Ch/Mz	200 g / 200 g / 200 g / 200g	99.66 a	0.34 a	0.34 a
Mz/CS/Mz/CS	200 g / 350 ml / 200 g / 350 ml	99.33 a	0.67 a	0.67 a
Mz/CS/CS/Mz	200 g / 350 ml / 200 g / 350 ml	98.00 a	2.00a	2.00a
Mancozeb (x4)	200 g	96.33 ab	0.67 a	0.67 a
Mz/Ch/Mz/Ch	200 g / 200 g / 200 g / 200 g	94.66 ab	4.00 a	4.00 a
Copstar (x4)	350 ml	91.0 b	7.60 a	1.40a
Control	-	19.33 c	20.33 b	60.34 b

^y Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004

Mz = Mancozeb

CS = Copstar

Ch = Champion

Table 4.5.9.5. Effect of different copper containing spray programmes as sprayed for CBS control on stipple formation on Valencia oranges as sprayed at Crocodile Valley Citrus Co. during the 2003/2004 season.

Treatment sequence	Rate / 100ℓ water	Stippling (%) ^z		
		No stippling	Mild stippling	Severe stippling
Ch/Mz/Ch/Mz	200 g/ 200 g/ 200 g/ 200 g	95.0 a	5.0 a	0 a
CS/Mz/CS/Mz	350 ml / 200 g/ 350 ml/ 200 g	93.4 a	6.6 ab	0 a
Ch/Mz/Mz/Ch	200 g/ 200 g / 200 g/ 200 g	86.8 ab	13.2 ab	0 a
CS/Mz/Mz/CS	350 ml/200 g/ 200 g/ 350 ml	86.8 ab	13.2 b	0 a
Mz/Ch/Ch/Mz	200 g/ 200 g / 200 g/ 200 g	78.4 bc	20.0 bc	1.6 a
Copstar (x4)	350 ml	70.0 c	30.0 cd	0 a
Mz/CS/CS/Mz	200 g/ 350 ml/ 350 ml/ 200 g	66.8 cd	33.2 cd	0 a
Champion (x4)	200 g / 200 g/ 200 g/ 20 g	53.0 d	43.4 d	3.6 a

^y 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.

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004

Mz = mancozeb

Ch = Champion

CS = Copstar

Table 4.5.9.6. Evaluation of Copper Count N (CCN), Cupro Plex and Mega Cop sprayed during the susceptible period from October to January 2003 and 2004 for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co., South Africa.

Treatment ^y	Concentration (product /hℓ water) ^y	No. of applications	No. of CBS lesions on fruit ^z		
			No lesions (%)	1-3 lesions (%)	4 or more lesions (%)
Mega Cop	1 ℓ	4	99.4 a	0.2 a	0.4 a
Cupro Plex	1 ℓ	4	99.2 a	0.8 abc	0.0 a
Mancozeb	200 g	4	95.2 ab	2.4 abc	2.4 a
CCN	1 ℓ	4	95.0 ab	2.2 abc	2.8 a
Mega Cop	500 ml	4	94.4 ab	2.2 abc	3.6 a
Cupro Plex	500 ml	4	84.2 ab	5.8 b	10.0 a
CCN	500 ml	4	80.4 b	5.2 bc	14.4 a
Control			39.6 c	14.6 b	45.8 b

^y Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004.

^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 4.5.9.7. Evaluation of Sporekill in tank mixtures with mancozeb sprayed during the susceptible period from October to January 2003 and 2004 for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment (number of applications)	Rate / 100ℓ water	Friedenheim Estates, Nelspruit ^y			Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^w	% fruit with 1 – 3 CBS lesions ^w	% fruit with four and more CBS lesions ^w	%Clean exportable fruit ^x	% fruit with 1 – 3 CBS lesions ^x	% fruit with four and more CBS lesions ^x
Sporekill + mancozeb(x4)	300 ml + 100 g	99.7 a	0.6 a	0.4 a	95.4 a	1.6 a	3.0 a
Sporekill + mancozeb(x4)	150 ml + 100 g	98.3 a	1.6 a	0.1 a	95.2 a	2.0 a	2.8 a
Sporekill + mancozeb(x4)	100 ml + 100 g	96.3 a	0.7 a	3.0 b	95.0 a	2.6 a	2.4 a
Mancozeb (x4)	200 g	99.0 a	0.6 a	0.4 a	95.0 a	3.0 a	2.0 a
Control		19.3 b	20.3 b	60.4 c	39.6 b	14.6 b	45.8 b

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004

Table 4.5.9.8. Evaluation of furfural sprayed during the susceptible period from October to January 2003 and 2004 for citrus black spot (CBS) control on Valencia oranges at Crocodile Valley Citrus Co., South Africa.

Treatment ^y	Concentration (product /hℓ water) ^y	No. of applications	No. of CBS lesions on fruit ^z		
			No lesions (%)	1-3 lesions (%)	4 or more lesions (%)
Mancozeb	200g	4	95.2 a	2.4 a	2.4 a
Furfural	1ℓ	4	64.2 b	15.0 b	20.8 ab
Furfural	5 ℓ	4	55.6 bc	18.8 b	25.6 bc
Furfural	2.5 ℓ	4	55.2 bc	14.6 b	30.2 bc
Control			39.6 c	14.6 b	45.8 c

^y Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004.

^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.

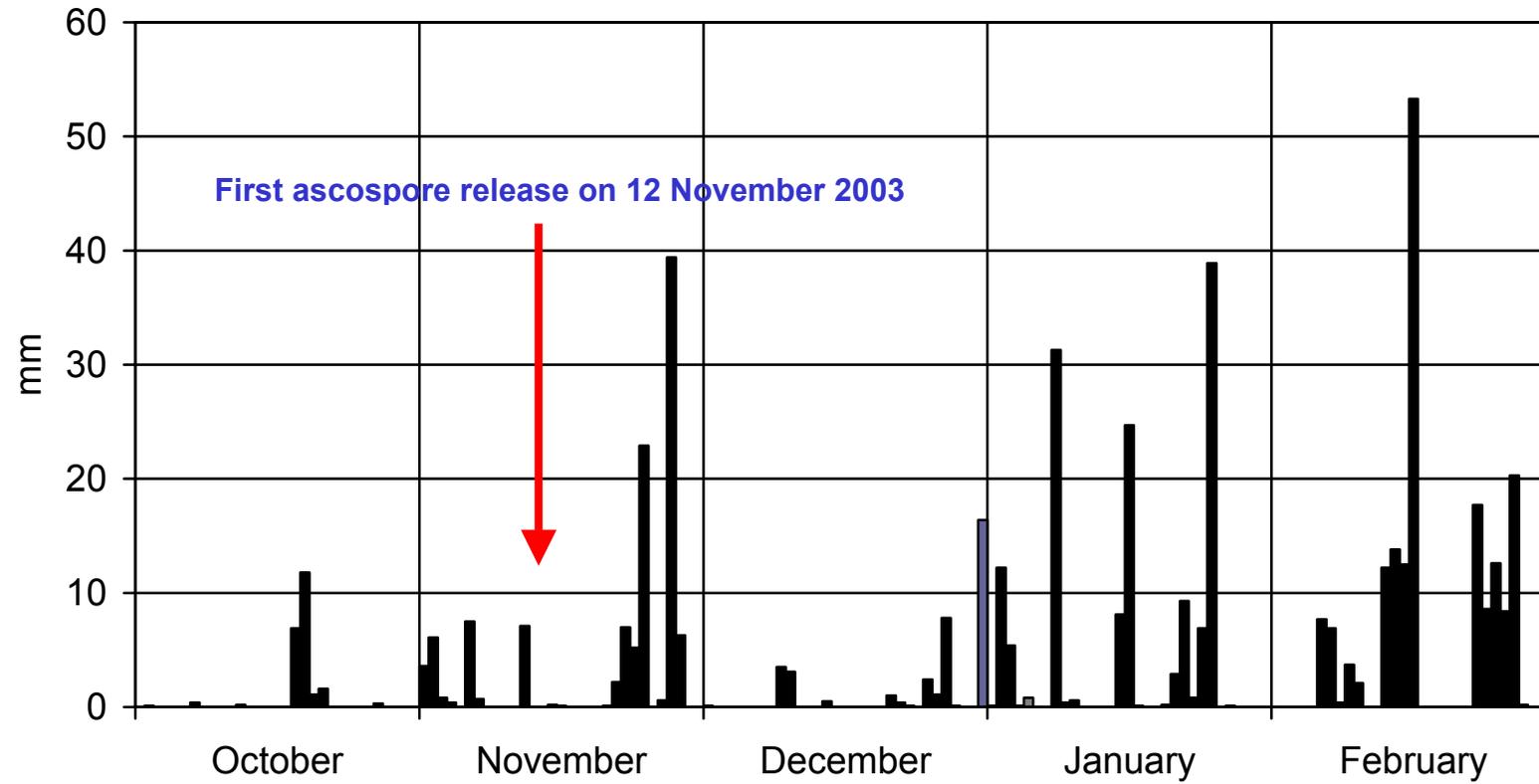


Fig. 4.5.9.1. Rainfall for the period October 2003 to February 2004 for the Nelspruit region.



Fig. 4.5.9.2. Mancozeb spray residue on Valencia oranges sprayed at the registered rate of 200 g/ hℓ water showing the uneven coverage.



Fig. 4.5.9.3. Mancozeb sprayed at half the registered rate of 100 g/hℓ water with Sporekill showing the good cover on the fruit surface.

4.5.10 Correlation between leaf drop and production of ascospore inoculum in citrus orchards Experiment QMS 2.1 by S.H. Swart (QMS)

Opsomming

Die doel van die studie is om seisoenale verskille in inokulum potensiaal, dit is die aantal beskikbare askospore van *Guignardia* spp, wat op dooie sitrus blare op die boordvloer geproduseer word, te bepaal. Die teenwoordigheid van askospore word met 'n askospoormonitor bepaal, wat spesifiek vir die monitoring *Guignardia* askospore ontwikkel is. Die effek van klimaatsverskille in verskillende geografiese areas word ook ondersoek. Die projek is in Augustus 2002 geïnisieër in 'n Navel boord op Letaba Landgoed, waar geen swamdoder bespuitings plaasvind nie. In Mei 2004 is Valencia boorde op Mahela, Letsitele, en Richmond, Hoedspruit, by die projek ingesluit. Resultate tot op datum dui daarop dat blare op enige stadium tydens die jaar kan val en as substraat vir die produksie van askospor inokulum kan dien, maar dat blare wat tussen Junie en Januarie val vir die oorgrote meerderheid inokulum verantwoordelik is. Die meerderheid van askospore word binne 4 maande na blaarval geproduseer tydens periodes van hoë reënval. Tydens droër en koel periodes neem die proses langer (4 tot 6 maande). In die meeste gevalle verweer blare totaal binne 6 maande onder natuurlike toestande in 'n boord. Seisoenale verskille in inokulum potensiaal is waargeneem en askosporgetalle was baie hoër tydens die 2003/2004 seisoen as tydens die 2002/2003 seisoen. Resultate dui ook dat die piek van askosporbesikbaarheid van Januarie en Februarie in 2003 verskuif het na Januarie, Februarie en Maart 2004. Dit hou verband met die reënval patroon tydens die 2 seisoene. Klimaat, veral reënval (aantal dae wat dit reën), en temperatuur speel 'n belangrike rol, veral met betrekking tot die tempo van die kompostering proses wat moontlik 'n direkte korrelasie met die produksie van askospore het. 'n Finale verslag sal in Augustus 2006 ingedien word.

Introduction

Management of plant diseases is mainly determined by the epidemiology of the pathogen. The sexual stage of *Guignardia citricarpa* produces perithecia and airborne ascospores on leaf litter, which is the major source of inoculum in South Africa (Kotzé, 1981). The aim of this study is to determine the seasonal variation in inoculum potential, that is the number of available ascospores produced on citrus leaf litter on the orchard floor. The effect of climatic conditions and geographical differences will be studied in different production areas.

Materials and methods

This project was initiated in August 2002 in an unsprayed Navel orchard on Letaba Estates. In May 2004, two Valencia orchards, one at Mahela, Letsitele, and one at Richmond, Hoedspruit, were included in the project in order to include commercial orchards in different production areas. All leaves are collected from an unsprayed Navel orchard, Plot L 57, planted in 1956 on Letaba Estates, near Tzaneen. Mature leaves are picked randomly in the orchard and placed between sets of two plastic mesh grids in order to cover approximately 700 cm². The 2 grids are then secured with cable ties and transported individual orchards. Each month, 6 grids of leaves are placed under each of three replicate trees in each orchard. Every four weeks a set of grids, representing the preceding 6 consecutive months are collected from all data trees in the 3 production areas.

Grids are submerged in hot water (40°C ± 2°C) for 5 min, allowed to drain for 5 min and then placed in an inoculum monitor (Quest Developments) for two hours. Ascospores are actively released from ripe asci into a chamber from where it is sucked by a vacuum pump and deposited on a Vaseline coated standard microscope slide. After two hours the slide is removed, stained with lactophenol cotton blue and covered with a cover slip. The number of deposited ascospores, resembling the morphology of *Guignardia citricarpa*, are counted under a light microscope at 400x magnification. The total number of ascospores in 4 lanes (0.5 mm x 45 mm) covering an area of approximately 90 mm² is recorded for each replicate.

Results and Discussion

Letaba Area

Leaves, picked on a monthly basis, and placed on an orchard floor for periods ranging from 2 to 6 months, were analysed for the ability to produce ripe ascospores after exposure to different climatic conditions. Data for leaves picked August 2002 to November 2004 and counted from September 2002 to December 2004 are presented in Table 4.5.10.1. Results suggested that naturally infected leaves that became detached between August 2002 and January 2003 and again from June 2003 until January 2004 were the major source of ascospore inoculum. However, leaves picked in March and May 2004 also produced relatively high numbers of ascospores. Unfortunately, no leaves were picked during April 2003. All leaves picked

between June 2004 and November 2004, with the exception of July and September 2004, produced relatively large numbers of ascospores

It also appears that naturally infected detached leaves take between 1 and 6 months to serve as substrate for perithecium development (Table 4.5.10.1). Leaves which dropped during the dry, cooler winter months (May, June, and July) took between 4 and 6 months to produce perithecia and ripe ascospores. Leaves which became detached between August and October took between 2 and 3 months to produce perithecia and ripe ascospores. Ascospores could be recovered within 1 to 3 months from leaves that was picked in November, December and January. However, data clearly showed that leaves that became detached in March 2004, already produced high numbers of ascospores in April. It generally appears that the majority of ascospores are produced within 4 months during periods when rain often occurs. During periods when conditions are drier it seems to take longer (4 to 6 months). In most cases leaves were totally composted after 6 months of exposure on the orchard floor.

The mean number of ascospores was calculated for each month by adding the total number of ascospore counted on leaves, detached and left on the orchard floor for 1, 2, 3, 4, 5, and 6 preceding months respectively, and divided by the number of months for which leaves were included (Fig. 4.5.10.1). Results show that leaves have the potential to produce ascospores from as early as September but generally from October in the Letaba area. Inoculum normally increases during November and December and peak in January and February. Inoculum declined in March and was relatively low in April. From June until August the number of available ascospores was very low. It is clear from the graph that there are seasonal differences and that the inoculum potential for the 2003/2004 season was higher than for the 2002/2003 season. Results also showed that the peak shifted from January and February in 2003 to January, February, and March in 2004.

Letsitele area

The first leaves were picked in May 2004 and grids evaluated from June 2004 until December 2004. Initial results show a pattern very similar to the Letaba area but with a higher peak of available inoculum during November 2004 (Fig. 4.5.10.2). However, this is a preliminary observation and more information will be gathered until March 2006.

Hoedspruit area

The first leaves were picked in May 2004 and grids evaluated from June 2004 until December 2004. Initial results show that inoculum potential in the Hoedspruit area has been very low up to now (Fig. 4.5.10.3). However, this is a preliminary observation and more information will be gathered until March 2006.

Conclusion

In general, it appears that ascospores can be produced on leaves that drop throughout the year. However, the major portion of inoculum is produced on leaves which drop between June and January. Leaves take longer to support ascospore production during the cooler winter months than the hot summer months. The ability of infected leaves to produce viable ascospores depends on the infection status of the leaves, that is climatic conditions and inoculum potential when leaves were young and susceptible to infection. Climate, especially rainfall (number of days with rain) and temperature play an important role, especially regarding the speed of the composting process which appears to be directly correlated with the production of perithecia. Normally most leaves were totally composted within 6 months after detachment. We presume that the type of irrigation could also play a significant role in the composting process due to the difference in the ability to wet leaf litter on the orchard floor.

Future research

To conclude this study, data will be generated until March 2006 in order to establish a general inoculum potential pattern compiled from data over three seasons for the Letaba area. This project will also include data generated over 2 seasons from commercial orchards in the Letsitele and Hoedspruit areas. The objective will be to answer the following questions:

Which leaf-drop is responsible for the first major ascospore releases in early summer?

Which leaf-drop is responsible for the major source of inoculum?

How long does it take for leaves to deteriorate in order to induce perithecia and ascospore development?

Until when is ascospore inoculum available during the season?

What is the effect of climatic differences, especially rain and temperature, on ascospore production in geographically different areas?

How can this information be utilized in reducing the inoculum potential in an orchard?

How can this information be utilized to compile an optimal management program?

References cited

- Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* 65: 945-950.
- Swart, S.H. 2002. The value of ascospore release monitoring and correlation with climatic conditions conducive to infection in citrus orchards. 2nd Citrus Symposium, Stellenbosch 2002.

Table 4.5.10.1. Summary of ascospore maturation – leaves picked August 2002 – July 2004.

		Month when leaves were picked												
		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Average ascospores monitored per month	2002													
	Sep 02	0	2002											
	Oct 02	7	6	2002										
	Nov 02	259	77	0	2002									
	Dec 02	48	194	0	0	2002								
	Jan 03	24	1215	1293	3	5	2003							
	Feb 03	16	0	19	1158	31	155	2003						
	Mar 03		0	0	1	0	3	2	2003					
	Apr 03			1	1	0	4	2	3	2003				
	May 03				2	12	0	66	0	0	2003			
	Jun 03					0	0	0	0	4	0	2003		
	Jul 03						0	0	0	0	0	0	2003	
	Aug 03	2003							0	0	12	0	0	
	Sep 03	0	2003							0	2	0	0	
	Oct 03	0	0	2003							0	0	0	
	Nov 03	4	214	0	2003							74	629	456
	Dec 03	250	61	123	46	2003							42	18
	Jan 04	2	0	3	6	1959	2004							2
	Feb 04	2	8	4002	2832	3634	3153	2004						
	Mar 04		0	0	0	2076	1704	0	2004					
	Apr 04			0	0	0	0	0	955	2004				
	May 04				0	0	0	0	-	-	2004			
	Jun 04					0	0	0	25	-	0	2004		
	Jul 04						0	0	178	-	0	0	2004	
	Aug 04	2004						0	0	-	0	0	0	
	Sep 04	0	2004							137	-	598	34	1
Oct 04	432	2	2004							-	25	0	0	
Nov 04	272	39	0	2004							46	231	0	
Dec 04	0	5	828	307	2004									
Jan 05						2005								
Feb 05							2005							

Letaba Estates (Biological plot)

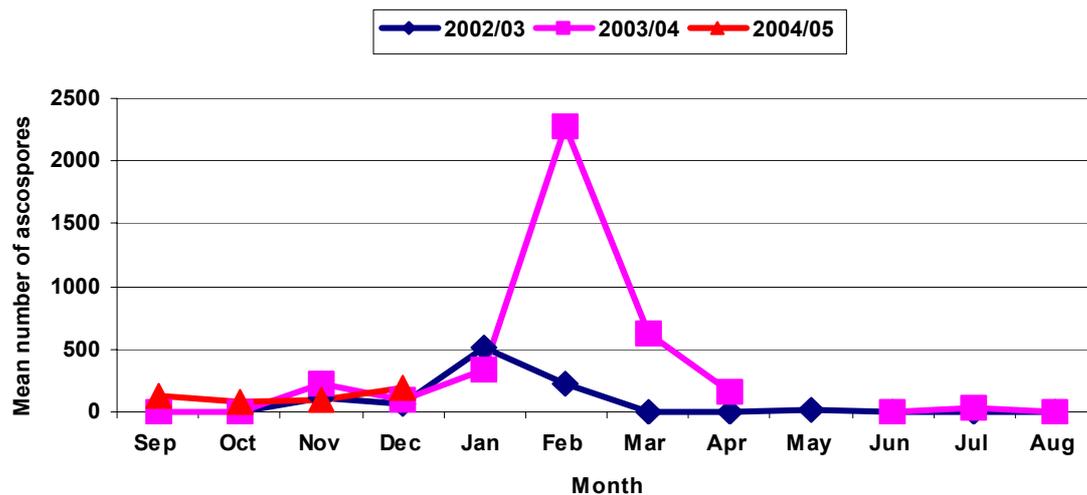


Fig. 4.5.10.1. Mean number of ascospores for each month, detected on leaves collected from an orchard at Letaba after being detached and left on the orchard floor for 1, 2, 3, 4, 5 and 6 months.

Letsitele

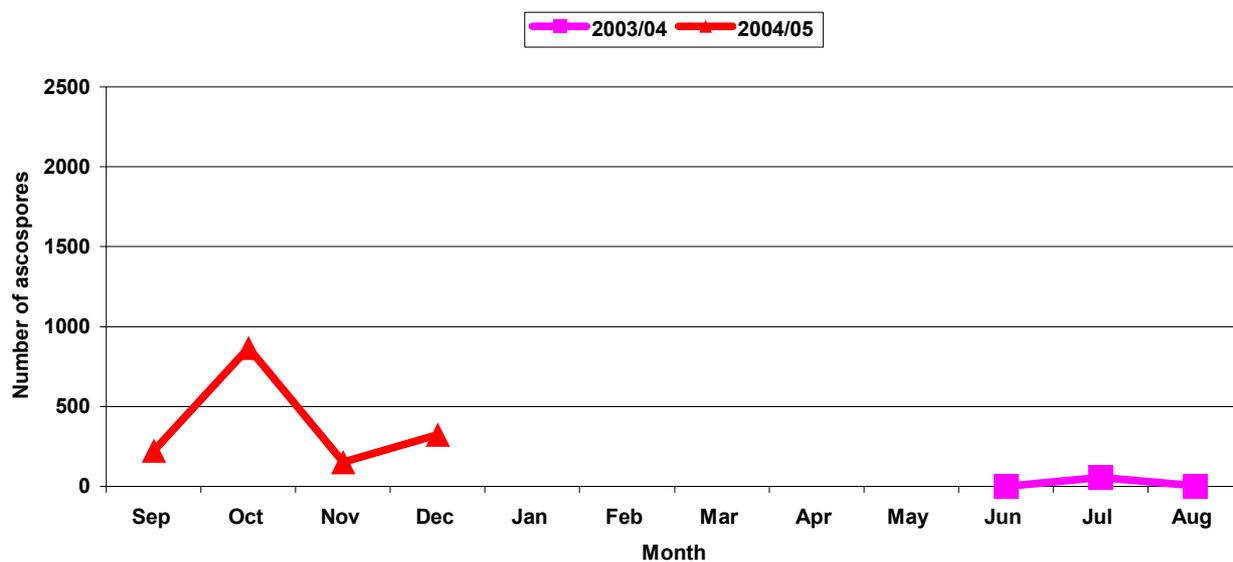


Figure 4.5.10.2. Mean number of ascospores for each month, detected on leaves collected from an orchard at Letsitele after being detached and left on the orchard floor for 1, 2, 3, 4, 5 and 6 months.

Hoedspruit

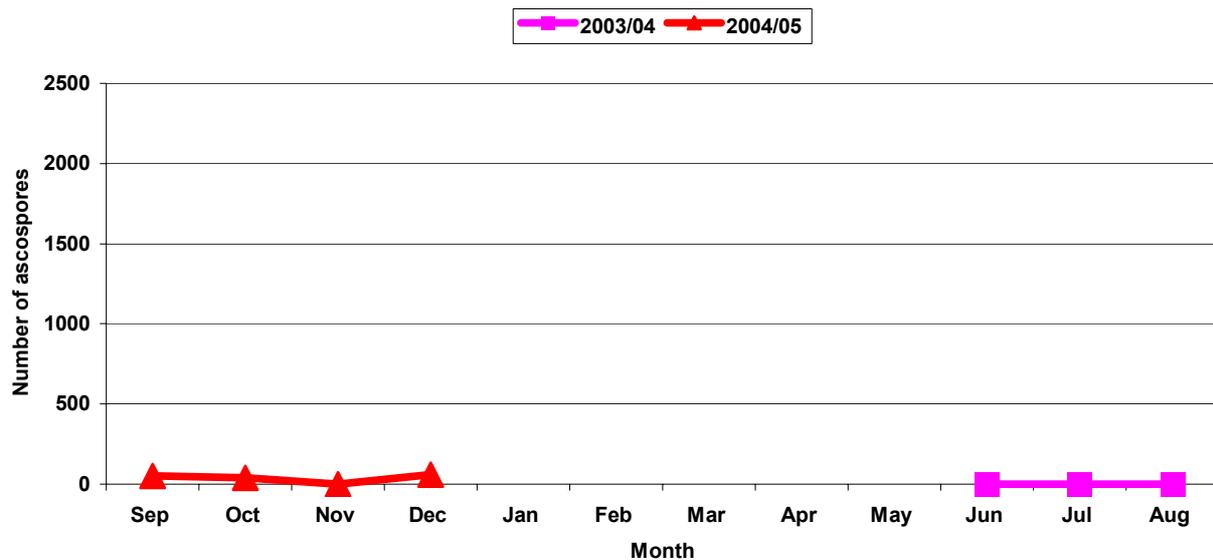


Figure 4.5.10.3. Mean number of ascospores for each month, detected on leaves collected from an orchard at Hoedspruit after being detached and left on the orchard floor for 1, 2, 3, 4, 5 and 6 months.

4.5.11 Determining if citrus leaves, naturally infected with *Guignardia citricarpa*, can produce inoculum while still positioned in a tree

Experiment QMS 2.1b by S.H. Swart (QMS)

Opsomming

Gedurende die 2003/2004 seisoen is groepe suurlemoene in 'n boord gevind met ernstige Swartvlek simptome. Hierdie infeksies is telkens met dooie takke geassosieer. Geen bron van inokulum kon egter op die takke gevind word nie, maar massas askospore is wel op dooie blare in die boom gevind. Die bevinding het die vermoede laat ontstaan dat askospore op dooie blare in die boom geproduseer kan word en dat die inokulum na vrugte kon versprei deur reën. In 'n vorige studie is takkies in suurlemoen bome geknak en daar gelaat om dooie blare te produseer. Baie min blare het egter behoue gebly wat in die askospoormonitor ontleed kon word. Enkele blaarmonsters in Maart 2003 het wel getoon dat askospore op dooie blare in die boom geproduseer kan word. In Junie 2004 is hierdie projek herhaal in 'n suurlemoen boord op Riverside, Letsitele, met die verskil dat takkies met volwasse en jong blare afgebreek is, en in groente sakkies in die bome opgehang is. Die blare is na onderskeidelik 2 en 3 en 4 maande uit bome verwyder en met die askospoormoniteerder ontleed vir teenwoordigheid van *Guignardia*-tipe askospore. Ontledings vanaf Julie tot Desember 2004 kon nog geen askospore aandui nie. Dit stem ooreen met data wat in die 2003/2004 seisoen ingesamel is. Ons verwag dat die eerste blare met inokulum vanaf Februarie gevind sal word. Die projek sal aanhou tot Mei 2005, waarna die laaste blare in Julie 2005 ontleed sal word. Resultate behoort aan te dui watter blare in 'n sitrus boom die hoofbron van inokulum is en hoe lank dit neem om inokulum op hierdie manier te produseer. Informasie sal bepaal watter tipe beheer maatreëls ingespan behoort te word om die potensiele bron van inokulum te verwyder of te verminder.

Summary

During the 2003/2004 season clusters of lemon fruits with Black Spot symptoms were found. These fruits were mostly associated with dead branches in trees. No source of inoculum could be found on the dead branches, but it was demonstrated that masses of ascospores were produced on dead leaves in the lemon trees. During a previous study, twigs were snapped and left in the tree to produce dead leaves. However, few leaves remained on branches which could be analysed with the ascospore monitor. Leaf samples taken in March 2003 did show that ascospores could be produced in this manner. In June 2004 this study was repeated with the difference that branches with mature and young leaves were picked, put in vegetable bags and hung in trees for 2, 3 and 4 months respectively. Leaves were analysed with an ascospore monitor for the production of *Guignardia*-like ascospores. Analyses from July to December 2004 showed no ascospore production on leaf samples. This corresponds with data gathered in the 2003/2004 season. We suspect that the first leaf samples with ascospores will be obtained from February. This project will continue until

May 2005 where after the last leaves will be analysed in July 2005. Results should be able to demonstrate which leaves are the major source of inoculum and how long it takes to produce inoculum in this manner. Information will determine which control measures should be put in place in order to remove or reduce this potential source of inoculum.

4.5.12 Developing a protocol for detecting CBS in citrus nurseries

Experiment QMS 5.3 by S.H. Swart (QMS)

Opsomming

Die doel van die studie was om die monster grootte van blare in 'n sitrus kwekery te bepaal, wat sal verseker dat *Guignardia*-agtige kulture wel opgespoor kan word, waar infeksie van blare plaasgevind het. In die algemeen is klein hoeveelhede *Guignardia*-agtige isolasies gevind tydens hierdie studies. Die grootste sukses (5%) is behaal waar monsters van 20 blare in die kwekery geselekteer is met klein (> 0.5 mm deursnee), swart, opgehewe letsels, en vir isolasies gebruik is. Indien blare ewekansig gepluk word en dan in die laboratorium vir letsels ondersoek word, was baie lae persentasie isolate gekry uit sub-monsters van 20 blare elk. Slegs blare met klein, swart, opgehewe letsels het isolate met *Guignardia*-agtige groei opgelewer. "PCR"-tegnieke kon betroubaar en herhaalbaar tussen *G. citricarpa* en *G. mangiferae* onderskei. Op hierdie stadium word te min isolate herwin om 'n afleiding te maak rakende die samestelling van die populasie dinamika van patogene en nie-patogene *Guignardia*-agtige organismes wat in sitrus kwekerye aangetref word.

Introduction

It has been noted that nurseries might play an important role in the spreading and manifestation of Citrus Black Spot (CBS) disease in areas that was previously free from the disease if climatic conditions support disease development. During the 2002/2003 season a project to detect *Guignardia* spp in citrus nurseries and to determine the magnitude of the problem, was initiated. Several *Guignardia*-like isolates were obtained from citrus leaves in local nurseries and from leaves in orchards close to Letsitele, Limpopo province. All but 1 isolate obtained from citrus leaves in nurseries and orchards were identified as *Guignardia mangiferae* by means of PCR tests, done by Plant Pathology Laboratories, University of Pretoria. All isolates obtained from fruit lesions tested positive for *Guignardia citricarpa*. Results showed that *Guignardia* spp were more frequently found on the oldest leaves of the scion than on leaves picked from the rootstock. Results also showed that infection was not confined to specific structures or blocks of trees in a nursery, but *Guignardia* spp were most often isolated from leaves in the oldest batch of trees in a specific structure. During our investigation eighty-three *Guignardia*-like isolates were found in 9 different blocks of citrus trees in 5 different shade net structures included in this investigation. No positive isolations could be made from trees grown in plastic tunnels. CBS isolates were more often obtained from Eureka lemon, Juval Valencia and Bahianinha navel trees than from Delta Valencia or Midnight Valencia cultivars. Twenty isolates obtained from 5 different blocks and 3 different cultivars during this evaluation, were identified as *G. citricarpa* according to PCR tests, done at Plant Pathology Laboratories, University of Pretoria.

Based on the percentage successful isolations of *Guignardia* spp during the above mentioned trials, it was concluded that further studies should be done on the oldest trees in a structure, preferably on Eureka, Juval, or Bahianinha scions. The oldest available leaves should be picked between January, and June. More isolates should be screened by means of PCR techniques in order to establish the composition of *Guignardia*-like populations found in citrus nurseries and also to verify the identity of isolates tested in 2002 (mostly *G. mangiferae*) and 2003 (all *G. citricarpa*).

The objective of this study was to develop a protocol for citrus nurseries in order to characterise lesions associated with *Guignardia* infections and to determine sample size that would ensure detection and possible quantification of *Guignardia* spp. in batches of trees. Since *Guignardia*-like isolates, obtained during different investigations were identified as different species on each of 2 occasions, we felt it necessary to recheck the identity of some of these isolations by means of PCR techniques and to include additional isolations obtained from citrus nurseries.

Materials and methods

a) Determining the sample size for detection of *Guignardia*-like lesions

Rough lemon seedlings were transplanted from seedling trays kept in plastic tunnels to 5 ℓ bags containing bark in March 2003 and placed in a standard shade net structure. These seedlings were grafted with Eureka lemon buds in October 2003. Sampling of the oldest leaves obtained from the scions of trees was done in

May and June 2004 and again in January 2005. Sampling was done in 5 different manners in a single block of approximately 3000 Eureka lemon trees.

1. Twenty leaves with minute, slightly protruding black lesions were selected for isolations.
2. Fifty leaves were picked randomly throughout the block of 3000 trees. Leaves with conspicuous lesions then sorted in the laboratory and 20 leaves were used for isolations.
3. One hundred leaves were picked randomly throughout the block of 3000 trees. Leaves with conspicuous lesions then sorted in the laboratory and 2 samples of 20 leaves each were used for isolations.
4. Two hundred leaves were picked randomly throughout the block of 3000 trees. Leaves with conspicuous lesions then sorted in the laboratory and 3 samples of 20 leaves each were used for isolations.
5. Five hundred leaves were picked randomly throughout the block of 3000 trees. Leaves with conspicuous lesions then sorted in the laboratory and 5 samples of 20 leaves each were used for isolations.

Leaves were surface sterilised and five isolations (0.5 mm in diameter) per leaf were plated on solidified Potato-dextrose-agar (PDA) containing 200 ppm chloramphenicol, in total 100 isolations per sub-sample. Culture media with isolations was incubated at $25\pm 2^{\circ}\text{C}$ under constant light. After 7 days incubation, cultures were evaluated and colonies with typical *Guignardia*-like morphology were sub-cultured on PDA slants and refrigerated. Isolates were sent to Plant Pathology Laboratories, University of Pretoria for identification.

b) Characterisation of lesions associated with *Guignardia*-like isolates

In August and September 2004, leaf samples were picked from Rough lemon trees in a nursery, close to Letsitele. Two types of lesions were identified. The one type represented small (approximately 0.5 mm diameter), black, protruding lesions (Fig. 4.5.12.1a) very often found on midrib of leaves (Fig. 4.5.12.1b), and the other type were slightly larger, flat and brownish in colour (Fig 4.5.12.2). Thirty leaves representing each of the 2 types of lesions were used for isolations.

Results and Discussion

a) Determining the sample size for detection of *Guignardia*-like lesions

No *Guignardia*-like isolates were obtained from any of the samples picked in May 2004, including isolations made from conspicuous lesions on 20 selected leaves as well as isolations made from leaves picked randomly. In the latter samples, between 70 and 95% of leaves had small black (Table 4.5.12.1).

From leaf samples picked in June 2004 some *Guignardia*-like isolates were obtained. Results show that 1 out of 100 isolations made from 20 leaves, picked due to conspicuous lesions, developed a *Guignardia*-like isolate, that is 1% of isolations made (Table 4.5.12.2). Between 45 and 57% of leaves picked randomly had conspicuous lesions. When 50 leaves were picked randomly, and 20 leaves with lesions selected, 3 isolates were recovered (3%). From a 100 leaves, 2 sub-samples of 20 leaves each were used for isolations (200 in total) and 1 isolate were found (0.5%). When 200 leaves were picked, 5 isolates were recovered from 1 out of 3 sub-samples, each containing 20 leaves, that is 5 out of 300 isolations (0.02%). In general 1% recovery was obtained when 20 leaves were selected in a block of nursery trees while only 0.007% *Guignardia*-like isolates were obtained when leaves were picked randomly.

From leaf samples picked in January 2005, only 5 isolates were recovered from the 20 leaves selected in the nursery, that is a 5% recovery (Table 4.5.12.3). None of the isolations made from lesions on randomly picked leaves developed *Guignardia*-like isolates in spite of the fact that between 57 and 68% of these leaves having conspicuous lesions.

b) Characterisation of lesions associated with *Guignardia*-like isolates

Three *Guignardia*-like isolate was obtained from 2 leaves during the August sampling. This was from leaves with small (<0.5 mm) conspicuous, protruding black lesions. Only 1 *Guignardia*-like isolate was obtained from 1 leaf during the September sampling. This was also from leaves with small (0.5 mm) conspicuous, protruding black lesions. No *Guignardia*-like isolates could be obtained from leaves with flat, light brown to black lesions.

PCR Results

In March 2004, 15 *Guignardia*-like isolates were send for PCR identification to Plant Pathology Laboratories, University of Pretoria. Results of these tests collaborated findings during previous PCR tests, therefore we

can conclude that both *G. citricarpa* and *G. mangiferae* can be isolated from leaves obtained from citrus nurseries.

Conclusions

In general, the overall recovery rate of *Guignardia*-like isolates is very low during all 3 sampling periods (0 to 0.0083%). Selecting leaves with conspicuous lesions in the nursery had the highest percentage recovery of *Guignardia*-like isolates (0 - 5%), while random selection gave variable results (0 – 0.5%). PCR is a reliable and repeatable technique to differentiate between *G. citricarpa* and *G. mangiferae*.

Future research

Leaves with conspicuous lesions should be selected in the nursery. This sample should be larger than the current 20 leaves per sample, perhaps 100 leaves, but this will mean 500 isolations per sample. However, the labour and cost of processing such large samples will render this practice impractical and uneconomical. The fact that morphological differences between pathogenic (slow-growing) and non-pathogenic (fast growing) *Guignardia* spp on PDA will not be accepted as a diagnostic distinction, can also pose a problem. Since PCR techniques are needed in any case for identification of *Guignardia*-like isolates, we suggest that leaves with conspicuous lesions should still be selected in the nursery, but that detection of *Guignardia* spp be attempted directly by means of PCR techniques.

Table 4.5.12.1. Samples taken and the number of *Guignardia*-like isolates recovered – May 2004.

Sample no.	Description	Leaf Samples	Perc. with lesions	Number isolations made	Total isolations made	Guignardia-like isolates obtained
1	20 leaves selected	1 x 20	100	1 x 100	100	0
2	50 leaves random	1 x 20	82	1 x 100	100	0
3	100 leaves random	2 x 20	89	2 x 100	200	0 ; 0
4	200 leaves random	3 x 20	70	3 x 100	300	0 ; 0 ; 0
5	500 leaves random	5 x 20	95	5 x 100	500	0 ; 0 ; 0 ; 0 ; 0
Total		12 x 20		12 x 100	1200	0

Table 4.5.12.2. Samples taken and the number of *Guignardia*-like isolates recovered – June 2004.

Sample no	Description	Leaf Samples	Perc. with lesions	Number isolations made	Total isolations made	Guignardia-like isolates obtained
1	20 leaves selected	1 x 20	100	1 x 100	100	1
2	50 leaves random	1 x 20	46	1 x 100	100	3
3	100 leaves random	2 x 20	57	2 x 100	200	1 ; 0
4	200 leaves random	3 x 20	45	3 x 100	300	5 ; 0 ; 0
5	500 leaves random	5 x 20	54	5 x 100	500	0 ; 0 ; 0 ; 0 ; 0
Total		12 x 20		12 x 100	1200	10

Table 4.5.12.3. Samples taken and the number of *Guignardia*-like isolates recovered – January 2005.

Sample no	Description	Leaf Samples	Perc. with lesions	Number isolations made	Total isolations made	Guignardia-like isolates obtained
1	20 leaves selected	1 x 20	100	1 x 100	100	5
2	50 leaves random	1 x 20	64	1 x 100	100	0

3	100 leaves random	2 x 20	57	2 x 100	200	0 ; 0
4	200 leaves random	3 x 20	61	3 x 100	300	0 ; 0 ; 0
5	500 leaves random	5 x 20	68	5 x 100	500	0 ; 0 ; 0 ; 0 ; 0
Total		12 x 20		12 x 100	1200	5



Figures 4.5.12.1a and 4.5.12.1b. Small (<0.5 mm) black, protruding lesions often concentrated round the midrib of citrus leaves in a nursery.



Figure 4.5.12.2. Larger (< 1 mm), flat light brown to black lesions on citrus leaves in a nursery.

4.5.13 The evaluation of several chemical programmes for the control of Citrus Black Spot Experiment QMS 2.3 by S.H Swart (QMS)

Opsomming

Die standaard praktyk in die Limpopo provinsie is om sitrus vrugte teen Swartvlek infeksie te beskerm vanaf Oktober tot die einde Januarie. Gedurende die 2002/2003 en 2003/2004 seisoene het vrugte, wat laat gepluk is, hoë insidensie van Sitrus Swartvlek getoon, ten spyte van spuitprogramme wat infeksie tot einde Februarie moes verhoed. Die doel van die proef was om standaard programme te evalueer ten opsigte van

hul vermoë om Swartvlek te verhoed, veral onder toestande wat laat infeksie bevorder. Blare van behandelde bome sal in Januarie 2005 gepluk word en in die boord gelaat word tot Maart 2005, waarna dit geëvalueer sal word vir die vermoë om askospoor vorming te ondersteun.

Die proef is in Oktober 2004 begin en sal in September 2005 geëvalueer word, waarna 'n finale verslag ingedien sal word.

Introduction

The control of CBS is mainly aimed at preventing fruit from getting infected. Popular belief is that fruit only needs to be protected from October until the end of February in the Limpopo province. During the 2002/2003 and 2003/2004 seasons several late hanging fruit showed high levels of Citrus Black Spot (CBS) symptoms in spite of programs that should have ensured adequate protection from early October until the end of February. In the Letaba, Letsitele and Hoedspruit areas ascospore discharges normally peak between November and March. Data recorded by QMS Agri Science show that during the 2002/2003 and 2003/2004 seasons, high numbers of ascospores were discharged between December and March and that climatic conditions were extremely favourable for infection, especially during March of both seasons. The occurrence of CBS infected fruit in several orchards raised the question whether standard registered programs can protect fruit adequately for markets that show zero tolerance to CBS infected fruit.

The aim of this project was to evaluate the efficacy of standard chemical programmes to prevent infection of citrus fruit, especially under conditions that favour late infection. Picked leaves of treated trees will be allowed to age in the orchard and screened with an ascospore monitor for their ability to produce inoculum in order to establish whether applied fungicides have the ability to reduce inoculum production due to a curative mode of action.

Materials and methods

A trial was conducted at Mahela, Letsitele, in a 52-year old Valencia orchard with a history of CBS infection. Eleven different programs were evaluated (Table 4.5.13.1). These include treatments with mancozeb (contact action) applied at 21, 23, 30, and 35-day intervals (treatments 2, 3, 4, 5), strobilurine fungicides (Ortiva, Flint and Cabrio) applied according to traditional programs (treatments 6, 7, 8), and benomyl (systemic) with curative action (treatment 9). Mancozeb (Dithane M45), pyraclostrobin (Cabrio) and benomyl (Benomyl) was also applied in programs to ensure protection until at least middle March (treatments 2, 3, 10, 11). Treatments were applied to single-tree plots, replicated 5 times in a randomised block design. Fruit will be evaluated for visible CBS symptoms in September 2005. Leaves of treated trees will be picked in January 2005 and evaluated in March 2005 for the ability to support ascospore development with the Quest ascospore monitor.

Results and discussion

This trial will be evaluated in September 2005.

Future research

Dependent on outcome of results.

Table 4.5.13.1. Treatments evaluated for their ability to control Citrus Black Spot.

Treatment no.	Program description	Program *	Active ingredient	Formulation	Dosage g or ml / 100 l
1	Control	-	-	-	-
2	7 x Dithane	M,M,M,M,M,M,M	mancozeb	800 g / kg, WP	200
3	6 x Dithane	M,M,M,M,M,M	mancozeb	800 g / kg, WP	200
4	5 x Dithane	M,M,M,M,M	mancozeb	800 g / kg, WP	200
5	4 x Dithane	M,M,M,M	mancozeb	800 g / kg, WP	200
6	Ortiva + Dithane + Citrex	M,AmO,AmO,M	azoxystrobin mancozeb oil	250 g / l, SC 800 g / kg, WP	20 200 / 150 300
7	Flint + Dithane + Citrex	M,TmO,TmO,M	trifloxistrobin mancozeb mineral oil	500 g / kg, WG 800 g / kg, WP	10 200 / 150 300

8	Cabrio + Dithane + Citrex	M,PmO,PmO,M	pyraclostrobin mancozeb mineral oil	250 g / l, EC 800 g / kg, WP	10 200 / 150 300
9	Benomyl + Dithane + Citrex	M,BmO,BmO,M	benomyl mancozeb mineral oil	500 g / kg, WP 800 g / kg, WP	50 200 / 150 300
10	3 x Benomyl + Dithane + Citrex	BmO, BmO, BmO	benomyl mancozeb mineral oil	500 g / kg, WP 800 g / kg, WP	50 150 300
11	3 x Cabrio + Dithane + Citrex	PmO, PmO, PmO	pyraclostrobin mancozeb mineral oil	250 g / l, EC 800 g / kg, WP	10 150 300

- M = mancozeb 200 g / 100l, m = mancozeb 150 g / 100 l, A = azoxystrobin, O = Citrex, T = trifloxistrobin, P = pyraclostrobin, B = benomyl

Table 4.5.13.2. Timing of fungicide application.

Treatment no.	Program description	Date of application						
		-	-	-	-	-	-	-
1	Control	-	-	-	-	-	-	-
2	7 x Dithane	13/10	03/11	24/11	15/12	05/01	26/01	16/02
3	6 x Dithane	16/10	05/11	02/12	23/12	13/01	07/02	
4	5 x Dithane	18/10	22/11		21/12	17/01		18/02
5	4 x Dithane	18/10	22/11		28/12		07/02	
6	Ortiva + Dithane + Citrex	18/10	05/11		22/12		07/02	
7	Flint + Dithane + Citrex	18/10	05/11		22/12		07/02	
8	Cabrio + Dithane + Citrex	18/10	05/11		22/12		07/02	
9	Benomyl + Dithane + Citrex	18/10	05/11		22/12		07/02	
10	3 x Benomyl + Dithane + Citrex	18/10	05/11		22/12		07/02	
11	3 x Cabrio + Dithane + Citrex	18/10	05/11		22/12		07/02	

4.5.14 Determine the correlation between increasing fruit age and resistance to infection by *Guignardia citricarpa*

Experiment QMS 03/68n by S.H. Swart (QMS)

Opsomming

Verskeie publikasies beweer dat sitrus vrugte hoogs vatbaar is vir infeksie deur *Guignardia citricarpa* vanaf vrugset, maar dat vrugte meer bestand raak teen infeksie met toenemende ouderdom tot ongeveer Februarie, waarna normaalweg geen beskerming meer nodig is nie. Data verskaf deur QMS Agri Science toon dat in die Letaba, Letsitele en Hoedspruit areas groot getalle askospore tussen Desember en Maart vrygestel kan word en dat kondisies na Februarie nog uiters gunstig vir infeksie kan wees. Verskeie boorde in die Letaba en Letsitele areas, veral die wat nog in September en Oktober 2004 gepluk is, het 'n hoë insidensie van Sitrus Swartvlek getoon. Die doel van hierdie studie was om vas te stel of sitrus vrugte wel bestand word teen infeksie na Januarie en of addisionele swamdoder bespuitings nodig is in Februarie of selfs Maart vir vrugte wat laat gepluk gaan word. 'n Proef waar nuut gesette vrugte met papiersakke bedek is, met die oog om vrugte van verskillende ouderdomme aan infeksie bloot te stel, het misluk vanweë die groot aantal vrugte wat binne 3 maande na bedekking afgespeen het. 'n Poging om volwasse vrugte tydens Maart 2004 met piknidiospore te besmet het ook geen simptome getoon tydens evaluasie in September 2004 nie. Op Mahela is nog 'n proef aangepak waar vrugte vanaf middel Oktober 2004 tot Maart 2005 elke 23 dae met 'n kontak swamdoder beskerm sal word. In die proef word een stel bome in elk van die 6 onderskeie maande wat die proef sal duur, onbeskermd gelaat. Vrugte sal in September 2005 gepluk word en die persentasie vrugte met swartvlek letsels sal bepaal word vir elke program. Daar sal dus gepoog word om te bepaal tot wanneer infeksie kon plaasvind en watter infeksie periode die grootste invloed op die

voorkoms van Sitrus Swartvlek gehad het. Na die evaluasie sal in September 2005 sal 'n volledige verslag ingehandig sal word.

Introduction

The control of CBS is mainly aimed at preventing fruit from getting infected. Kotzé (1981), reported that ascospores produced on dead leaves on the orchard floor are the primary source of CBS infection. According to published data, citrus fruit is highly susceptible to infection by *G. citricarpa* from fruit set but become more resistant to infection as fruit mature and needs only to be protected until February (Kotzé, 1981; 1988, 1996). In the Letaba, Letsitele and Hoedspruit areas ascospore discharges normally peak from November to March. During the 2002/2003 and 2003/2004 seasons high numbers of ascospores were discharged between December and March and climatic conditions were extremely favourable for infection during March of both seasons according to data recorded by QMS Agri Science. Several orchards in the area where fruit was picked in September and October 2004 showed high incidence of Citrus Black Spot. Popular belief is that fruit only needs to be protected from October until the end of January in the Limpopo province. However, the occurrence of high levels of infected fruit, in spite of spray programs which ensured protection from early October until the end of February, raised some questions regarding acquired resistance with fruit maturity.

The aim of this study was to determine whether fruit do become more resistant to infection by *G. citricarpa* with increasing maturity and whether additional protection is needed until the end of February and even March for late hanging cultivars.

Materials and methods

a) Covering and exposing fruit during periods conducive to infection

During the 2003/2004 season, fruit in an unsprayed Navel orchard at Letaba Estates was covered with paper bags soon after fruit set. Fruit was supposed to be uncovered at monthly intervals and exposed to artificial infection conditions with overhead irrigation installed in the trees.

b) Artificially inoculated fruit

In another trial at Letaba Estates, 50 Delta Valencia fruit were inoculated with a suspension of artificially produced pycnidiospores obtained from 3-week-old laboratory grown *G. citricarpa* cultures on carnation-leaf-agar. Inoculation was done during March 2004. Fruit was covered with plastic bags containing moisturised cotton wool for 4 days in order to enhance infection.

c) Protecting fruit with a contact chemical

A trial was set up in a 52-year old Valencia orchard at Mahela, Letsitele. In this trial, chemical treatments with 200 g mancozeb / 100 ℓ water commenced middle October 2004 and will continue until middle March 2005. The trial consisted of 7 groups of trees, which will be sprayed every 23 days, but 1 group will be left unprotected in each of the 6 consecutive months. Each treatment will be replicated 5 times in a randomised block design.

Results and discussion

a) Covering and exposing fruit during periods conducive to infection

Most of the fruit became detached within 3 months after being covered just after fruit set. There were also problems with large populations of insects, especially mealybug, which colonised the fruit stems where paper bags were attached. This trial was discontinued in November 2003.

b) Artificially inoculated fruit

No signs of Black spot symptoms could be detected on fruit when the trial was evaluated in September 2004. The artificial infection process with pycnidiospores was unsuccessful on mature fruit during March 2004. It is unclear whether fruit was resistant against infection at the time of inoculation or whether the artificial infection process failed.

c) Protecting fruit with a contact chemical

This trial will be evaluated in August/September 2005 in order to allow for maximum development of Black spot symptoms.

Conclusions

The covering of small fruit with paper bags are very laborious and large numbers of fruit become detached due to this practice. This practice will have to be modified for future work.

March was too late to induce artificial infections with pycnidiospores on mature fruit. This strategy should be attempted at an earlier stage by using ascospores producing leaves and artificially produced pycnidiospores.

Future research

Generating data from the trial where fruit was protected for the whole period in which infection could take place, but left unprotected for specific periods of 4 weeks. Evaluation August / September 2005.

Set up a trial to attempt artificial infection earlier stage, possibly with ascospores (leaf litter) and pycnidiospores (artificially produced). Overhead irrigation will be used to ensure a microclimate conducive to infection.

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4.6 PROJECT: POST-HARVEST PATHOLOGY

Project Co-ordinator: K.H. Lesar (CRI)

4.6.1 Project summary

A number of new formulations of the post-harvest fungicides imazalil and thiabendazole have been evaluated and submitted for registration. The post-harvest fungicide, prochloraz, has also been evaluated for registration in a post-harvest dip treatment. All these registrations are pending. A potentially new fungicide was evaluated but poor efficacy results were recorded. A number of organic products as well as new quaternary ammonium compounds were also evaluated with poor efficacy results being recorded. The search for new safe products for post-harvest disease control is ongoing and such products will continuously be evaluated. Screening of *Penicillium* fungal spore samples revealed imazalil resistant spores in the Eastern Cape production areas.

Projekopsomming

Verskeie nuwe formulasies van die naoes swamdoders imazalil en thiabendazole is ge-evalueer en die resultate is vir registrasie ingehandig. Die naoes swamdoder, prochloraz, is ook ge-evalueer vir registrasie in 'n naoes doopbehandeling. Al die registrasies is in afwagting van 'n antwoord. 'n Moontlike nuwe swamdoder is ge-evalueer maar geen goeie resultate is verkry nie. 'n Paar organiese produkte asook nuwe kwaternêre ammonium middels is ook ge-evalueer sonder om goeie resultate vir effektiwiteit te toon nie. Die soek vir nuwe, veilige produkte vir naoes siektebeheer is aangaande en sulke produkte sal voortdurend ge-evalueer word. Die evaluering van *Penicillium* swamspoormonsters het imazalil bestande spore uit die Oos-Kaap produksie gebiede getoon.

4.6.2 The evaluation of three formulations of the post-harvest fungicide imazalil against post-harvest diseases for the purpose of registration

Experiment 123 by K.H. Lesar (CRI)

Opsomming

Drie generiese formulasies van imazalil 750 WSP en WSG en 500 EC is ge-evalueer vir effektiwiteit teen die naoes sitrus patogeen *Penicillium digitatum* (groenskimmel). Albei die 750 WSP en die 750 WSG formulasies het goeie beheer van die patogeen, in vergelyking met die standard Fungazil 750 WSP, getoon. Die 500 EC formulasie het onaanvaarbare beheer van die patogeen getoon. Dié evaluasie sal herhaal moet word. Geen fitotoksiteit is op die behandelde vrugte waargeneem nie.

Introduction

Three new formulations of the post-harvest fungicide, imazalil, were submitted to CRI by ICA International Chemicals and AG Chem Africa. The two formulations from ICA were an imazalil sulphate 750 WSG (granules) and a 500 EC formulation, and the formulation from AG Chem Africa was an imazalil sulphate WSP. These formulations were evaluated for efficacy against *P. digitatum*. The 750 WSG and 750 WSP formulations were evaluated in a post-harvest water dip treatment and were compared with the standard Fungazil 750 WSP at the recommended commercial rate of 500 g/kg. These two formulations were evaluated at the rates of 250 g/kg ($\frac{1}{2}x$), 500 g/kg (x) and 1000 g/kg ($2x$). The 500 EC formulation was evaluated in citrus wax in a dip treatment at the rates of 1500 g/l ($\frac{1}{2}x$), 3000 g/l (x) and 6000 g/l ($2x$). This formulation was compared with the standard Fungazil 800 EC at the standard rate of 3000 g/l.

Materials and methods

A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Valencia oranges from Crocodile Valley Citrus Co., and lemons from Larten, were obtained in bulk. For the purpose of inoculation, blemish-free, sound fruit was selected and randomised. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

All the treatments with the ICA imazalil sulphate 750 WSG and the AG Chem Africa 750 WSP were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 and 16 hours after inoculation (to simulate a short and long delay after harvesting prior to packhouse treatments) with the chemical compound being evaluated. Each treatment was immersed in the water bath for 3 minutes. After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay. The treatments with the imazalil 500 EC were done by dipping the inoculated fruit into the fungicide incorporated in a citrus wax. The inoculated fruit was treated 4 hours after inoculation and each treatment was immersed in the wax incorporating the fungicide for 3 minutes. After treatment the wax was allowed to dry on the fruit overnight and the fruit was then incubated in paper packets as above and evaluated, and the results recorded in the same way as the imazalil sulphate 750 WSG.

Treatments – Imazalil sulphate 750 WSG

1. Untreated control (*P. digitatum*).
2. Treated control – Fungazil WSP 500 g/kg – 4 hrs after inoculation.
3. Treated control – Fungazil WSP 500 g/kg – 16 hrs after infection.

4. ICA Imazalil sulphate 750 WSG – 250 g/kg – 4 hrs after infection.
5. ICA Imazalil sulphate 750 WSG – 250 g/kg – 16 hrs after infection.
6. ICA Imazalil sulphate 750 WSG – 500 g/kg – 4 hrs after infection.
7. ICA Imazalil sulphate 750 WSG – 500 g/kg – 16 hrs after infection.
8. ICA Imazalil sulphate 750 WSG – 1000 g/kg – 4 hrs after infection.
9. ICA Imazalil sulphate 750 WSG – 1000 g/kg – 16 hrs after infection.

Treatments – Imazalil 500 EC

1. Untreated control (*P. digitatum*).
2. Treated control – Fungazil 800 EC (3000 g/l) – 4 hrs after infection.
3. ICA Imazalil 500 EC – 1500 g/l - 4 hrs after infection.
4. ICA Imazalil 500 EC – 3000 g/l - 4 hrs after infection.
5. ICA Imazalil 500 EC – 6000 g/l - 4 hrs after infection.

Three trials were conducted with the ICA imazalil using Valencia oranges in all three trials. All concentrations designated g/kg refer to the a.i. of imazalil.

Results

No phytotoxicity was evident on the fruit treated at the highest concentration (1000 g/kg) of the ICA imazalil sulphate.

Table 4.6.2.1. The effect of ICA imazalil 750 WSG on *P. digitatum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ – 4 hrs	Nil
3. Treated control 500 g/kg Fungazil SO ₄ – 16 hrs	Nil
4. 250 g/kg ICA Imazalil – 4 hrs	30
5. 250 g/kg ICA Imazalil – 16 hrs	20
6. 500 g/kg ICA Imazalil – 4 hrs	Nil
7. 500 g/kg ICA Imazalil – 16 hrs	Nil
8. 1000 g/kg ICA Imazalil – 4 hrs	Nil
9. 1000 g/kg ICA Imazalil – 16 hrs	Nil

Table 4.6.2.2. The effect of ICA Imazalil 750 WSG on *P. digitatum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ – 4 hrs	Nil
3. Treated control 500 g/kg Fungazil SO ₄ – 16 hrs	Nil
4. 250 g/kg ICA Imazalil – 4 hrs	10
5. 250 g/kg ICA Imazalil – 16 hrs	10
6. 500 g/kg ICA Imazalil – 4 hrs	Nil
7. 500 g/kg ICA Imazalil – 16 hrs	Nil
8. 1000 g/kg ICA Imazalil – 4 hrs	Nil
9. 1000 g/kg ICA Imazalil – 16 hrs	Nil

Table 4.6.2.3. The effect of ICA Imazalil 750 WSG on *P. digitatum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ – 4 hrs	Nil
3. Treated control 500 g/kg Fungazil SO ₄ – 16 hrs	Nil
4. 250 g/kg ICA Imazalil – 4 hrs	Nil
5. 250 g/kg ICA Imazalil – 16 hrs	Nil

6. 500 g/kg ICA Imazalil – 4 hrs	Nil
7. 500 g/kg ICA Imazalil – 16 hrs	Nil
8. 1000 g/kg ICA Imazalil – 4 hrs	Nil
9. 1000 g/kg ICA Imazalil – 16 hrs	Nil

Conclusion

The Imazalil sulphate 750 WSG submitted by ICA International demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil sulphate 750 WSP. Fruit samples will be submitted for residue analyses.

Treatments – Imazalil sulphate 750 WSP

1. Untreated control (*P. digitatum*).
2. Treated control – Fungazil WSP 500 g/kg – 4 hrs after inoculation.
3. Treated control – Fungazil WSP 500 g/kg – 16 hrs after infection.
4. AG Chem Imazalil - 250 g/kg – 4 hrs after infection.
5. AG Chem Imazalil - 250 g/kg – 16 hrs after infection.
6. AG Chem Imazalil - 500 g/kg – 4 hrs after infection.
7. AG Chem Imazalil - 500 g/kg – 16 hrs after infection.
8. AG Chem Imazalil - 1000 g/kg – 4 hrs after infection.
9. AG Chem Imazalil - 1000 g/kg – 16 hrs after infection.

Three trials were conducted with the AG Chem Africa imazalil, i.e. two with Valencia oranges and one with lemons.

Treatments – Imazalil 500 EC

1. Untreated control (*P. digitatum*).
2. Treated control – Fungazil 800 EC (3000 g/l - 4 hrs after infection).
3. ICA Imazalil 500 EC - 1500 g/l - 4 hrs after infection.
4. ICA Imazalil 500 EC - 3000 g/l - 4 hrs after infection.
5. ICA Imazalil 500 EC - 6000 g/l - 4 hrs after infection.

All concentrations designated g/kg refer to the a.i. of imazalil.

Results

No phytotoxicity was evident on the fruit treated at the highest concentration (1000 g/kg) of the AG Chem imazalil.

Table 4.6.2.4. The effect of AG Chem Imazalil 750 WSP on *P. digitatum*

LEMONS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ 4hrs	Nil
3. Treated control 500 g/kg Fungazil SO ₄ 16hrs	Nil
4. 250 g/kg AG Chem Imazalil – 4 hrs	Nil
5. 250 g/kg AG Chem Imazalil – 16 hrs	Nil
6. 500 g/kg AG Chem Imazalil – 4 hrs	Nil
7. 500 g/kg AG Chem Imazalil – 16 hrs	Nil
8. 1000 g/kg AG Chem Imazalil – 4 hrs	Nil
9. 1000 g/kg AG Chem Imazalil – 16 hrs	Nil

Table 4.6.2.5. The effect of AG Chem Imazalil 750 WSP on *P. digitatum*

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ 4hrs	Nil

3. Treated control 500 g/kg Fungazil SO ₄ 16hrs	Nil
4. 250 g/kg AG Chem Imazalil – 4 hrs	Nil
5. 250 g/kg AG Chem Imazalil – 16 hrs	Nil
6. 500 g/kg AG Chem Imazalil – 4 hrs	Nil
7. 500 g/kg AG Chem Imazalil – 16 hrs	Nil
8. 1000 g/kg AG Chem Imazalil – 4 hrs	Nil
9. 1000 g/kg AG Chem Imazalil – 16 hrs	Nil

Table 4.6.2.6. The effect of AG Chem Imazalil 750 WSP on *P. digitatum*

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil SO ₄ 4hrs	Nil
3. Treated control 500 g/kg Fungazil SO ₄ 16hrs	Nil
4. 250 g/kg AG Chem Imazalil – 4 hrs	Nil
5. 250 g/kg AG Chem Imazalil – 16 hrs	Nil
6. 500 g/kg AG Chem Imazalil – 4 hrs	Nil
7. 500 g/kg AG Chem Imazalil – 16 hrs	Nil
8. 1000 g/kg AG Chem Imazalil – 4 hrs	Nil
9. 1000 g/kg AG Chem Imazalil – 16 hrs	Nil

Conclusion

The generic formulation of imazalil submitted by AG Chem Africa demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil 750 WSP. Fruit samples will be submitted for residue analyses.

Results

No phytotoxicity was evident on the fruit treated at the highest concentration (6000 g/l) of the ICA imazalil 500 EC.

Table 4.6.2.7. The effect of ICA Imazalil 500 EC on *P. digitatum*.

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control 3000 g/kg Fungazil 800 EC	10
3. 1500 g/l ICA Imazalil 500 EC	30
4. 3000 g/l ICA Imazalil 500 EC	20
5. 6000 g/l ICA Imazalil 500 EC	20

Table 4.6.2.8. The effect of ICA Imazalil 500 EC on *P. digitatum*.

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control 3000 g/kg Fungazil 800 EC	Nil
3. 1500 g/l ICA Imazalil 500 EC	20
4. 3000 g/l ICA Imazalil 500 EC	20
5. 6000 g/l ICA Imazalil 500 EC	10

Conclusion

The Imazalil 500 EC submitted by ICA International did not exhibit good control of the pathogen, *P. digitatum*, compared to the standard Fungazil 800 EC. This work will have to be repeated.

4.6.3 The evaluation of the post-harvest fungicide Prochloraz against the *Penicillium* organisms for the purpose of registration as a post-harvest dip treatment

Opsomming

Twee formulasies van die naoes swamdoder, prochloraz, is ge-evalueer vir effektiwiteit teen die naoes patogeen *P. digitatum* vir die registrasie van dié formulasies in 'n naoes doopbehandeling. Albei middels het goeie beheer teen die patogeen, in vergelyking met die standard Fungazil 750 WSP, getoon. Geen fitotoksisiteit is op die behandelde vrugte waargeneem nie.

Introduction

Prochloraz is currently registered on citrus as a spray-on over a bed of brushes (total loss system) at 1500 ppm for the control of green and blue mould. Since the advent of hot water fungicide baths, packhouses have moved away from the registered application and are no longer equipped for this method of application. This scenario has thus justified the evaluation of prochloraz in a hot water dip treatment for the purpose of registration thereof.

Samples of prochloraz were submitted by Villa Crop Protection and AgChem Africa for evaluation against the post-harvest citrus pathogen *Penicillium digitatum* (green mould) to determine the effective concentration of the product in a dip treatment.

Materials and methods

A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good sound, untreated Valencia and navel oranges (Crocodile Valley Citrus Co.) and lemons (Larten) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

Treatments

1. Untreated control (*P. digitatum*)
2. Treated control – Fungazil WSP (500 ppm) – 4 hrs after infection
3. Treated control – Fungazil WSP (500 ppm) – 16 hrs after infection
4. Prochloraz – 500 ppm – 4 hrs after infection
5. Prochloraz – 500 ppm – 16 hours after infection
6. Prochloraz – 1000 ppm – 4 hrs after infection
7. Prochloraz – 1000 pm – 16 hrs after infection
8. Prochloraz – 2000 ppm – 4 hrs after infection
9. Prochloraz – 2000 ppm – 16 hrs after infection

All concentrations designated ppm refer to the a.i. of the product.

All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C for three minutes, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 and 16 hours after inoculation (to simulate a short and long delay period after harvesting prior to packhouse treatments) with the chemical compound being evaluated. After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

No phytotoxicity was evident on the fruit treated at the highest concentration (2000 ppm) of both formulations of prochloraz.

Table 4.6.3.1. The effect of Villa prochloraz on *P. digitatum* inoculated lemons.

LEMONS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	60
5. Prochloraz 500 ppm – 16 hrs after infection	100
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	40
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	Nil

Table 4.6.3.2. The effect of Villa prochloraz on *P. digitatum* inoculated navels.

NAVELS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	30
5. Prochloraz 500 ppm – 16 hrs after infection	100
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	40
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	20

Table 4.6.3.3. The effect of Villa prochloraz on *P. digitatum* inoculated Valencias.

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	30
5. Prochloraz 500 ppm – 16 hrs after infection	70
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	60
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	40

Table 4.6.3.4. The effect of AG Chem prochloraz on *P. digitatum* inoculated lemons.

LEMONS	
Treatments (a.i.)	% Decay
1. Untreated control	90
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	40
5. Prochloraz 500 ppm – 16 hrs after infection	50
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	50
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	Nil

Table 4.6.3.5. The effect of AG Chem prochloraz on *P. digitatum* inoculated navels.

NAVELS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	50
5. Prochloraz 500 ppm – 16 hrs after infection	100
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	40
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	30

Table 4.6.3.6. The effect of AG Chem prochloraz on *P. digitatum* inoculated Valencias.

VALENCIAS	
Treatments (a.i.)	% Decay
1. Untreated control	100
2. Treated control – 4 hrs after infection	Nil
3. Treated control – 16 hrs after infection	Nil
4. Prochloraz 500 ppm – 4 hrs after infection	30
5. Prochloraz 500 ppm – 16 hrs after infection	60
6. Prochloraz 1000 ppm – 4 hrs after infection	Nil
7. Prochloraz 1000 ppm – 16 hrs after infection	50
8. Prochloraz 2000 ppm – 4 hrs after infection	Nil
9. Prochloraz 2000 ppm – 16 hrs after infection	20

Discussion

For any prospective post-harvest fungicide to be effective, it is essential to determine how soon after harvest it must be applied, what the influence of fruit temperature is during the delay between infection and fungicide application and what concentration of the fungicide is needed for effective control.

Prochloraz is a fungicide with the same mode of action as imazalil. Good results have been obtained from these trials demonstrating how effective prochloraz is in controlling infections by green mould (*P. digitatum*), as well as blue mould (*P. italicum*), which was demonstrated in previous trial work conducted by Outspan in the years 1985-1990.

One very important attribute of prochloraz is that it effectively controls sporulation, as does imazalil. This is important in preventing soilage in packed cartons of fruit.

However, one advantage that imazalil has over prochloraz is the time delay between infection and fungicide application. Imazalil can be applied to fruit up to 72 hours delay (maximum), under ideal conditions, and still achieve good control of infections. Prochloraz, on the other hand, must be applied within **12 hours** from harvest to achieve effective control.

Conclusion

Both formulations of prochloraz demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil 750 WSP. The data have been submitted for registration of the two formulations.

4.6.4 The evaluation of three formulations of the post-harvest fungicide, thiabendazole, against post-harvest diseases for the purpose of registration

Opsomming

Drie formulasies van die naoes swamdoder, thiabendazole, is ge-evalueer vir effektiwiteit teen die latente patogene *Diplodia natalensis* (*Diplodia* stingelentvrot) en *Colletotrichum gloeosporioides* (Anthracnose) vir registrasie doeleindes. Die drie formulasies het goeie beheer oor Anthracnose, wat die oorheersende

patogeen was tydens evaluasie, getoon in vergelyking met die standard Tecto 500 SC. Geen fitotoksisiteit is waargeneem nie.

Introduction

Three formulations of the post-harvest fungicide, thiabendazole, were submitted to CRI by Villa Crop Protection, ICA International Chemicals and H.G. Molenaar, for efficacy trials against post-harvest pathogens for the purpose of registration. The three formulations were evaluated *in vitro* and *in vivo* against the post-harvest pathogens *Colletotrichum gloeosporioides* (Anthracnose) and *Diplodia natalensis* (Diplodia stem-end rot). These products were compared, *in vivo*, with the standard thiabendazole (Tecto 500 SC) at the recommended commercial rate of 4000 ppm in wax and at 2000 ppm in a water dip treatment. The trial formulations were evaluated at the rates of 4000 ppm and 8000 ppm (for phytotoxicity).

In vitro evaluation

Materials and methods

Isolations of both pathogens (Anthracnose and Diplodia) were made from infected citrus fruit onto Potato Dextrose agar (PDA) and incubated as stock cultures for the purpose of this evaluation. Stock solutions of both the standard recommended Tecto 500 SC and the other three formulations of thiabendazole 500 SC were made up at a concentration of 10 000 ppm in sterile deionised water.

Both stock solutions were then diluted down further in sterile deionised water to concentrations of 0.1, 1.0 and 10.0 ppm. These concentrations of the two products were then incorporated into pour plates (petri dishes) of PDA. Three plates of each dilution of the standard Tecto and the other three TBZ formulations were poured for both pathogens. PDA plates without fungicide were also poured as control plates. The pour plates were allowed to stall and set prior to seeding with the pathogens. The stock cultures of the two pathogens were prepared for this evaluation by plugging 9 mm agar plugs of the pathogens on the PDA culture plates by means of a cork borer. The PDA petri dishes incorporating the three thiabendazole formulations were then seeded with the two pathogens by placing a single 9 mm plug of each of the pathogens in the centre of three PDA plates of each concentration of the fungicides. Three control plates, per fungicide, were seeded likewise. The plates were then incubated at 25°C until such time as the control cultures had completely grown over the entire surface of the petri dishes. The diameter growth of the pathogens on each plate was then measured in millimetres (mm) and the average growth for the three plates for each concentration, recorded as average mm growth. The percentage inhibition of the pathogens by the fungicides was calculated and the following results were recorded.

Results

The results in the tables below indicate effective inhibition of the two latent citrus pathogens by the three thiabendazole formulations. These results compare favourably with the standard thiabendazole formulation.

Table 4.6.4.1. Villa thiabendazole.

Concentration. (ppm) a.i.	Growth in mm			
	Standard TBZ		Villa TBZ	
	Diplodia	Anthracnose	Diplodia	Anthracnose
Control x 3	51,0	51,0	51,0	51,0
0,1	44,0	41,0	20,0	19,0
0,1	40,0	43,0	25,0	21,0
0,1	48,0	43,0	24,0	14,0
Ave	44,0	42,3	23,0	18,0
1,0	2,0	0	6,0	0
1,0	0	0	7,0	0
1,0	4,0	0	3,0	0
Ave	2,0	0	5,3	0
10,0	0	0	0	0
10,0	0	0	0	0
10,0	0	0	0	0

Ave	0	0	0	0
% INHIBITION				
0,1	13,7	17,1	54,9	64,7
1,0	95,5	100	89,6	100
10,0	100	100	100	100

Table 4.6.4.2. ICA thiabendazole.

Growth in mm				
Concentration. (ppm) a.i.	Standard TBZ		ICA TBZ	
	Diplodia	Anthraco ⁿ ose	Diplodia	Anthraco ⁿ ose
Control x 3	51,0	51,0	51,0	51,0
0,1	44,0	41,0	41,0	42,0
0,1	40,0	43,0	44,0	43,0
0,1	48,0	43,0	47,0	45,0
Ave	44,0	42,3	44,0	43,3
1,0	2,0	0	2,0	0
1,0	0	0	3,0	0
1,0	4,0	0	2,0	0
Ave	2,0	0	2,3	0
10,0	0	0	0	0
10,0	0	0	0	0
10,0	0	0	0	0
Ave	0	0	0	0
% INHIBITION				
0,1	13,7	17,1	13,7	15,7
1,0	95,5	100	95,5	100
10,0	100	100	100	100

Table 4.6.4.3. Molenaar thiabendazole.

Growth in mm				
Concentration. (ppm) a.i.	Standard TBZ		Molenaar TBZ	
	Diplodia	Anthraco ⁿ ose	Diplodia	Anthraco ⁿ ose
Control x 3	51,0	51,0	51,0	51,0
0,1	44,0	41,0	49,0	51,0
0,1	40,0	43,0	51,0	51,0
0,1	48,0	43,0	49,0	51,0
Ave	44,0	42,3	49,7	51,0
1,0	2,0	0	2,0	0
1,0	0	0	2,0	0
1,0	4,0	0	2,0	0
Ave	2,0	0	2,0	0
10,0	0	0	0	0
10,0	0	0	0	0
10,0	0	0	0	0
Ave	0	0	0	0
% INHIBITION				
0,1	13,7	17,1	2,5	0
1,0	95,5	100	96,1	100
10,0	100	100	100	100

In vivo evaluation

Materials and methods

Good, sound, untreated navel oranges (Crocodile Valley Citrus Co.) and lemons (Larten Estates) were obtained for the purpose of this evaluation. Blemish-free fruit was selected and randomised. Fruit with non-abscised, live green calyxes were selected. The fruit was then divided up into lots of 20 fruit each per treatment. Thereafter all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was allowed to dry prior to treatment.

The following treatments were conducted.

1. Untreated control dipped in clean water.
2. Treated control – Standard Tecto 500 SC water dip at 2000 ppm.
3. Treated control – Standard Tecto 500 SC wax dip at 4000 ppm.
4. Villa Tecto 500 SC water dip at 2000 ppm.
5. ICA Tecto 500 SC water dip at 2000 ppm.
6. Molenaar Tecto 500 SC water dip at 2000 ppm.
7. Villa Tecto 500 SC wax dip at 4000 ppm.
8. ICA Tecto 500 SC wax dip at 4000 ppm.
9. Molenaar Tecto 500 SC wax dip at 4000 ppm.

Three trials were conducted with the three test thiabendazole formulations, i.e. two with navel oranges and one with lemons. All concentrations designated ppm refer to the a.i. of the product. Each treatment was dipped in water at ambient temperature and wax for three minutes. The wax treatments were allowed to dry overnight. The treatments were then stored in paper packets under simulated shipping conditions at 8°C for 6 weeks, and then at ambient (20°C) for 1 week.

After simulated shipping storage the treatments were evaluated by determining the incidence of either or both of the two latent pathogens by means of isolations from the calyx material from the fruit onto Potato dextrose agar (PDA). The isolations were incubated at 25°C for 7-10 days. Thereafter the percentage infection/control was calculated for each treatment.

A representative sample of 10 fruit per treatment (50%) was used for pathogen isolation purposes. The following results were recorded.

Results

Navel oranges were also treated with 8000 ppm ICA thiabendazole in the wax for the purpose of determining the phytotoxicity status on the fruit. The standard recommended Tecto (Control) was also applied at the rate of 8000 ppm in the wax. No phytotoxicity was observed on any of the treated fruit after 14 days incubation of the at ambient temperature.

Table 4.6.4.4. The effect of Villa Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated lemons.

Lemons	
Treatments (a.i.)	% Decay
1. Untreated control	Nil
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. Villa Tecto – 2000 ppm water dip	Nil
5. Villa Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.5. The effect of Villa Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navel oranges.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	90
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	10

4. Villa Tecto – 2000 ppm water dip	Nil
5. Villa Tecto – 4000 ppm wax dip	20

Table 4.6.4.5. The effect of Villa Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navel oranges.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	80
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. Villa Tecto – 2000 ppm water dip	Nil
5. Villa Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.6. The effect of ICA thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated lemons.

Lemons	
Treatments (a.i.)	% Decay
1. Untreated control	Nil
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. ICA Tecto – 2000 ppm water dip	Nil
5. ICA Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.7. The effect of ICA thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navels.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	90
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	10
4. ICA Tecto – 2000 ppm water dip	Nil
5. ICA Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.8. The effect of ICA thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navel oranges.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	80
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. ICA Tecto – 2000 ppm water dip	Nil
5. ICA Tecto – 4000 ppm wax dip	10

Table 4.6.4.9. The effect of Molenaar Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated lemons.

Lemons	
Treatments (a.i.)	% Decay
1. Untreated control	Nil
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. Molenaar Tecto – 2000 ppm water dip	Nil
5. Molenaar Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.10. The effect of Molenaar Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navel oranges.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	90
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	10
4. Molenaar Tecto – 2000 ppm water dip	Nil
5. Molenaar Tecto – 4000 ppm wax dip	Nil

Table 4.6.4.11. The effect of Molenaar Thiabendazole on the latent pathogens (Anthracnose and Diplodia) on treated navel oranges.

Navels	
Treatments (a.i.)	% Decay
1. Untreated control	80
2. Treated control – Std. Tecto 2000 ppm water dip	Nil
3. Treated control – Std. Tecto 4000 ppm wax dip	Nil
4. Molenaar Tecto – 2000 ppm water dip	Nil
5. Molenaar Tecto – 4000 ppm wax dip	10

Discussion

Good control of both pathogens by the three thiabendazole formulations compared to the standard recommended formulation is indicated in the *in vitro* evaluation, i.e. 95.5-100% inhibition at 1.0 ppm and 100% inhibition at 10.0 ppm.

In the above *in vivo* evaluations the lemons used were harvested from young vigorous trees. Neither of the latent pathogens were quiescent in and around the calyx area of the fruit due to low or nil spore load, therefore no pathogen was isolated from these lemons. On the other hand, the pathogen Anthracnose was cultured from all the isolations made from the navel oranges in the other two trials. These navels were old fruit and less vigorous, which predisposed the fruit to possible infection by either one or the other, or both latent pathogens. In this instance Anthracnose was quiescent in and around the calyx area of the navel oranges. The above results of the two navel trials indicated good control of this pathogen by the three thiabendazole formulations compared to the standard formulation.

The above *in vivo* treatments were conducted with the thiabendazole in a water dip as well as in a wax treatment.

Conclusion

The three formulations of thiabendazole submitted by Villa Crop Protection, ICA International Chemicals and Molenaar, demonstrated good control of the citrus pathogens, *c. gloeosporioides* and *D. natalensis*, compared to the standard thiabendazole. These products can therefore be recommended by CRI for registration as a wax application at a concentration of 4000 g/l.

4.6.5 The evaluation of the potentially new post-harvest fungicide Fludioxonil against the post-harvest citrus pathogen, *P. digitatum*

Opsomming

'n Potensieële nuwe naoes swamdoder, Fludioxonil vanaf Syngenta, is ge-evalueer vir effektiwiteit teen die naoes patogeen *P. digitatum*. Die evaluering en herevaluering van die middel het nie aanvaarbare beheer van dié patogeen gewys nie, in vergelyking met die standard Fungazil 750 WSP nie. Verdere proewe met dié middel sal uitgeoef word.

Introduction

A new generation of post-harvest fungicides, fludioxonil from Syngenta, and pyrimethanil from Janssen Pharmaceutica, are currently being evaluated in the USA for the purpose of eventual registration against post-harvest citrus pathogens. These compounds have been classified in the USA by the EPA as safe and

reduced risk chemicals. Both these chemicals have been reported to effectively control green mould (*P. digitatum*), however, it is reported that the new chemicals appear less effective than imazalil and TBZ for the control of green mould and stem-end rot respectively.

Fludioxonil was obtained from Syngenta during 2004 and the first trials have already been done, indicating limited success against the control of green mould (*P. digitatum*) when applied in a water dip treatment and incorporated into citrus wax. This evaluation has now been repeated.

Materials and method

A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

Fludioxonil was evaluated in a hot water bath dip treatment at 40°C, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated after inoculation (to simulate a short delay after harvesting prior to packhouse treatments) with the chemical compound being evaluated. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Inoculated fruit was also treated with fludioxonil incorporated in a citrus wax by dipping the fruit in the mixture for 3 minutes. The inoculated fruit was also treated 4 hours after inoculation, as above. After treatment the wax was allowed to dry on the treated fruit overnight. The fruit was then incubated in paper packets as above and evaluated and the results recorded in the same way as for the water dip treatments.

Treatments

Dip treatments

1. Untreated control (*P. digitatum*).
2. Treated control – 500 g/kg Fungazil 750 WSP.
3. Fludioxonil – 500 g/l.
4. Fludioxonil – 1000 g/l.
5. Fludioxonil – 2000 g/l

Wax treatments

1. Untreated control (*P. digitatum*).
2. Treated control – 3000 g/l Fungazil 800 EC.
3. Fludioxonil – 3000 g/l.
4. Fludioxonil – 4000 g/l.

All concentrations designated g/kg or g/l refer to a.i. of the product.

Results

No phytotoxicity was evident on the fruit treated at the highest concentrations of Fludioxonil in the dip (2000 g/l) and wax (4000 g/l) treatments.

Table 4.6.5.1. The effect of Fludioxonil on *P. digitatum* in a water dip treatment.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control 500 g/kg Fungazil 750 WSP	Nil
3. Fludioxonil 500 g/l	80
4. Fludioxonil 1000 g/l	80
5. Fludioxonil 2000 g/l	70

Table 4.6.5.2. The effect of Fludioxonil on *P. digitatum* in a wax treatment.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control 3000 g/l Fungazil 800 EC	10
3. Fludioxonil 3000 g/l	100
4. Fludioxonil 4000 g/l	80

Conclusions

Fludioxonil demonstrated poor control of the pathogen *P. digitatum* compared to the standard Fungazil sulphate 750 WSP and Fungazil 800 EC. These trials will be repeated using the same citrus cultivar as above and different citrus cultivars.

4.6.6 The screening of organic compounds, Citrofresh, DM31 and KanLife, against post-harvest fungal pathogens causing diseases of citrus fruits

Opsomming

Drie organiese middels, Citrofresh, DM31 en KanLife is ge-evalueer vir swamdoerende eienskappe teen die sitruspatogeen, *P. digitatum* en *Geotrichum candidum* (suurvrot). Nie een van die drie middels het die twee patogeen infeksie beheer nie.

Introduction

The organically certified compounds, Citrofresh, DM31 and KanLife, were screened *in vivo* against the post-harvest citrus pathogens *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot) to determine whether the products have any fungicidal properties. The post-harvest fungicides, imazalil sulphate and guazatine which are recommended for the control of these two pathogens were used as the treated controls at the standard rates of 500 ppm a.i. and 1000 ppm a.i., respectively.

Materials and methods

Spore suspensions of both of these pathogens were made up by suspending spores, grown on Potato Dextrose agar plates, in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 10^6 spores / ml spectrophotometrically.

Good, sound, untreated Valencia and navel oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 10 fruit each, per treatment, and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially (20 inoculations per treatment) on opposite sides to each other. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

All the treatments were dipped in water at ambient temperature. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds. Each treatment was immersed in the water bath for 3 minutes. After treatment the fruit was incubated in paper packets as well as plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

No phytotoxicity was observed at any of the concentrations of Citrofresh or DM31 evaluated.

Table 4.6.6.1. The effect of Citrofresh on *P. digitatum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control (500 ppm Imazalil sulphate)	Nil
3. 2% Citrofresh	100
4. 3% Citrofresh	100
5. 4% Citrofresh	100
6. 5% Citrofresh	100

Table 4.6.6.2. The effect of Citrofresh on *G. candidum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	90
2. Treated control (1000 ppm guazatine)	Nil
3. 2% Citrofresh	100
4. 3% Citrofresh	100
5. 4% Citrofresh	100
6. 5% Citrofresh	100

Table 4.6.6.3. The effect of DM31 on *P. digitatum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control (500 ppm Imazalil sulphate 750 WSP)	Nil
3. 250 ppm DM31	100
4. 500 ppm DM31	100
5. 1000 ppm DM31	100

Table 4.6.6.4. The effect of DM31 on *G. candidum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control	100
2. Treated control (1000 ppm guazatine)	Nil
3. 250 ppm DM31	100
4. 500 ppm DM31	100
5. 1000 ppm DM31	100

Table 4.6.6.5. The effect of KanLife on *P. digitatum*.

NAVELS	
Treatments	% Decay
1. Untreated control	100
2. Treated control - 500 ppm Imazalil sulphate 750 WSP	Nil
3. KanLife 2.5 ml/l	100

4. KanLife 5 mℓℓ	100
5. KanLife 10 mℓℓ	100

Table 4.6.6.6. The effect of KanLife on *G. candidum*

NAVELS	
Treatments	% Decay
1. Untreated control	100
2. Treated control - 1000 ppm guazatine	Nil
3. KanLife 2.5 mℓℓ	100
4. KanLife 5 mℓℓ	100
5. KanLife 10 mℓℓ	100

Conclusion

As can be seen from the above results, the three organic compounds did not inhibit the two post-harvest citrus pathogens at any of the concentrations evaluated, thus demonstrating no fungicidal properties.

4.6.7 **The *in vitro* and *in vivo* screening of the quaternary ammonium compounds, Sanicide and Ambicide, against the post-harvest diseases of citrus green mould, *Penicillium digitatum*, and sour rot, *Geotrichum candidum*, to determine the efficacy of the compound in killing the fungal spores**

Opsomming

Twee nuwe formulasies van kwaternêre ammonium verbinding middels, Sanicide en Ambicide, is ge-evalueer *in vitro* en *in vivo* teen *P. digitatum* en *G. candidum*. Die twee kwats het geen swamdodende eienskappe vir die beheer van die twee patogene getoon nie. Nietemin, dié twee middels het gewys dat hulle wel doeltreffende ontsmettingsmiddels is deurdat hulle oppervlakkige swamspore van die twee patogene op die vrugskil in oplossing doodmaak. Loodsproewe sal moet in pakhuis dompelbaddens gedoen word om die produk konsentrasie toetsapparaat te evalueer voor die produkte aanbeveel word vir gebruik as saniteermiddels in sitruspakhuis dompelbaddens.

A. *In vitro* evaluation

Materials and methods

In order to study the effect of Chlorine and Sanicide on germination of *P. digitatum* and *G. candidum*, spore suspensions of both these organisms were made by suspending spores, grown on Potato dextrose agar plates, in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores/mℓ spectrophotometrically.

A stock solution of chlorine (HTH) was made up at a concentration of 200 ppm a.i. (i.e. available chlorine) as recommended for citrus packhouses. A stock solution of Sanicide was made up at a concentration of 150 mℓ/100 ℓ water (1500 ppm product) for the purpose of this evaluation.

For this evaluation, 1 mℓ of each fungal spore suspension was added to 9 mℓ of chlorine stock solution as well as to 9 mℓ of solution of the compound being evaluated. After the fungal spores had been exposed to the chlorine for time periods of 10 sec., 30 sec., 1 min., 5 min., and 10 mins, a 1 mℓ sample from each dilution was added separately to 0.1 mℓ of 0.1 molar Sodium thiosulphate in order to then inactivate the chlorine. Thereafter, 1mℓ from each inactivated chlorine suspension was added to a PDA plate. Similarly a 1 mℓ sample from each time exposure of the test compound to the fungal spores was added to a PDA plate. A 1 mℓ sample of spore suspension was also added to PDA plates as controls.

All media plates were incubated at 25°C for 7 days or until such time as the controls grew and the results were then recorded as spore germination or no spore germination.

Results

Sanicide and Ambicide demonstrated good fungal spore kill qualities of both post-harvest citrus pathogens compared to the control, chlorine. Spore germination did however occur with both pathogens at the lower exposure rates as seen in the results. It is always advisable to use a longer exposure period in a citrus packhouse dumptank system.

Table 4.6.7.1. The effect of Sanicide on spore germination of *G. candidum*.

Product name	Concentration	Exposure time (sec.)	Spore germination
Sanicide	1500 ppm product	10	+
		30	-
		60	-
		300	-
		600	-
Chlorine	200 ppm a.i.	10	+
		30	-
		60	-
		300	-
		600	-
<i>G. candidum</i> control – positive + = spore germination - = no spore germination			

Table 4.6.7.2. The effect of Sanicide on spore germination of *P. digitatum*.

Product name	Concentration	Exposure time (sec.)	Spore germination
Sanicide	1500 ppm product	10	+
		30	-
		60	-
		300	-
		600	-
Chlorine	200 ppm a.i.	10	-
		30	-
		60	-
		300	-
		600	-
<i>P. digitatum</i> control – positive + = spore germination - = no spore germination			

Table 4.6.7.3. The effect of Ambicide on spore germination of *G. candidum*.

Product name	Concentration	Exposure time (sec.)	Spore germination
Ambicide	1500 ppm product	10	+
		30	-
		60	-
		300	-
		600	-
Chlorine	200 ppm a.i.	10	-
		30	-
		60	-
		300	-
		600	-
<i>G. candidum</i> control – positive + = spore germination - = no spore germination			

Table 4.6.7.4. The effect of Ambicide on spore germination of *P. digitatum*.

Product name	Concentration	Exposure time (sec.)	Spore germination
Ambicide	1500 ppm product	10	+
		30	+
		60	-
		300	-
		600	-
Chlorine	200 ppm a.i.	10	-
		30	-
		60	-
		300	-
		600	-
<i>P. digitatum</i> control – positive + = spore germination - = no spore germination			

Conclusion

Prior to this product or suchlike sanitizing product being recommended for use in citrus packhouse fruit washing systems, a pilot trial will have to be conducted to evaluate the monitoring of the product concentration (reliable test kit) and correlating these readings with the micro-organism counts in water samples from the system.

One of the criteria for use of such a sanitizing product in a citrus packhouse washing system is a reliable titration type test kit (not dipsticks) to be able to measure the product concentration, at the recommended concentration for effective fungal spore kill, and to then top the system up with the correct concentration, as the product is lost out of the system.

B. *In vivo* evaluation

Materials and methods

Sanicide and Ambicide were also evaluated for any phytotoxic reaction on the fruit. Spore suspensions of both of these pathogens were made up by suspending spores grown on Potato Dextrose agar plates, in

sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores /ml, spectrophotometrically.

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other. Each treatment thus consisted of 40 inoculation sites. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

Treatments

The fruit was treated with the following compounds at the given (recommended) concentrations.

Imazalil sulphate WSP	-	500 ppm a.i.
Deccotine (guazatine)	-	1000 ppm a.i.
Sanicide & Ambicide	-	1000 ppm product
		1500 ppm product (recommended concentration)
		2000 ppm product

All the treatments were dipped in water at ambient temperature for 3 minutes. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds.

After treatment the fruit was incubated in paper packets as well as plastic bags at 20°C for 7-10 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

No phytotoxicity was observed in any of the fruit treated with the three concentrations of Sanicide and Ambicide. However, prior to final recommendation of the product for use in a citrus dump tank washing system, the phytotoxicity of the product will have to be evaluated on citrus that is exposed to the product for an extended period of time, at the recommended concentration, as all the other quats evaluated "burn" sensitive fruit upon longer than normal exposure to the product in such a system. Sanicide and Ambicide have, however, good sanitizing properties but cannot be recommended for use in a dumptank system at this stage as an appropriate test kit to measure the product concentration is not available. Once an appropriate test kit is available this kit will have to be evaluated in a pilot trial in a citrus packhouse dump tank washing system.

Table 4.6.7.5. The effect of Sanicide on *P. digitatum* inoculated into Valencia oranges.

Treatments	% Infected fruit
1. Untreated control	100
2. Treated control – 500 ppm Imazalil sulphate	Nil
3. Sanicide – 1000 ppm product	100
4. Sanicide – 1500 ppm product	100
5. Sanicide – 2000 ppm product	100

Table 4.6.7.6. The effect of Sanicide on *G. candidum* inoculated into Valencia oranges.

Treatments	% Infected fruit
1. Untreated control	90
2. Treated control – 1000 ppm guazatine	Nil
3. Sanicide – 1000 ppm product	100
4. Sanicide – 1500 ppm product	100
5. Sanicide – 2000 ppm product	100

Table 4.6.7.7. The effect of Ambicide on *P. digitatum* inoculated into Valencia oranges.

Treatments	% Infected fruit
1. Untreated control	100
2. Treated control – 500 ppm Imazalil sulphate	Nil
3. Ambicide – 1000 ppm product	100
4. Ambicide – 1500 ppm product	100
5. Ambicide – 2000 ppm product	100

Table 4.6.7.8. The effect of Ambicide on *G. candidum* inoculated into Valencia oranges.

Treatments	% Infected fruit
1. Untreated control	100
2. Treated control – 500 ppm guazatine	Nil
3. Ambicide – 1000 ppm product	100
4. Ambicide – 1500 ppm product	100
5. Ambicide – 2000 ppm product	90

Conclusion

Sanicide and Ambicide did not demonstrate any fungicidal activity against either of the citrus pathogens.

4.6.8 Evaluation of the two sanitizing agents, Sterifect and Prasin (a quaternary ammonium compound) in a hot water fungicide bath

Opsomming

Twee saniteermiddels, Sterifect en Prasin ('n kwaterenere ammonium verbinding) is ge-evalueer in 'n warmwater bad om die moontlike effektiwiteit van die produkte te bepaal om die sisteem (die bad) steriele en skoon te hou sonder die opbou van mikro-organismes. Geen waardevolle gevolgtrekkings kon bepaal word van enige van die vier proewe op dié twee middels. Resultate was te wisselvallig en die noodsaaklike vermindering in swamspoorlading is nie bereik nie.

Introduction

Sterifect, an electrically charged water product (oxidant) and Prasin, a quat, are two sanitizing agents currently being evaluated in a hot water bath to determine the possible efficacy of the products in maintaining a sterile system, free of micro-organism build-up and then, secondly, if the products demonstrate efficacy in maintaining a sterile, clean system, to screen the two products in combination with the fungicides to determine compatibility and evaluate for possible phytotoxicity.

Materials and methods

A spore suspension of this pathogen was made up by suspending spores in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 1×10^6 spores / ml spectrophotometrically.

Good, sound, untreated Valencia oranges from Crocodile Valley Citrus Co. were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other, giving a total of 40 inoculation sites or injury sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

All the treatments were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse.

4.6.9 The effect of citrus waxes applied post-harvest to citrus sprayed with Kaolin in the orchards for the prevention of sunburn

Opsomming

'n Kaolin-sitruswaks pakhuis proef is uitgevoer op kaolin besproeide Star Ruby pomelos. Die doel van hierdie proef was om die effek van kaolienresidu op die vrugte op die waksaanwending op die vrugte te bepaal. Star ruby pomelobome is met kaolien gespuit vir die voorkoming van sonbrand op die vrugte. Die vrugte is geoes en dan behandel op die paklyn by CRI. Hier is die vrugte eers gewas deur die hoëdrukspuit met 'n doeltreffende saniteermiddel, en dan beweeg deur die warmwaterbad (40°C) en dan afgedroog in die droogtonnel. Carnaubawaks is daarna op die vrugte aangewend. Na droging van die waks is die vrugte verpak en opgeberg onder gesimuleerde verskepingstoestande.

Die vermoede afbraak van die waks op die vrugte na verskeping, as gevolg van die kaolienresidu op die vrugte, is nie waargeneem nie. Die moontlike rede hiervoor is dat die kaolienresidu is van die vrugoppervlak afgewas deur die hoëdrukspuit en warmwaterbad.

Dié proef is herhaal op Valencia lemoene vanaf Crocodile Valley Sitrus Maatskappy. Die spuitbehandeling van die vrugte by CRI is soos in die vorige proef uitgevoer. Die vrugte is dan behandel met verskeie Carnauba en polyetileenwakse en opgeberg onder verskepingstoestande soos in die vorige proef. Na opberging is die afbraak van die waks weer nie waargeneem nie soos in die vorige proef.

Introduction

Kaolin is a compound sprayed on citrus fruit pre-harvest for the prevention of sunburn during extremely hot conditions in certain production areas. It has been reported that the presence of kaolin on the fruit leads to a breakdown of the citrus wax formulation on the fruit. The purpose of this work was to determine the effect of kaolin residue, on the fruit, on the waxing of the fruit.

Materials and methods

In the first trial Kaolin was sprayed on trial trees of Star ruby grapefruit at Tomahawk Estates in the Onderberg. The fruit was harvested and treated at CRI on the packline. Here the fruit was washed in the high pressure spray with a suitable sanitizer (Prasin, a quat), exposed to the hot water bath (40°C) for 3 minutes and then dried.

The fruit was then waxed with a Carnauba wax in a three minute dip treatment. The waxed fruit was left to dry overnight and then packed into cartons and stored under simulated shipping conditions for 4 weeks at 8°C.

Results

After simulated shipping the reported wax breakdown was not observed visually on this fruit.

Conclusion

The possible reason for the good uniform application of the wax, after shipping, was that the kaolin residue on the fruit was suitably washed off during the washing process in the packline and also on exposure to the hot water bath.

A larger scale trial was repeated on Valencia oranges on trees sprayed at Crocodile Valley Citrus Co. The materials and methods were the same as for the first trial except that in this case the fruit was waxed with a range of carnauba and polyethylene waxes. The wax treatments were as follows:

1. Carnauba Tropical – Sasol wax.
2. Stafresh – FMC wax (polyethylene).
3. Carnauba Natural – Sasol wax.
4. Carnauba citrus – Sasol wax.
5. Quick Dry Poly – Sasol wax.
6. Qualifresh (Carnauba) – Novon wax.
7. Deccowax – Citrashine wax (polyethylene) – Control.

Results

The breakdown of the wax after simulated shipping was also not evident on the Valencia oranges as in the first trial. A good even distribution of the wax was still seen on the fruit.

Conclusion

Sufficient evidence was obtained in these two trials to indicate that the kaolin was adequately washed off the surface of the fruit and a good even distribution of wax was retained.

4.6.10 The evaluation of the efficacy of guazatine formulated into citrus waxes (i.e. CitriWax and Deccowax)

Opsomming

CRI is deur die Spaanse sitrusbedryf in kennis gestel dat die effektiwiteit van guazatine, geformuleer in sitruswaxse (Deccowaks en Citriwaks), afneem sodra die guazatine-waks formulاسie verouër oor 'n tydperk. Sitrusnavorsers in Australië het ook geraporteer dat guazatine vinnig afbreek tydens meng in hoë alkaliese mengsels (wakse). Hierdie moontlike afbraak van guazatine word tans opgevolg deur proewe op nuutgeformuleerde Citriwaks om te bepaal hoe lank na formulering van die mengsel die guazatine se effektiwiteit afneem. Die eerste evaluasie het volle effektiwiteit van die guazatine getoon.

Introduction

It has been indicated to CRI via the Spanish citrus industry that the efficacy of guazatine, formulated into citrus waxes, decreases as the guazatine-wax formulation ages over a period of time. This scenario is being investigated at present on a newly formulated batch of Citriwax, in order to determine after what period this breakdown in efficacy of guazatine occurs. This information will then be conveyed to the industry. The producers will then be advised as to how soon such a drum of wax needs to be used up before a loss of efficacy of guazatine occurs.

The first evaluation of this Citriwax has been conducted for efficacy of the guazatine against the two citrus pathogens *P. digitatum* (green mould) and *G. candidum* (sour rot).

Materials and methods

Spore suspension of both pathogens were made up by suspending spores in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good sound, untreated Valencia oranges (Crocodyle Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 10 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

The inoculated fruit was treated 4 hours after inoculation by dipping the fruit in the CitriWax for 3 minutes. After treatment the fruit was incubated in paper packets and plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Treatments

1. Untreated control (*P. digitatum*).
2. Treated control 500 g/kg fungazil 750 WSP.
3. Untreated control (*G. candidum*).
4. Treated control 1000 g/l guazatine.
5. Guazatine-Wax 3000 g/l (*P. digitatum*).
6. Guazatine-Wax 3000 g/l (*G. candidum*).

Results

The first evaluation of this wax-guazatine formulation, CitriWax, indicated 100% control of the two pathogens by the guazatine.

Table 4.6.10.1. The effect of guazatine in citrus wax on *P. digitatum* and *G. candidum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control (<i>P. digitatum</i>)	100
2. Treated control 500 g/kg Fungazil 750 WSP	Nil
3. Untreated control (<i>G. candidum</i>)	100
4. Treated control 1000 g/l guazatine	10
5. Guazatine wax 3000 g/l (<i>P. digitatum</i>)	Nil
6. Guazatine wax 3000 g/l (<i>G. candidum</i>)	Nil

Conclusion

This wax will continue to be screened at 3-4 weekly intervals until such time as breakdown and decrease in efficacy of the guazatine is detected.

4.6.11 The *in vivo* screening of *P. digitatum* (green mould) spores for resistance to the post-harvest fungicide Imazalil

Opsomming

Een-en-dertig swamspoor monsters van *P. digitatum*, geisoleer vanaf navel en Valencia lemoene en suurlemoene en vanaf ander sitruskultivars vanuit die Oos- en Wes-Kaap en Mpumalanga produksie gebiede, is ge-evalueer teen imazalil vir bestandheid. Vier van die monsters vanaf die Oos-Kaap het bestaande spore gewys. Dié spore is ge-evalueer teen die ander naoes swamdoders sowel as teen 'n hoër konsentrasie van imazalil om 'n strategie van behandeling te kan bepaalin die pakhuis om die bestaande spore te kan vernietig tydens die nuwe produksie seisoen. Al die ander swamspoormonsters is sensitief vir imazalil.

Introduction

The screening of post-harvest pathogens for resistance to the post-harvest fungicides is done randomly on an ongoing basis from season to season. Thirty-one fungal spore samples of *P. digitatum*, isolated from navel and Valencia oranges and lemons and also isolated from undisclosed citrus cultivars, from production areas in the Eastern and Western Cape and Mpumalanga, were screened for resistance to the post-harvest fungicide, imazalil.

Materials and methods

Spore suspensions of all the samples were made up by suspending the spores in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

Blemish-free, sound untreated navel and Valencia oranges (Crocodile Valley Citrus Co.) were selected and randomised for inoculation. The fruit was divided up into lots of 10 fruit per treatment. All the fruit was washed in clean water and surface sterilized by dipping into a 1% NaOCl solution for a few minutes. The fruit was allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides to each other. Each injury was then infected with the spores being screened by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette. The inoculated fruit was then treated 4 hours after inoculation.

After treatment the fruit was incubated in paper packets at 20°C for 7 days or until the untreated controls had grown. The treatments were evaluated and the results recorded as percentage decay. All the treatments (excluding the controls) were treated with 500 g/kg imazalil sulphate.

Results

P. digitatum spores screened for resistance.

TREATMENTS		% DECAY
1.	Untreated control Kirkwood orchard	100 Nil
2.	Untreated control Kirkwood – after packing	100 Nil
3.	Untreated control Hermitage orchard	100 Nil
4.	Untreated control ALG, Citrusdal	100 Nil
5.	Untreated control Citrus Select ALG Citrusdal	100 Nil
6.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
7.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
8.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
9.	Untreated control Stellenpack	Samples contaminated
10.	Untreated control Stellenpack	
11.	Untreated control Stellenpack	
12.	Untreated control Patensie Co-op, Britz	100 80
13.	Untreated control Patensie Co-op, reject fruit	100 70
14.	Untreated control Patensie Co-op crates	100 90
15.	Untreated control Patensie Co-op crates	100 30
16.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
17.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
18.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
19.	Untreated control Crocodile Valley Citrus Co. - Valencias	100 Nil
20.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
21.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
22.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil
23.	Untreated control Crocodile Valley Citrus Co. - navels	100 Nil

24.	Untreated control Crocodile Valley Citrus Co. - Valencias	100 Nil
25.	Untreated control Crocodile Valley Citrus Co. - Valencias	100 Nil
26.	Untreated control Larten Estate - Lemons	100 Nil
27.	Untreated control Larten Estate - Lemons	90 Nil
28.	Untreated control Larten Estate - Lemons	100 Nil
29.	Untreated control Tomahawk (Onderberg) – Star Ruby grapefruit	100 Nil
30.	Untreated control Tomahawk (Onderberg) – Star Ruby grapefruit	100 Nil
31.	Untreated control Tomahawk (Onderberg) – Star Ruby grapefruit	100 Nil

Conclusion

Resistant spores were detected in the four spore samples (*P. digitatum*) from the Eastern Cape production area. These spores were screened further against the existing post-harvest fungicides, Tecto 500 and guazatine, as well as increased concentrations of imazalil for the purpose of recommending a strategy in the specific packhouse to eradicate any resistant spores. A higher concentration of imazalil (1000 ppm), as well as guazatine at the standard concentration, eliminated the resistant spores in these four spore samples. All the other spore samples submitted for screening were sensitive to imazalil.

4.6.12 CRI Diagnostic Centre (Laura Huisman and Timothy Zulu)

Diagnostic tests conducted

	Citrus Nurseries	Commercial samples	Contract work	Research samples	Other crops
Nematode :Roots		366	183	1196	26
Soil		28	111	1116	122
<i>Phytophthora</i>	1198	243			141
Nursery water	78				
Black spot resistance to benzimidazole		7			
Redscale resistance to organophosphate		7			
Packhouse water		2			
Internal fruit quality		32			

Citrus Accredited Nurseries

There are currently 19 accredited citrus nurseries countrywide. It is compulsory for the accredited nurseries to submit *Phytophthora* samples on a quarterly basis. Of the 1198 samples received, 8,8% tested positive for *Phytophthora*.

Commercial Samples

Samples were sent to the DC from most of the citrus growing areas. In 65% of the nematode samples analysed, the citrus nematode female counts per 10 grams of roots exceeded the threshold value. Forty percent of the *Phytophthora* soil samples tested positive. Seven samples were received for black spot resistance tests against benzimidazole and seven for redscale resistance test against organophosphates.

5 PROGRAMME: CROP LOAD AND FRUIT QUALITY MANAGEMENT

5.1 PROGRAMME SUMMARY

By Tim G. Grout (Research & Technical Manager: CRI)

Although one of the three projects within this programme deals specifically with fruit quality enhancement, all research was either directly or indirectly involved in improving the quality and therefore marketability of southern African citrus. Although previous results with MAP for lowering fruit acidity had appeared promising, the 2004 results were not and the approach to this research will therefore need to be broadened. Negative results were also obtained in the attempt to elongate lemons by the use of gibberellic acid. However, double sprays of kaolin (Surround) were successful in not only eliminating sunburn from Satsuma mandarins but also increasing sugar content. Rather than cool night temperatures improving colour by directly causing chlorophyll breakdown and carotenoid synthesis it now appears that cool temperatures stop vegetative growth and this indirectly improves colour development. However, research with gibberellin biosynthesis inhibitors that should suppress vegetative growth did not produce very promising results. Although fertigation provides various production-related improvements, it can also result in delayed colouration. Studies are being conducted to overcome this draw-back but results this year were inconclusive.

Most funding in this programme went towards research on rind condition and the progress achieved continued to emphasise the complex interaction of both pre- and post-harvest factors. Preliminary results with creasing research confirmed known relationships with calcium and other minerals but results of foliar sprays of various nutrients and minerals will only be known in 2005. Research by both CRI and ExperiCo on rind breakdown confirmed that higher storage temperatures (7.5°C) resulted in more rind breakdown than lower storage temperatures (-0.6°C). Results also confirmed those found a few years ago that Nules Clementines were more susceptible to rind breakdown than Oroval, although the latter selection was more susceptible to puffiness. Techniques for anatomical research were developed that will be used further during 2005. Different researchers found different results when the susceptibility to rind breakdown of inside versus outside fruit was compared. Research on cooling rates and both land and sea transport resulted in some practical recommendations for reducing losses and further results concerning CO₂ levels are expected as this work continues.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

Alhoewel een van die drie projekte binne die program spesifiek handel oor verbetering van vrugkwaliteit, is alle navorsing direk of indirek gerig op die verbetering van kwaliteit en derhalwe bemerkbaarheid van suider-Afrika sitrus. Ofskoon vorige navorsing om vrugsuurheid te verlaag belowend gelyk het, was dit nie die geval in 2004 nie en die benadering tot hierdie navorsing sal dus verbreed moet word. Negatiewe resultate is ook verkry in 'n poging om suurlemoene te verleng met gibberellinesuur. Dubbel bespuitings met kaolien (Surround) het nietemin sonbrand by Satsuma mandarin voorkom en ook die suikerinhoud verhoog. Dit wil nou voorkom of koel nagtemperatuur vegetatiewe groei verminder en sodoende kleurontwikkeling indirek verbeter, eerder as direk deur chlorofil-afbraak en karotenoïedsintese. Navorsing met gibberellin-biosintese inhibeerders wat veronderstel is om vegetatiewe groei te onderdruk, het egter nie belowende resultate gelewer nie. Alhoewel bemesting d.m.v. besproeiing verskeie produksie-verbetering teweegbring, kan dit ook verkleuring vertraag. Studies word uitgevoer om hierdie nadeel te oorbrug, maar resultate vir die jaar was nie afdoende nie.

Meeste befondsing in hierdie program is toegewys aan navorsing oor skilkondisie en die vordering wat gemaak is beklemtoon die komplekse wisselwerking tussen beide voor- en na-oesfaktore. Voorlopige resultate met rimpel-navorsing het die bekende verwantskap met kalsium en ander minerale bevestig, maar resultate oor blaarbespuitings met verskeie voedinstowwe en minerale sal eers in 2005 bekend wees. Navorsing by beide CRI en ExperiCo op skilneerstorting het bevestig dat hoër bergingstemperatuur (7.5°C) meer skilneerstorting veroorsaak as laer temperatuur (-0.6°C). Resultate het ook vorige waarnemings bevestig dat Nules Clementines meer vatbaar is vir skilneerstorting as Oroval, hoewel laasgenoemde meer vatbaar is vir pofferigheid. Tegnieke vir anatomiese navorsing is ontwikkel wat gedurende 2005 verder gebruik sal word. Verskillende navorsers het verskillende resultate verkry met betrekking tot die vatbaarheid vir skilneerstorting van binnekantste en buitekantste. Navorsing op verkoelingstempo's en beide land- en seevervoer het gelei tot 'n aantal praktiese aanbevelings vir die vermindering van verliese en verdere resultate oor CO₂-vlakke word verwag namate die werk voortgaan.

5.2 PROJECT: FRUIT QUALITY ENHANCEMENT Project Co-ordinator: Graham H. Barry (CRI/SU)

5.2.1 Project summary

The requirement of meeting minimum quality specifications and being able to successfully market citrus fruit products has now been superseded by the necessity to produce a product of superior quality, in terms of appearance and eating quality. When the supply of citrus products exceeds demand, product differentiation becomes increasingly important to ensure that sales rates are maintained. The goal of Citrus Research International's Fruit Quality Enhancement project is to provide Southern African citrus producers with cultural practices that assist in producing superior product quality.

Sunburn of Satsuma mandarin (*Citrus unshiu* Marc.) fruit and fruit of other sunburn-susceptible cultivars of *Citrus* can result in commercially significant crop losses. Therefore, reducing sunburn incidence and severity, thereby improving packout, will have a direct and positive effect on improving grower returns. The application of Surround® particle (kaolin) film as an early, double spray during mid-summer effectively eliminated sunburn incidence on Miho Wase Satsuma mandarin fruit. In addition, sugar content was also higher in fruit from Surround®-treated trees (5.2.2).

During the 2003-04 season, various concentrations of MAP applied to Delta Valencia and Midnight Valencia oranges did not reduce acidity. This result is contrary to previous results where 1% MAP applied 6 weeks after full bloom did reduce TA. Double applications or combinations with other substances should be investigated in the future (5.2.3).

Premium prices are obtained in the Japanese market for elongated lemons with a length-to-diameter ratio (L:D) exceeding 1.25:1. Various GA sources (ProGibb®, Promalin®, Provide®, Perlan®, Falgro®) and a gibberellin biosynthesis inhibitor (Regalis®) were applied to Eureka lemon in the Gt. Drakenstein area of the Western Cape during flower differentiation (19 Jun. 2003) and 6 weeks after full bloom (mid-Nov. 2003). None of the treatments applied increased L:D ratio of similar sized lemon fruit. Some of the products applied resulted in leaf abscission (Ethrel) or reduced bloom (GAs), indicating that the chemical products did have a physiological effect (5.2.4).

Rind colour development of citrus is a problem in South Africa, especially for early maturing cultivars, because autumn temperatures are too high for chlorophyll breakdown and carotenoid synthesis. Citrus fruit require night temperatures of <13°C for optimal colour development. It is unlikely that cool night temperature *per se* is the cause of chlorophyll breakdown and carotenoid synthesis. Rather, the cool night temperature probably plays a direct role in halting vegetative growth. The latter is antagonistic to chloroplast conversion to chromoplasts, thereby delaying chlorophyll breakdown and carotenoid synthesis. Therefore, techniques to reduce vegetative vigour during late summer and early autumn may play a role in enhancing rind colour development (5.2.5).

Endogenous gibberellins promote vegetative growth in plants. Gibberellin biosynthesis inhibitors effectively reduce vegetative growth and, in turn, may promote the conversion of chloroplasts to chromoplasts. Previous exploratory research (2002) on Navelina Navel orange trees treated with prohexadione calcium (Ph-Ca), a new gibberellin biosynthesis inhibitor with less persistence than triazoles, had fruit that were better coloured than the control by more than half a colour plate. However, subsequent treatment of Palmer Navel orange with Ph-Ca did not significantly improve rind colour, although there was a trend towards enhanced colour (5.2.6). The objective of this research was to determine the dose response of citrus trees to Ph-Ca. An experimental orchard of Miho Wase Satsuma and Nules Clementine mandarins in Stellenbosch, and commercial orchards of Palmer Navel orange in Wellington and Eureka lemon in Gt. Drakenstein were used. Ph-Ca at 0, 100, 200, 400 and 800 ppm was applied as a foliar spray 3 months before anticipated harvest in late 2003/early 2004. The treatments did not improve rind colour as it is thought that treatment timing was not optimal.

The effects of tree nutrition and post-harvest treatments on concentration of the most important colour imparting carotenoids in physiologically mature citrus fruit forms the basis of the PhD research of Molipa Mosoeunyane at UNP. Within the year 2004 the following activities were carried out at the University of KwaZulu-Natal:

- (a) Project proposal.
- (b) A literature review concentrating on carotenoid biosynthesis and its possible regulation.
- (c) Determination of total carotenoid concentration of *Citrus* exocarp extractable with different organic solvents.

Projekopsomming

Die behoefte om aan die minimum verlangte kwaliteit vereistes te voldoen om suksesvol sitrus produkte te bemark word nou vervang deur die behoefte om produkte van superieure kwaliteit, met die klem op voorkoms en eetbaarheid te produseer. Sodra die aanbod van sitrus die aanvraag oortref word produk differensiasie al meer belangrik om gewaarborgde verkope te handhaaf. Die vrugkwaliteit verbeterings program van die CRI staan te doel om die Suider Afrikaanse sitrus produsent met verbouings praktyke te voorsien wat kan dien as hulpmiddel in die produksie van superieure kwaliteit produkte.

Sonbrand kan lei tot ernstige kommersiële verliese van Satsuma mandaryn (*Citrus unshiu* Marc.) en ander sonbrandsensitiewe kultivars. As die insidensie en graad van sonbrand verminder kan word, sal dit lei tot verhoogde uitpakkings, wat 'n direkte en positiewe effek op die produsent se omset sal het. Die aanwending van 'n Surround® partikel-laag dmv vroeë dubbel spuit toedienings gedurende die mid-sommer kan effektief sonbrand skade elimineer op Miho Wase mandaryn vrugte. 'n Bykomende voordeel was 'n verhoging in die suiker inhoud in Surround® behandelde bome (5.2.2).

Gedurende die 2003-04 seisoene was daar verskillende konsentrasies MAP toegedien op Delta en Midnight Valencia lemoene, maar het nie die suur vlakke verlaag nie. Die resultate is teenstrydig met vorige resultate waar MAP toegedien 6 weke na vol blom wel die suur inhoud verlaag het. Dubbele toediening MAP asook kombinasies met ander middel moet in die toekomst ondersoek word (5.2.3).

'n Premie word in die Japanse mark betaal vir suurlimoene met 'n lengte-deursnee verhouding (L:D) van 1.25:1 en meer. Die gibberellin behandeling is in die vorm van 'n verskeidenheid produkte (ProGibb®, Promalin®, Perlan®, Falgro®) asook 'n gibberellin biosintese inhibeerder (Regalis®) toegedien op Eureka suurlimoen in die Gt. Drakenstein area van die Wes Kaap, gedurende blom differensiasie (19 Jun 2003) en 6 weke na volblom (mid Nov 2003). Geen een van die behandelings het die L:D verhouding verhoog in vergelyking met suurlimoene van dieselfde groter nie. Die toediening van die produkte het tot blaarafsnoering (Ethrel) of 'n verlaagde blom (GA's) gelei., wat 'n aanduiding is dat die chemiese produkte wel 'n fisiologiese effek tot gevolg het (5.2.4).

Skilkleur ontwikkeling van sitrus in Suider Afrika is veral 'n probleem in die vroeë kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karoteen sintese. Vir optimale kleur ontwikkeling van sitrus word 'n nagtemperatuur van 13°C verlang. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nag temperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n bekende antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karoteen sintese vertraag. Om die rede kan maatstawwe wat die oordadige vegetatiewe groei gedurende die laat somer en vroeë herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleur te verbeter (5.2.5).

Interne gibberellin bevorder vegetatiewe groei in plante en om daardie rede kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Gedurende 2002 is daar voeler proewe met prohexadione kalsium (Ph-Ca) op Navelina Navel lemoen bome gedoen. Ph-Ca is 'n nuwe produk wat gibberellin biosintese inhibeer en het korter nawerkings effek as die triazole en die toediening daarvan het die vrugkleur verbeter met 'n halwe kleurkaart. Opvolg behandeling van Ph-Ca op Palmer Navel lemoenbome het egter in geen betekenisvolle verbetering van kleur tot gevolg gehad nie alhoewel daar aan die einde wel 'n neiging na kleur verbetering was (5.2.6). Die doel met die navorsing was om te bepaal wat die respons van *Citrus* bome sal wees op verskillende dosisse Ph-Ca. Vir die doeleinde is eksperimentele Miho Wase Satsuma en Nules Clementine mandaryn boorde in Stellenbosch, asook komersiële boorde gebruik (Palmer Navel lemoene in Wellington en Eureka suurlimoen in Gt. Drakenstein). Die Ph-Ca is toegedien teen 0, 100, 200, 400 en 800 dpm as blaar bespuitings 3 maande voor die verwagte oes datum gedurende laat 2003/vroeë 2004. Die behandelings het nie 'n effek op skil kleur getoon nie (data word nie aangebied nie) en daar word vermoed dat die tyd van behandeling nie optimaal was nie.

Gedurende 2004 was die volgende aktiwiteite by die Universiteit van KwaZulu-Natal uitgevoer:

- a) Projek voorstel.
- b) 'n Literatuur oorsig wat fokus op karoteen biosintese en die moontlikheid om dit te beheer.
- c) Bepalings van die totale karoteen konsentrasie van *Citrus* eksokarp soos geëkstraheer met verskillende organiese oplosmiddels.

5.2.2 Reduction in sunburn incidence of Satsuma mandarin

Experiment SUN01/04 by Graham H. Barry (SU/CRI)

Opsomming

Sonbrand kan lei tot ernstige kommersiële verliese van Satsuma mandaryn (*Citrus unshiu* Marc.) en ander sonbrandsensitiewe kultivars. As die insidensie en graad van sonbrand verminder kan word, sal dit lei tot verhoogde uitpak, wat 'n direkte en positiewe effek op die produsent se omset sal het. Die aanwending van 'n Surround® partikel-laag dmv vroeë dubbel spuit toedienings gedurende die mid-sommer kan effektief sonbrand skade elimineer op Miho Wase mandaryn vrugte. 'n Bykomende voordeel was 'n verhoging in die suiker inhoud in Surround® behandelde bome.

Introduction

Sunburn of Satsuma mandarin (*Citrus unshiu* Marc.) fruit and fruit of other sunburn-susceptible cultivars of *Citrus* can result in commercially significant crop losses. Sunburn incidence of Satsuma mandarin in the Western Cape Province of South Africa typically results in loss of packout by about 10%. Frequently this loss is not even quantified or accounted for as fruit are removed prior to harvest or selected out at harvest and not sent to the packinghouse (pers. obs.). Reducing sunburn incidence and severity, thereby improving packout, will have a direct and positive effect on improving grower returns.

Sunburn invariably develops on fruit exposed to late afternoon sun, i.e. on the west side of trees, on the sun-facing side of fruit. The most damaging period of intense solar radiation appears to be between the summer solstice and autumnal equinox, i.e. between 22 December and 22 March in the southern hemisphere. Evidently, nonacclimated fruit or nonacclimated portions of exposed fruit are prone to sunburn damage during hot, dry summer conditions.

Glenn et al. (2002) showed that particle film technology using processed kaolin (Surround®) suppressed sunburn of apples (*Malus sylvestris* var. *domestica*) through reduced fruit temperature, possibly via increased reflection of the UV wavelengths. Various reflective substances and whitewashes have previously been tested in an attempt to reduce sunburn in Satsuma mandarin in South Africa (E. Rabe, J.P. Wahl, pers. comm.). However, these products were ineffective, or did not wash off easily in the packinghouse. Surround®, however, appears to have the right specifications for the protection of fruit against sunburn.

In the 2003 season, a preliminary trial showed that sunburn incidence could be reduced and juice Brix increased following the application of Surround in mid-summer. The objectives of the current research were to ameliorate the incidence of sunburn and to increase juice Brix of Satsuma mandarin fruit using particle film technology and to corroborate the earlier research results.

Materials and methods

A Miho Wase Satsuma mandarin orchard planted in Nov. 1991 at Welgevallen Experimental Farm in Stellenbosch was used.

Surround® was applied as an "early" and "late" treatment, and compared with an untreated control. Surround was applied as an early, double application on 4 Dec. 2003 at 6 kg/100 ℓ water and on 2 Jan. 2004 at 3 kg/100 ℓ water, i.e. 6% + 3%, or as a later, double application on 18 Dec. 2003 at 6 kg/100 ℓ water and on 15 Jan. 2004 at 3 kg/100 ℓ water. However, due to a rain event of 22 mm on 25 Dec. 2003, the coverage of the second treatment appeared to be less than satisfactory. Therefore a third application was applied on 15 Jan. 2004 at 6 kg/100 ℓ water resulting in a final application of 6% + 3% + 6%. In all cases 25 ml Agral 90®/100 ℓ was included in the spray mixture as an adjuvant.

Approximately 7.5 ℓ of spray material was applied per tree as a full-cover spray using a high-pressure hand-held spray gun, resulting in an application rate of 675 or 1125 kg Surround per hectare for the two treatments, respectively. Eight single-tree replicates were used in a randomised complete block experimental design.

Prior to maturity, on 8 and 15 Mar. 2004, and again on 29 Mar. 2004 when the final fruit harvest occurred, 10 fruit from each of the east and west sides of trees were randomly selected for internal fruit quality determination. At maturity, fruit were selectively harvested according to colour development on 23 and 29 Mar. 2004 when yield (total weight and fruit number) and sunburn incidence (fruit weight and number) were determined.

Following a general observation of pest status, a detailed citrus red scale assessment was conducted by Mr. Johan Ackermann where percentage leaves with citrus red scale was determined. Statistical analysis involved analysis of variance, and treatment means were compared by least significant difference.

Results and discussion

Sunburn incidence of untreated control trees was relatively low ($\approx 4\%$ of total fruit yield) in the 2003-04 season (Table 5.2.2.1). Between Dec. 2003 and Mar. 2004 there were only three periods of relatively high maximum air temperature and low relative humidity, viz. 4 Jan. 2004 (38.5°C, 42% RH), 21/22 Jan. 2004 (34.5°C, 62% RH) and 9 Feb. 2004 (37°C, 53% RH). Therefore, the summer of 2003-04 can be classified as mild, and resulted in a low incidence of sunburn on Miho Wase Satsuma mandarin fruit. Nevertheless, the number of sunburnt fruit on the “early” Surround-treated trees was half that of the untreated control trees (at $P=0.1$) and effectively eliminated in the “late” Surround-treated trees (Table 5.2.2.1). Furthermore, large fruit, on average, were sunburnt in the “early” Surround treatment, whereas large and medium sized fruit were sunburnt on the untreated control trees.

Sugar content of fruit from Surround-treated trees at maturity was significantly higher than that of fruit from untreated control trees by 0.7 °Brix (Table 5.2.2.2; Fig. 5.2.2.1). Titratable acidity (TA) was also significantly higher in fruit from Surround-treated trees than fruit from untreated trees, resulting in slightly higher ratio of Brix-to-TA in untreated trees. There was no significant difference in juice content among treatments. The higher sugar content is of commercial significance to both fresh and processing citrus industries and appears to be a consistent horticultural response to the treatment of Surround. However, the higher sugar content does not result in earlier fruit maturity due to the concomitant increase in TA. Nevertheless, the increased sugar content resulted in improved product quality as evidenced by superior flavour (informal taste panel response).

Although the incidence of citrus red scale was higher for Surround-treated trees than untreated trees (4.2 vs. 2.1% infestation), the incidence of red scale was not of commercial significance.

A detailed assessment of residue removal in the packinghouse was not conducted. However, fruit from Surround-treated trees were inspected after commercial packing, and residue was not noticeable.

Conclusion

The application of Surround® particle film as an early, double spray during mid-summer effectively eliminated sunburn incidence on Miho Wase Satsuma mandarin fruit. However, due to the relatively mild summer conditions experienced in Stellenbosch during the 2003-04 season the incidence of sunburn on Surround-treated trees still needs to be ascertained under severe sunburn conditions of a hot, dry summer.

The increase in sugar content of fruit from Surround-treated trees is meaningful to both fresh and processing citrus industries, and provides an environmentally-safe way to enhance product quality. Furthermore, the increase in TA may be important in the production of low acid citrus cultivars or in production regions which produce fruit of relatively low acidity.

The response of increased sugar accumulation is also of physiological importance as these data provide evidence for improved horticultural responses to Surround treatment, and are supported by physiological responses (Jifon and Syvertsen, 2003), lending further credence to the claim that Surround could increase net leaf carbon uptake efficiency.

References cited

- Glenn, D.M., E. Prado, A. Erez, J. McFerson, and G.J. Puterka. 2002. A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury to apple. *J. Amer. Soc. Hort. Sci.* 127:188-193.
- Jifon, J.L. and J.P. Syvertsen. 2003. Kaolin particle film applications can increase photosynthesis and water use efficiency of ‘Ruby Red’ grapefruit leaves. *J. Amer. Soc. Hort. Sci.* 128:107-112.

Table 5.2.2.1. Fruit yield and sunburn incidence of Miho Wase Satsuma mandarin fruit on 29 March 2004 of untreated control and “early” (Surround 1) and “late” (Surround 2) Surround-treated trees.

Treatment	Total yield (kg/tree)	Fruit number per tree	Mean fruit weight (g)	Sunburn incidence (kg/tree)	Sunburn incidence (fruit no./tree)	Mean fruit weight (g)
Control	55.2 ns ^z	793 ns	69.6	1.95 a	28 a	69.6
Surround 1	72.4	974	74.3	1.57 a	15 a	104.7
Surround 2	73.2	983	74.5	0.00 b	0 b	-
<i>P</i> -values	0.2414	0.4271		0.0067	0.0030	
LSD	24.6	340		1.17	14.3	

^z Means within columns or within rows with different letters are significantly different at $P=0.05$; $n=8$.

Table 5.2.2.2. Internal fruit quality of Miho Wase Satsuma mandarin fruit on 29 March 2004 from east and west canopy positions of untreated control and Surround-treated trees.

Treatment	Fruit weight (g)			Juice content (%)			Brix (°)			TA (%)			Brix:TA Ratio		
	East	West	Mean	East	West	Mean	East	West	Mean	East	West	Mean	East	West	Mean
Control	94.1	90.9	92.6 ns ^y	47.5	48.7	48.1 ns	9.1	9.0	9.1 a	1.10	1.14	1.12 a	8.30	7.91	8.12 a
Surround 1	89.4	91.4	90.4	49.4	49.7	49.5	10.0	9.7	9.9 b	1.30	1.32	1.31 b	7.71	7.44	7.58 ab
Surround 2	91.0	89.2	90.1	49.0	49.6	49.3	9.9	9.7	9.8 b	1.33	1.31	1.32 b	7.48	7.46	7.47 b
<i>P-values</i> ^z															
Trmnt x CP			0.7729			0.8930			0.9394			0.6550			0.8052
Trmnt			0.7813			0.3079			0.0078			0.0001			0.0711
CP			0.7536			0.3750			0.4207			0.7222			0.3446

^z Trmnt x CP = treatment x canopy position interaction; Trmnt = treatment effect; CP = canopy position effect.

^y Means within columns with different letters are significantly different at $P=0.05$; n=8; ns=non-significant.

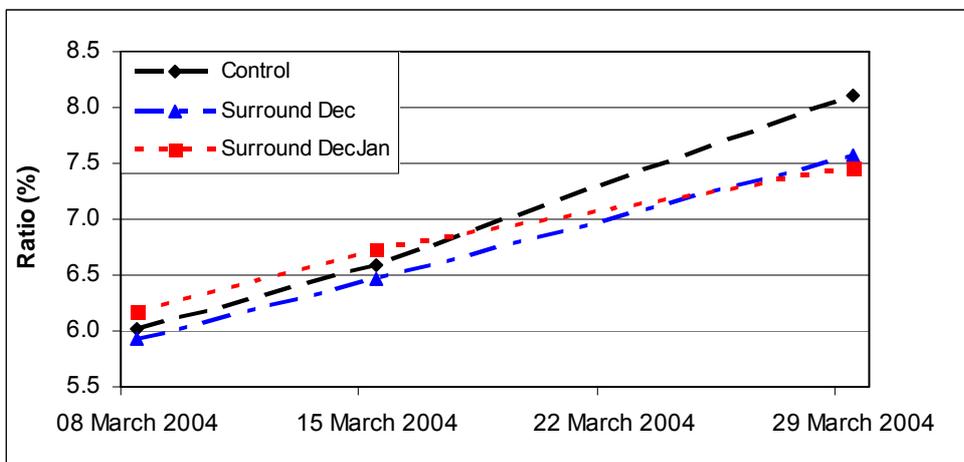
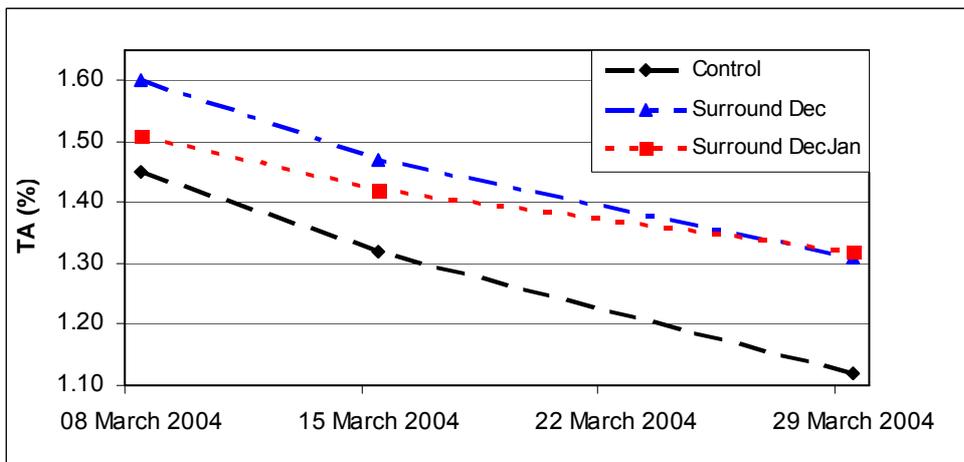
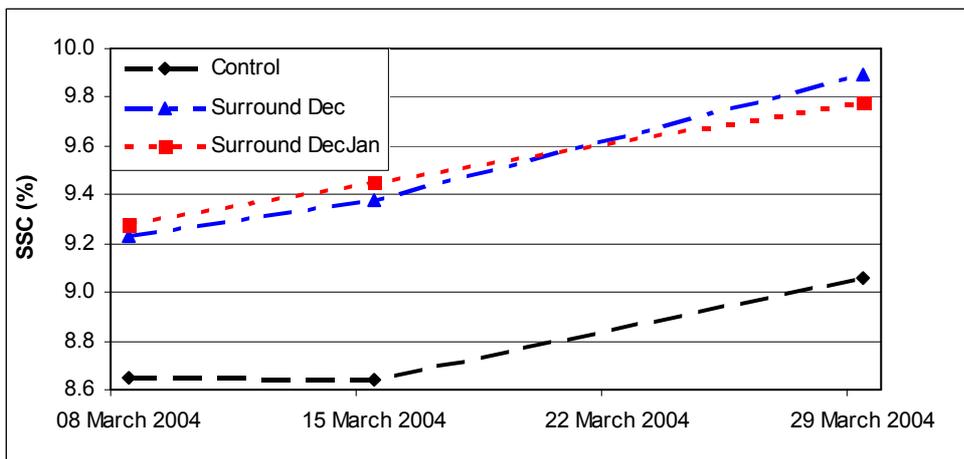


Fig. 5.2.2.1. Changes in internal fruit quality of Miho Wase Satsuma mandarin fruit during March 2004 averaged across east and west canopy positions of untreated control and Surround-treated trees.

5.2.3 Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate
 Experiment ACID 01/02 by Graham H. Barry (SU/CRI)

Opsomming

Gedurende die 2003-04 seisoene was daar verskillende konsentrasies MAP toegedien op Delta en Midnight Valencia lemoene, maar het nie die suur vlakke verlaag nie. Die resultate is teenstrydig met vorige resultate waar MAP toegedien 6 weke na vol blom wel die suur inhoud verlaag het. Dubbele toediening MAP asook kombinasies met ander middel moet in die toekoms ondersoek word.

Introduction

Following promising results in the 2002 and 2003 seasons (see 2002 and 2003 CRI Annual Research Reports), various concentrations of monoammonium phosphate (MAP) were tested to determine the optimal treatment concentration to reduce acidity.

Materials and methods

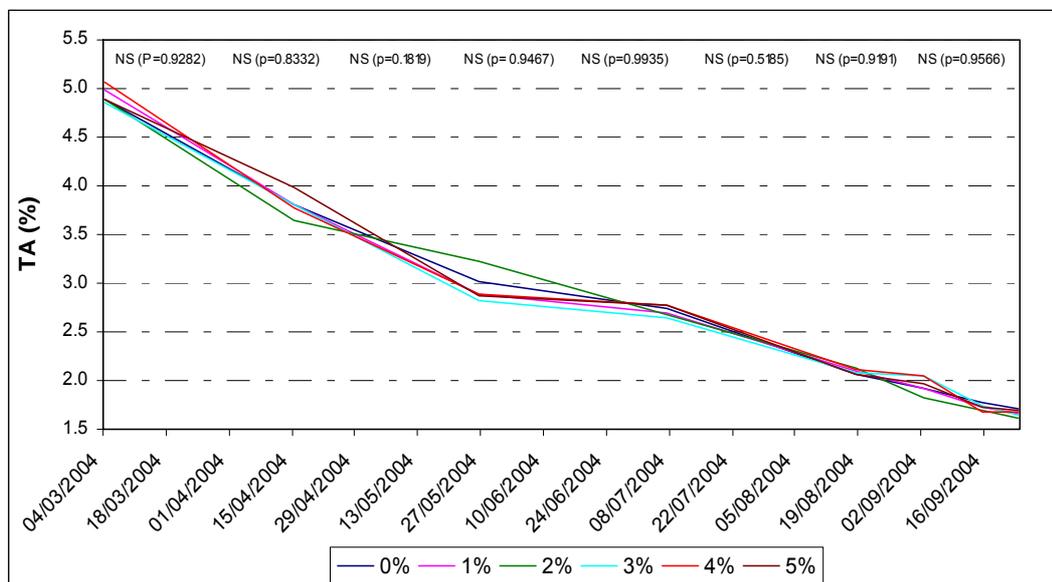
Plant material and treatments. A Delta Valencia orange orchard at Paardekop and a Midnight Valencia orange orchard at Boontjiesrivier, Citrusdal were used in this study. The trees used were selected for uniformity in tree size and health.

Treatments were applied 6 weeks after full bloom (WAFB) on 28 November 2003. A randomized complete block design with six single-tree replicates was used. Treatments included 0, 1, 2, 3, 4 and 5% MAP. A medium-cover spray was used to apply spray material until just before run-off. On average, 7.5 l of spray material was applied per tree.

Data collection and statistical analysis. Fruit acidity was determined every 6 weeks from 4 Mar. 2004 until maturity to map seasonal changes in acidity. Ten fruit of similar size were sampled from the east side of trees. At maturity on 22 Sept. 2004, samples of 12 fruit per replicate were taken for juice quality analysis. Juice content, Brix (by refractometer), titratable acidity (TA) and ratio were determined using standard procedures. Leaf samples were taken 6 weeks after spray applications in Feb 2004. These samples were used for N, P and K analysis. In each treatment, three replicates were sampled and analysed. Data were analysed using SAS.

Results and discussion

In this progress report, only the acidity data are presented (Figs. 5.2.3.1 and 5.2.3.2). There was a numerical difference in TA among all treatments for both Delta Valencia and Midnight Valencia oranges (Figs. 5.2.3.1 and 5.2.3.2), and 2% MAP tended to result in the lowest TA. However, there were no significant differences between any of the treatments compared to the control for both Midnight Valencia and Delta Valencia oranges.



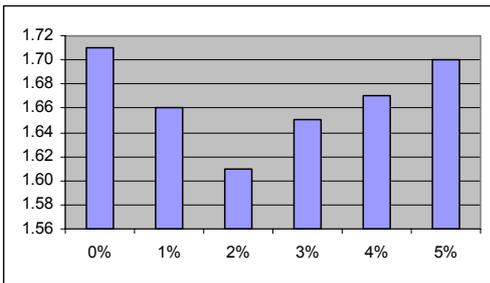
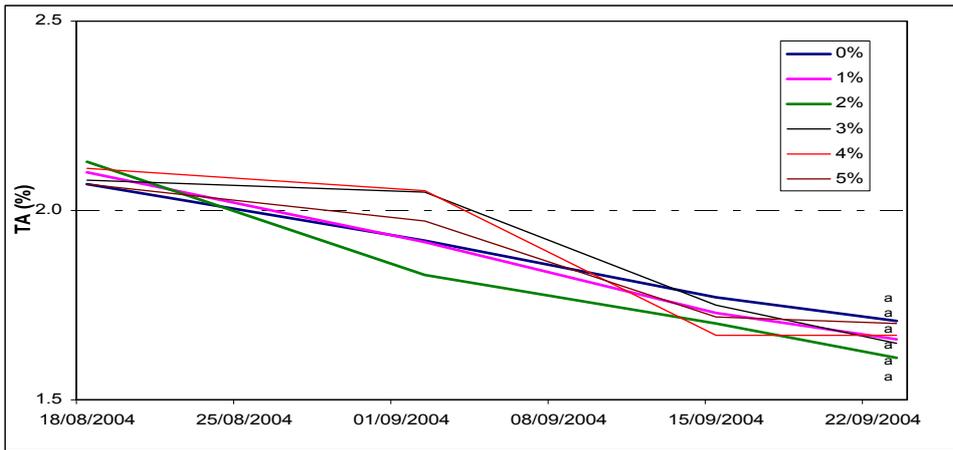
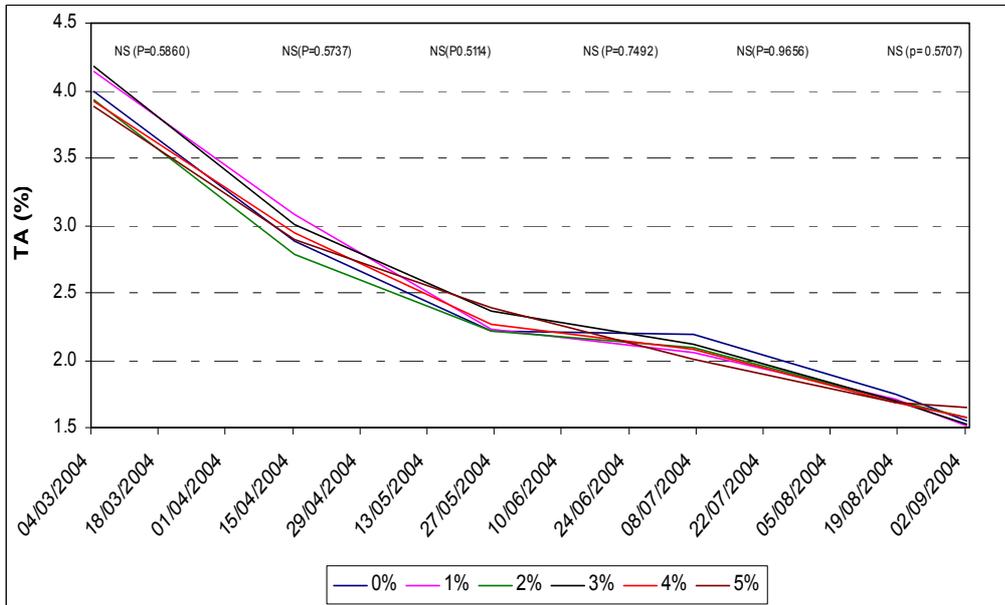


Figure 5.2.3.1. Effect of various concentrations of MAP on fruit acidity of Delta Valencia orange. Treatments were applied on 28 November 2003 and fruit were sampled six-weekly from early March to demonstrate changes in acidity over time and to overcome issues of sampling error. The second graph shows only the last six sampling times to decrease the Y-axis scale and thereby allow the reader to more clearly see the data points. (Means followed by different letters differed at $P=0.1$; $n=6$). The lower bar graph shows the TA response to concentration.



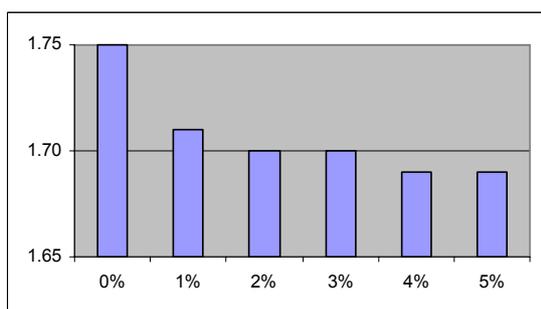
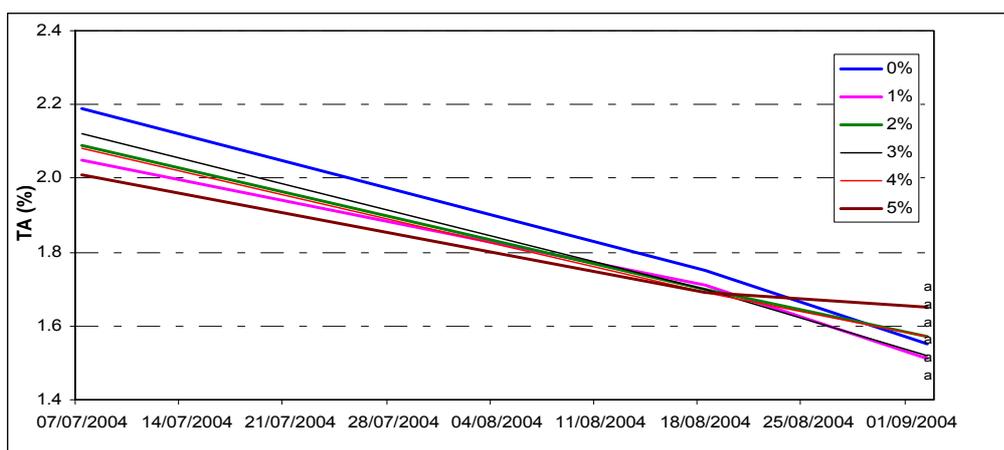


Fig. 5.2.3.2. Effect of MAP on fruit acidity of Midnight Valencia orange. Treatments were applied on 28 November 2003 and fruit were sampled every six weeks from early March to demonstrate changes in acidity over time and to overcome issues of sampling error. The second graph shows only the last 2 months of sampling to decrease the Y-axis scale and thereby allow the reader to more clearly see the data points. (Means do not differ significantly among treatments at any sampling date). The lower bar graph shows the TA response to concentration.

Leaf analysis of N, P and K showed that MAP concentration did not affect leaf N, P or K levels. However, N and P levels were relatively low in the Delta Valencia orchard, and K level was low in the Midnight Valencia orchard. With the relatively low leaf P levels, a treatment response to increasing P application would be expected. However, these leaf samples were taken in February, after application of MAP. Leaf P levels prior to MAP application could differ from those reported here.

Table 5.2.3.1. Leaf analysis data (%) for Delta Valencia and Midnight Valencia oranges.

% MAP	Delta Valencia			Midnight Valencia		
	N	P	K	N	P	K
0	2.09	0.11	1.30	2.52	0.12	0.78
1	2.07	0.12	1.47	2.35	0.12	0.62
2	1.99	0.11	1.17	2.45	0.13	0.71
3	1.91	0.10	1.37	2.38	0.12	0.66
4	1.94	0.11	1.41	2.21	0.12	0.53
5	2.06	0.10	1.61	2.38	0.12	0.63
P-value	0.45	0.05	0.56	0.40	0.82	0.51
Norms	2.2-2.6	0.11-0.15	0.9-1.8	2.2-2.6	0.11-0.15	0.9-1.8

Conclusion

During the 2003-04 season, various concentrations of MAP applied to Delta Valencia and Midnight Valencia oranges did not reduce TA. This result is contrary to previous results where 1% MAP applied 6 weeks after full bloom did reduce TA. Double applications or combinations with other substances should be investigated in the future.

5.2.4 Lemon fruit shape

Experiment SHAPE 01/03 by Graham H. Barry (SU/CRI)

Opsomming

'n Premie word in die Japanese mark betaal vir suurlemoene met 'n lengte-deursnee verhouding (L:D) van 1.25:1 en meer. Die gibberelien behandeling is in die vorm van 'n verskeidenheid produkte (ProGibb®, Promalin®, Perlan®, Falgro®) asook 'n gibberelien biosintese inhibeerder (Regalis®) toegedien op Eureka suurlemoen in die Gt. Drakenstein area van die Wes Kaap, gedurende blom differensiasie (19 Jun 2003) en 6 weke na volblom (mid Nov 2003). Geen een van die behandelings het die L:D verhouding verhoog in vergelyking met suurlemoene van dieselfde groter nie. Die toediening van die produkte het tot blaarfsnoering (Ethrel) of 'n verlaagde blom (GAs) gelei, wat 'n aanduiding is dat die chemiese produkte wel 'n fisiologiese effek tot gevolg het.

Introduction

Premium prices are obtained in the Japanese market for elongated lemons with a length-to-diameter ratio (L:D) exceeding 1.25:1. Increasing the proportion of a lemon crop with elongated fruit will potentially improve grower returns. Goosen (2002) conducted a thorough study on the factors affecting fruit shape in lemon, from which the role of gibberellins was highlighted.

In the apple industry, gibberellins are used to produce elongated fruit. Initial results using GAs on lemons resulted in variable results, and Goosen (2002) recommended that earlier application timings be attempted to increase L:D in lemon.

Materials and methods

Various GA sources (ProGibb®, Promalin®, Provide®, Perlan®, Falgro®) and a gibberellin biosynthesis inhibitor (Regalis®) were applied to Eureka lemon in the Gt. Drakenstein area of the Western Cape during flower differentiation (19 Jun. 2003) and 6 weeks after full bloom (mid-Nov. 2003). Fruit were sampled in 2, 4, 6, and 8 weeks after treatment application and again the following winter to determine return bloom effects on fruit shape. In addition, other PGRs (auxins, ethylene and cytokinins) and girdling were also evaluated.

Fruit length and diameter was measured of fruit of similar size, from which the L:D ratio was calculated.

Results and discussion

Auxins

Fruit samples taken from a lemon orchard treated with Citrimax® and Maxim® as fruit thinning treatments did not differ in L:D ratio (Table 5.2.4.1).

Table 5.2.4.1. Affect of auxins on L:D ratio of similar sized Eureka lemon.

Treatment	Length (mm)	Diameter (mm)	L:D ratio
Control	74.2	59.2	1.25
Citrimax	74.5	59.8	1.25
Maxim	74.8	60.4	1.24
<i>P</i> -values	0.92	0.41	0.66
CV	5.5	4.4	3.9

Ethylene

Treatment with ethylene releasing and ethylene inhibiting compounds did not affect L:D ratio of Eureka lemon (Table 5.2.4.2). However, return bloom fruit (May 2004) treated with the high rate of Ethrel had significantly longer fruit than fruit treated with an ethylene-action inhibitor. However, excessive leaf drop would probably eliminate such a high Ethrel application.

Table 5.2.4.2. Affect of ethylene (Ethrel®) and an ethylene inhibitor (ReTain®) applied in June 2003 on L:D ratio of similar size Eureka lemon. Fruit were sampled 2, 4, 6 and 8 weeks after treatment and again the following winter (May 2004).

Trt.	Length (mm)					Diameter (mm)					Ratio				
	2	4	6	8	Ma y04	2	4	6	8	May 04	2	4	6	8	May 04
Ethrel 0 ppm	70.6	68.7	68.5	75.7	72.7	56.1	55.1	55.2	60.5	58.8	1.26	1.25	1.24	1.25	1.24
Ethrel 200 ppm	68.5	69.4	67.5	84.0	72.4	55.6	56.0	55.7	67.7	59.2	1.23	1.24	1.21	1.24	1.23
Ethrel 400 ppm	73.5	67.6	68.3	81.2	75.3	57.4	55.5	55.2	65.2	58.8	1.28	1.22	1.24	1.25	1.28
ReTain 0 ppm	69.1	67.9	68.8	81.8	70.7	55.8	55.3	55.8	64.9	58.6	1.24	1.23	1.23	1.27	1.21
ReTain 200 ppm	71.7	68.7	71.3	82.2	70.3	57.2	56.6	57.9	67.1	58.8	1.25	1.21	1.23	1.23	1.20
ReTain 400 ppm	70.2	69.7	70.5	78.0	70.2	57.7	56.9	57.1	64.0	58.0	1.22	1.23	1.24	1.22	1.21
<i>P</i> -values	0.17	0.94	0.66	0.03	0.08	0.36	0.75	0.47	0.07	0.98	0.28	0.74	0.92	0.75	0.08
CV	4.8	6.1	6.5	5.4	4.5	3.6	4.4	4.9	6.4	4.0	3.7	3.6	3.7	4.5	4.1

Gibberellins and cytokinins

Treatment with various sources of gibberellins, cytokinins and a gibberellin-biosynthesis inhibitor did not affect L:D ratio of Eureka lemon (Table 5.2.4.3).

Table 5.2.4.3. Affect of gibberellins, cytokinins and a gibberellin-biosynthesis inhibitor (Regalis®) applied in June 2003 on L:D ratio of similar size Eureka lemon. Fruit were sampled 2, 4, 6 and 8 weeks after treatment and again the following winter (June 2004).

Trt.	Length (mm)					Diameter (mm)					Ratio				
	2	4	6	8	Jun 04	2	4	6	8	Jun 04	2	4	6	8	Jun 04
Control	70.3	72.6	73.0	69.5	74.6	56.3	57.8	58.3	55.1	60.4	1.25	1.25	1.25	1.26	1.23
ProGibb	70.7	75.3	72.3	71.2	73.7	55.4	61.9	57.5	60.0	60.2	1.28	1.22	1.26	1.20	1.22
Fallgro	71.5	76.1	72.6	69.4	72.4	55.9	61.9	58.5	57.9	59.0	1.28	1.23	1.24	1.20	1.23

Promalin	-	73.0	-	71.3	72.7	-	59.6	-	57.5	58.8	-	1.22	-	1.24	1.24
Perslan	-	75.9	-	71.6	71.7	-	62.3	-	58.2	59.9	-	1.22	-	1.23	1.20
Provide	-	74.2	-	73.2	76.3	-	58.7	-	59.0	61.3	-	1.26	-	1.24	1.25
BA	-	74.2	-	73.1	71.6	-	59.4	-	58.0	58.3	-	1.25	-	1.26	1.23
Regalis	71.2	71.9	71.7	67.6	70.1	56.9	59.0	58.2	55.6	57.7	1.25	1.22	1.24	1.22	1.22
<i>P</i> -values	0.90	0.67	0.91	0.18	0.15	0.41	0.12	0.89	0.08	0.19	0.41	0.32	0.89	0.16	0.80
CV	3.9	6.5	4.3	5.4	5.1	2.8	5.3	3.9	4.9	4.1	3.2	3.3	4.2	3.9	3.9

Girdling, urea and thiosulphate

Various treatments which can affect blossom intensity and fruit set did not affect L:D ratio of Eureka lemon (Table 5.2.4.4).

Table 5.2.4.4. Affect of gibberellins, girdling, a gibberellin-biosynthesis inhibitor (Regalis®), urea prior to bloom, and ammonium thiosulphate (ATS) on L:D ratio of similar size Eureka lemon.

Treatment	Length (mm)			Diameter (mm)			Ratio		
	Hazyview	Hoedspruit	Nov 03	Hazyview	Hoedspruit	Nov 03	Hazyview	Hoedspruit	Nov 03
Control	76.9	67.5	74.0	62.6	52.9	59.7	1.23	1.28	1.24
ProGibb	71.8	66.7	77.7	58.0	53.8	61.3	1.24	1.24	1.28
Girdling	70.9	67.9	77.8	58.3	52.8	62.4	1.22	1.29	1.25
ProG + Girdling	-	67.6	-	-	52.8	-	-	1.28	-
Promalin	-	-	74.9	-	-	61.0	-	-	1.23
Regalis	-	-	76.1	-	-	60.0	-	-	1.27
Urea	-	-	76.1	-	-	61.7	-	-	1.23
ATS 0.5%	-	-	74.1	-	-	60.0	-	-	1.24
ATS 1.0%	-	-	76.1	-	-	61.4	-	-	1.24
<i>P</i> -values	0.06	0.97	0.28	0.13	0.89	0.38	0.87	0.49	0.67
CV	4.4	5.5	4.1	5.3	4.3	3.6	4.3	3.6	3.5

Conclusions

None of the treatments applied increased L:D ratio of similar sized lemon fruit. Some of the products applied resulted in leaf abscission (Ethrel) or reduced bloom (GAs), indicating that the chemical products did have a physiological effect.

5.2.5 Physiological aspects of rind colour development Experiment COL01/02 by Graham H. Barry (SU/CRI)

Opsomming

Skilkleur ontwikkeling van sitrus in Suider Afrika is veral 'n probleem in die vroeë kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karoteen sintese. Vir optimale kleur ontwikkeling van sitrus word 'n nagtemperatuur van <13°C verlang. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nag temperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n bekende antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karoteen sintese vertraag. Om die rede kan

maatstawwe wat die oordadige vegetatiewe groei gedurende die laat somer en vroeg herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleur te verbeter.

Interne gibberellin bevorder vegetatiewe groei in plante en om daardie rede kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Gedurende 2002 is daar voeler proewe met prohexadione kalsium (Ph-Ca) op Navelina navel lemoen bome gedoen. Ph-Ca is 'n nuwe produk wat gibberellin biosintese inhibeer en het korter nawerkings effek as die triazole en die toediening daarvan het die vrugkleur verbeter met 'n halwe kleurkaart. Opvolg behandeling van Ph-Ca op Palmer Navel lemoenbome het egter in geen betekenisvolle verbetering van kleur tot gevolg gehad nie alhoewel daar aan die einde wel 'n neiging na kleur verbetering was.

Die doel met die navorsing was om te bepaal wat die respons van *Citrus* bome sal wees op verskillende dosisse Ph-Ca. Vir die doeleinde is eksperimentele Miho Wase Satsuma en Nules Clementine mandaryn boorde in Stellenbosch, asook kommersieel boorde gebruik (Palmer navel lemoene in Wellington en Eureka suurlimoen in Gt. Drakenstein). Die Ph-Ca is toegedien teen 0, 100, 200, 400 en 800 dpm as blaar bespuitings 3 maande voor die verwagte oes datum gedurende laat 2003/vroeg 2004. Die behandelings het nie 'n effek op skil kleur getoon nie (data word nie aangebied nie) en daar word vermoed dat die tyd van behandeling nie optimaal was nie.

Summary

Rind colour development of citrus is a problem in South Africa, especially for early maturing cultivars, because autumn temperatures are too high for chlorophyll breakdown and carotenoid synthesis. Citrus fruit require night temperatures of <13°C for optimal colour development. It is unlikely that cool night temperature *per se* is the cause of chlorophyll breakdown and carotenoid synthesis. Rather, the cool night temperature probably plays a direct role in halting vegetative growth. The latter is antagonistic to chloroplast conversion to chromoplasts, thereby delaying chlorophyll breakdown and carotenoid synthesis. Therefore, techniques to reduce vegetative vigour during late summer and early autumn may play a role in enhancing rind colour development.

Endogenous gibberellins promote vegetative growth in plants. Gibberellin biosynthesis inhibitors effectively reduce vegetative growth and, in turn, may promote the conversion of chloroplasts to chromoplasts. Previous exploratory research (2002) on Navelina Navel orange trees treated with prohexadione calcium (Ph-Ca), a new gibberellin biosynthesis inhibitor with less persistence than triazoles, had fruit that were better coloured than the control by more than half a colour plate. However, subsequent treatment of Palmer Navel orange with Ph-Ca did not significantly improve rind colour, although there was a trend towards enhanced colour.

The objective of this research was to determine the dose response of citrus trees to Ph-Ca. An experimental orchard of Miho Wase Satsuma and Nules Clementine mandarins in Stellenbosch, and commercial orchards of Palmer Navel orange in Wellington and Eureka lemon in Gt. Drakenstein were used. Ph-Ca at 0, 100, 200, 400 and 800 ppm was applied as a foliar spray 3 months before anticipated harvest in late 2003/early 2004. The treatments did not improve rind colour, perhaps because treatment timing was not optimal.

5.2.6 Effects of tree nutrition and post-harvest treatments on concentration of the most important colour-imparting carotenoids in physiologically mature citrus fruit

By Isa Bertling, John Bower, Renate Oberholster, Molipa Mosoeunyane and Tony Bruton (UKZNP)

Opsomming

Gedurende 2004 was die volgende aktiwiteite by die Universiteit van KwaZulu-Natal uitgevoer:

- a) Projek voorstel.
- b) 'n Literatuur oorsig wat fokus op karoteen biosintese en die moontlikheid om dit te beheer.
- c) Bepalings van die totale karoteen konsentrasie van *Citrus* eksokarp soos geëkstraheer met verskillende organiese oplosmiddels.

Research directions emanating from the literature reviewed

Literature indicates that many researchers have investigated the expression of genes encoding for enzymes involved in β , β -xanthophyll biosynthesis in the flavedo of certain citrus cultivars. Contrary to that information, this study will focus on answering the following questions:

- Which enzymes play a critical role in key colour-imparting carotenoid biosynthesis?
- Will pre-harvest supply of certain micro-nutrients and a brief post-harvest exposure of citrus flavedo of early 'Valencia' and navel oranges to low temperature regulate activities of certain enzymes involved in biosynthesis of key colour-imparting carotenoids in citrus flavedo?
- Can change of temperature post-harvest and varying supply of micro-nutrients to citrus trees impact on red-orange colour-imparting carotenoid in early 'Valencia' and navel oranges?

An attempt to answer the aforementioned questions involves carrying out the following experiments.

Materials and methods

Biochemical analysis:

- isolation of chromoplasts from citrus flavedo
- extraction of chromoplast protein from citrus flavedo
- isolation and purification of enzymes from flavedo of both green and fully-degreened fruit
- quantification of key colour-imparting carotenoids in treated citrus flavedo

Postharvest treatment:

- Hydro-cooling fruit in butan-(2)-ol at 4°C
- Hydro-warming fruit in water bath at 22°C

Preharvest treatment:

- Application, by side dressing and foliar feeding, of molybdenum and tungsten to navel and Valencia trees at flowering and colour-break.

Different organic solvents have been used to extract pigments from various *Citrus* cultivars and have performed remarkably well. On the basis of that information, this experiment was planned to compare the efficiency of different organic solvents in extracting total carotenoid concentration from exocarp of two *Citrus* cultivars.

Results

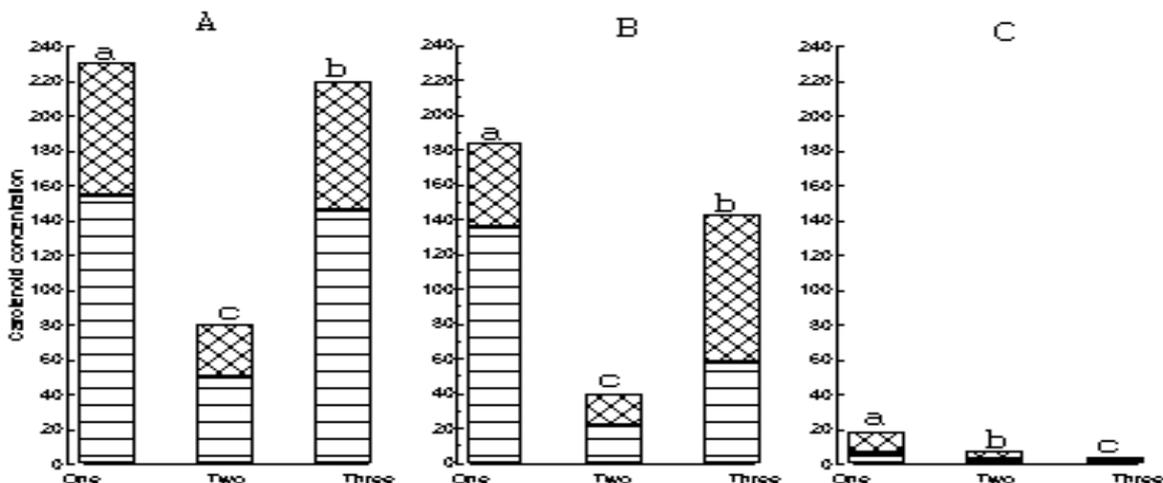


Figure 5.2.6.1. Comparative efficacy of three organic solvents in extracting total carotenoids ($\mu\text{g g}^{-1}$ tissue mass) from 1 g dry (A) as well as 1 g (B) and 5 g (C) fresh *Citrus* exocarp. Solvents numbers: One = 95% ethanol (v/v); two = 90% methanol (v/v) and three = 100% methanol. Total carotenoids extracted by each solvent comprised the concentration determined spectrophotometrically in the initial (homogenized; lined bars) extracts and re-extracted (vortexed; checked bars) extracts combined together. Different lower case letters on top of the bars denote significant difference in total carotenoid concentration extracted by each solvent ($P=0.05$).

Conclusion

These experimental data allow the following inference to be made: re-extraction of pigmented material for pigment quantification is necessary as 95% (v/v) ethanol out-competed methanol (90% and 100%) in extracting carotenoids from both dry and fresh exocarp. This solvent will be utilised for extraction of pigments, preferably from freeze-dried material.

5.3 PROJECT: CROP LOAD MANAGEMENT

Project Co-ordinator: Graham H. Barry (SU/CRI)

5.3.1 Project summary

Daily fertigation could be considered as one of the most important recent advances to citricultural practices. Direct benefits of managing plant water relations and mineral nutrition through such a system includes, *inter alia*, i) increased precocity, i.e. earlier fruit bearing potential, ii) improved fruit yield, and iii) larger fruit size. However, there is also a biological “cost” to these benefits, *viz.* delayed rind colouration and sub-optimal sugar accumulation.

The advantages and disadvantages of daily fertigation are in the process of being quantified, and detailed physiological studies are being conducted to fine-tune current recommendations and industry practices. Part of this research aims to prove that reduced midday depression of leaf gas exchange by daily fertigation results in increased C fixation, thereby providing extra photosynthates, which are translocated to the fruit, and hence improved internal fruit quality in terms of juice content, Brix and acidity.

Differential irrigation treatments (1/2X, X and 2X) were applied to Nules Clementine mandarin trees from the end of stage 1 of fruit development until maturity to quantify the ecophysiological responses and benefits of daily fertigation, as well as the effects of this technology on crop load and fruit quality. Although soil water content was relatively low, treatment differences in ψ_{stem} were not sufficiently large to cause differences in sugar accumulation and other fruit quality variables (5.3.2).

Projek opsomming

Daaglikse toediening van bemesting deur middel van die besproeiingsstelsel (“fertigation”), is een van die belangrikste vorderings wat onlangs gemaak is in die verbouing van sitrus. Daar is direkte voordele aan verbonde deur die plant water verhouding en minerale voeding so te bestuur, naamlik, i) verkorting van die boom se jeugfase en dus die vermoë om vroeër vrugbaar te wees, ii) verhoging in opbrengs, en iii) verbeterde vruggrootte. Daar is egter ’n negatiewe biologiese prys wat verhaal word, naamlik vertraagde kleurontwikkeling en sub-optimale suiker akkumulاسie.

Verskillende besproeiingsbehandelings (1/2X, X en 2X) is toegedien op ‘Nules Clementine’ mandaryn bome vanaf fase I van vrugontwikkeling tot vrugvolwassenheid om die ekofisiologiese reaksies asook die voordele wat verkry word met die gebruik van die tegnologie op opbrengs en vrugkwaliteit te bepaal. Ten spyte van ’n relatiewe lae grondwaterinhoud was verskille in stamwaterpotensiaal tussen behandelings te klein om verskille in suikerinhoud en ander vrugkwaliteitsaspekte te weeg te bring.

Die voor- en nadele verbonde aan daaglikse bemesting deur middel van die besproeiingstelsel is in die proses om gekwantifiseer te word. Gedetailleerde fisiologiese studies word tans onderneem om huidige aanbevelings en verbouingspraktyke te verfyn. ’n Deel van die navorsingsprojek poog om te bewys dat indien ’n verdere verlaging in die mid-dag daling van die gasuitruiling van die blaar bewerkstellig kan word met die toediening van bemesting deur die besproeiingstelsel (fertigation), ’n verhoogde koolstof vaslegging verkry kan word. Gevolglik sal vrugkwaliteit verbeter in terme van sapinhoud, Brix en sure as gevolg van die verhoogde translokasie van fotosintetiese produkte na die vrug (5.3.2).

5.3.2 Ecophysiological responses and changes in sugar accumulation due to altered plant water relations of *Citrus* trees

Experiment TSS 01/02 by Jandr  A. Prinsloo (SU) and Graham H. Barry (SU/CRI)

Opsomming

Verskillende besproeiingsbehandelings (1/2X, X en 2X) is toegedien op ‘Nules Clementine’ mandaryn bome vanaf fase I van vrugontwikkeling tot vrugvolwassenheid om die ekofisiologiese reaksies asook die voordele wat verkry word met die gebruik van die tegnologie op opbrengs en vrugkwaliteit te bepaal. Ten spyte van ’n

relatiewe lae grondwaterinhoud was verskille in stamwaterpotensiaal tussen behandelings te klein om verskille in suikerinhoud en ander vrugkwaliteitsaspekte te weeg tebring.

Introduction

The daily fertigation method of irrigation and fertilisation promises to increase orchard productivity and ultimately profitability through improved and timeous application of water and mineral nutrients. However, these promises are not necessarily supported by facts. For example, increases in fruit yield and fruit size have been reported for citrus trees grown under daily fertigation. However, delayed rind colour development and inadequate sugar accumulation are being reported in the citrus industry. Therefore, it is essential to quantify the ecophysiological responses and benefits of daily fertigation, as well as the effects of this technology on crop load and fruit quality.

Materials and methods

Nules Clementine mandarin orchards at two commercial farms (Backsberg and Greendale) in Simondium, Western Cape province, were used. Differential irrigation consisting of 2X, X and 1/2X was applied at the end of stage I (mid-December) of fruit development. Six replicates each consisting of six trees were used at each orchard in a randomized complete blocks design.

Stem water potential (WP) was determined by using a pressure chamber. Two leaves per replicate on the west side of trees were bagged before sunrise with small black bags and covered with tinfoil. Water potential measurements were taken from 11H00 at Greendale and from 12H00 at Backsberg. In December 2003, 10 fruit were tagged and fruit size measurements taken with an electronic caliper. From March 2004, 10 fruit were randomly harvested for internal fruit quality analysis. The fruit that were tagged for fruit growth measurements were harvested at maturity (May 2004) and also analyzed for internal fruit quality purposes. Fruit quality analysis consisted of fruit size and fresh weight, juice weight and percentage, Brix (using an electronic refractometer) and titratable acidity. All sampling was made from the middle four trees of each replicate to reduce possible influences from adjacent treatments. Soil samples were taken and weighed at each evaluation period to determine gravimetric soil water content (SWC).

Results and discussion

Soil water content (SWC). The SWC in the 2003-2004 season at both Backsberg and Greendale was much lower than in the 2002-2003 season, i.e. the soil was much drier. The average SWC for all treatments at Greendale was 6.1% in 2002-2003 and 2.6% in 2003-2004. The difference at Backsberg was even greater, with the average for the 2002-2003 season being 12.0%, and 3.5% for 2003-2004.

As can be expected, the 2X treatment (which received double the amount of water of the X treatment) had consistently the highest SWC and the 1/2X treatment had the lowest SWC (Figs. 5.3.2.1 and 5.3.2.2). The only exceptions were on 6 February 2004 at Backsberg and on 16 April 2003 at Greendale. At Backsberg, the average SWC during the season ranged between 2.0% and 5.0%, and between 1.6% and 4.1% at Greendale. The soils at Greendale are compact, which resulted in irrigation water tending to run off the ridges. The trees at Backsberg are larger than at Greendale and these trees are probably capable of obtaining water from greater depths.

Stem water potential. At Backsberg and Greendale, the 2X treatment generally had the highest (least negative) ψ_{stem} and the 1/2X treatment had the lowest ψ_{stem} (Figs 5.3.2.3 and 5.3.2.4). The average ψ_{stem} fluctuated between -0.80 MPa and -1.40 MPa at Backsberg and between -0.93 MPa and -1.35 MPa at Greendale.

At Backsberg it took 8 weeks to reach a difference of 0.16 MPa between treatments. This difference lasted more than 2 weeks, after which time it decreased. A difference of 0.2 MPa was reached only after 2 months at Greendale and did not even last for 2 weeks.

Fruit size. At Backsberg, fruit size among treatments did not differ (Fig. 5.3.2.5), and the average fruit size at harvest (52.4 mm) was smaller than in the 2002-2003 season, probably due to the lower SWC during the 2003-2004 season.

Fruit size at Greendale was only significantly different at harvesting (Fig. 5.3.2.16). The X treatment had the largest fruit. At harvesting, the average fruit size was 51.8 mm.

Internal fruit quality of tagged fruit. At Backsberg, significant ($P < 0.05$) differences in treatments were measured for SSC and juice percentage (Table 5.3.2.1). Fruit weight averaged 67.8 g. The highest juice content was measured for the X treatment (58.0%) and the 2X treatment had the lowest juice content (51.3%). The 1/2X treatment had the highest SSC (10.8 °Brix) compared with the 2X treatment which had the lowest SSC (10.5 °Brix). Average acidity was 1.22% and ratio was 9.3.

There were no significant differences in fruit quality at Greendale (Table 5.3.2.2). The average fruit weight was lower (66.4 g) than in 2002-2003. Juice content was higher (57.5%) than in the previous season, SSC averaged 10.7 °Brix, acidity was 1.08%, and ratio was 10.1, which was lower than in the 2002-2003 season.

Internal quality of fruit sampled on a two-weekly basis. At Backsberg, fruit size on 30 April 2004 was higher than that of the tagged fruit (Table 5.3.2.3). However, fruit size, fruit weight, juice content, SSC, acidity and ratio did not significantly differ among treatments. However, acidity tended to be lower ($P = 0.0863$) for the 2X treatment than for the 1/2X treatment, with a resultant tendency to higher ratio ($P = 0.1387$) for the 2X treatment.

At Greendale, average fruit diameter was higher than for the tagged fruit (Table 5.3.2.4). The 1/2X had larger fruit (53.7 mm) than the X treatment (52.0 mm), but fruit weight was not significantly different among treatments. Juice content, SSC, acidity and ratio did not significantly differ among treatments.

Conclusion

It was expected that fruit from trees receiving the 2X treatment would be larger than fruit from other treatments because trees received more water. Although SWC was relatively low, treatment differences in ψ_{stem} were not sufficiently large to cause differences in sugar accumulation and other fruit quality variables.

A statistical difference in fruit quality among treatments was not observed. A lower SSC value was expected for the 2X treatment due to a dilution effect that occurs when more water is available.

Therefore, to demonstrate differences in plant water status, differences in ψ_{stem} should be of a sufficient intensity of between 0.16-0.3 MPa. This difference should be maintained for a sufficient duration of between 4 and 6 weeks. Differential irrigation should be applied relatively early in fruit development. This must be during the sugar accumulation stages which is between middle December to end of January.

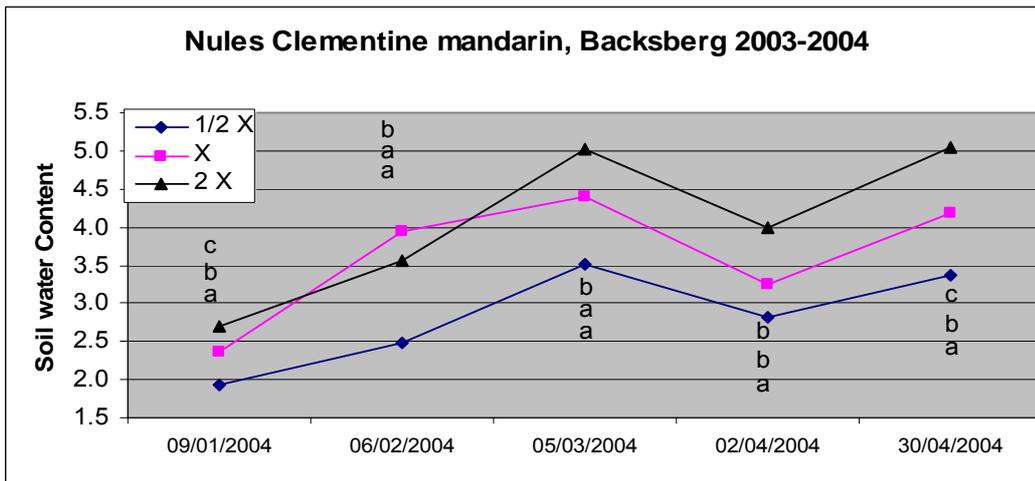


Figure 5.3.2.1. Gravimetric soil water content of Nules Clementine mandarin at Backsberg.

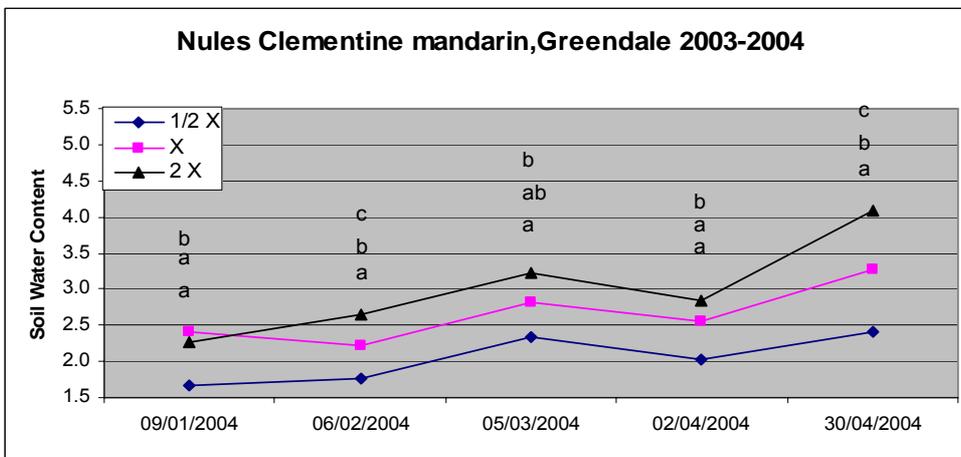


Figure 5.3.2.2. Gravimetric soil water content of Nules Clementine mandarin at Greendale.

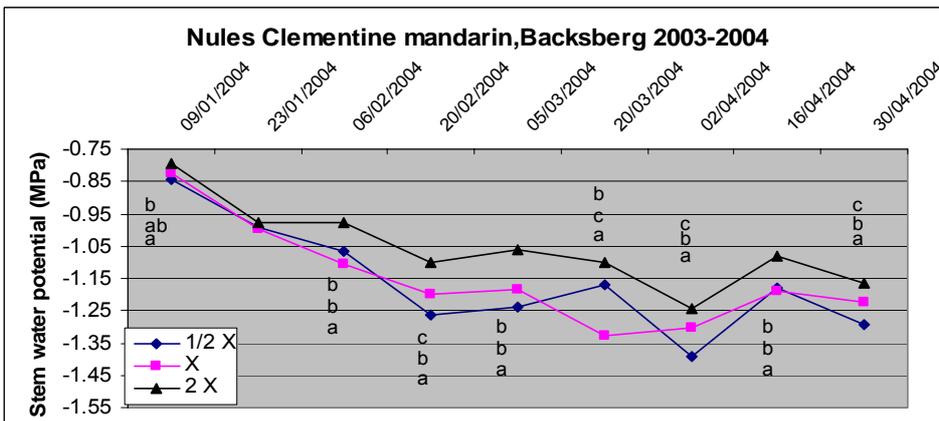


Figure 5.3.2.3. Stem water potential (MPa) measurements taken of Nules Clementine mandarin over a period of 5 months at Backsberg. Leaves were bagged on the west side of trees before sunrise and measurements were taken from 12h00. There were 6 replicates with 2 leaves in each replicate.

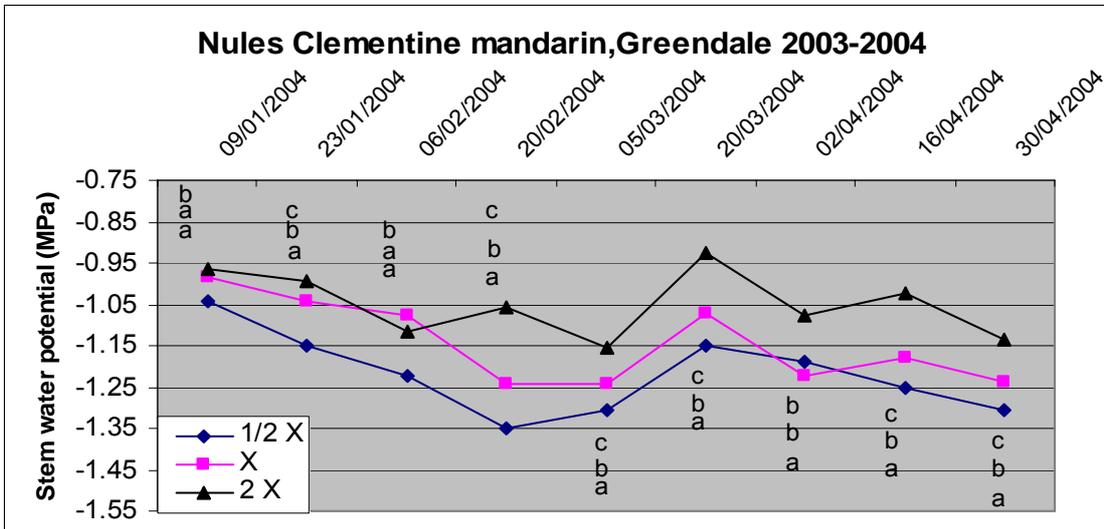


Figure 5.3.2.4. Stem water potential (MPa) measurements taken of Nules Clementine mandarin over a period of 5 months at Greendale. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 11h00. There were 6 replicates with 2 leaves in each replicate.

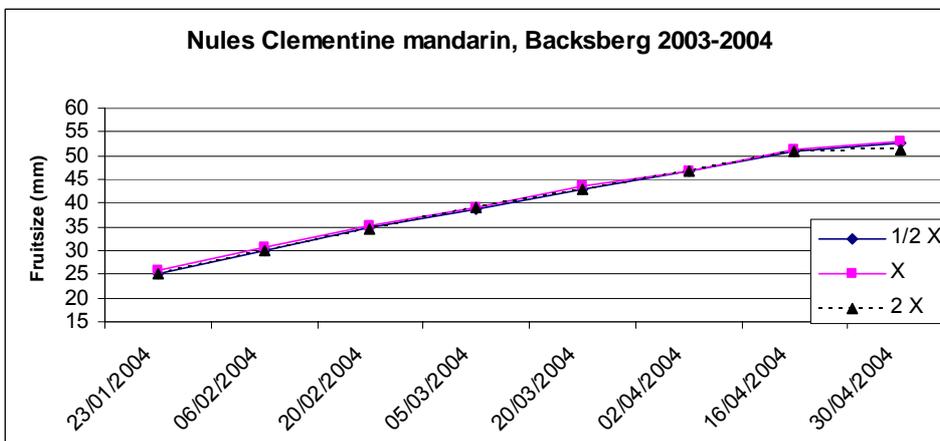


Figure 5.3.2.5. Tagged fruit size measurements of Nules Clementine mandarin over a period of 5 months at Backsberg.

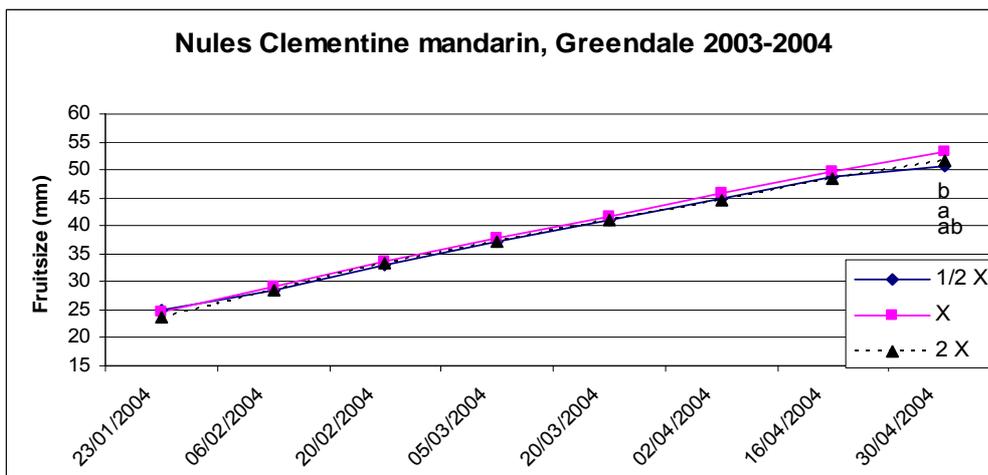


Figure 5.3.2.6. Tagged fruit size measurements of Nules Clementine mandarin over a period of 5 months at Greendale.

Table 5.3.2.1. Fruit quality of tagged Nules Clementine mandarin fruit harvested on 7 May 2003 at Backsberg.

Treatment	Fruit size		Fruit weight		Juice content		SSC		Acid		Ratio	
1/2 X	52.7	a	69.2	a	55.7	ab	10.8	a	1.20	a	9.33	a
X	53.1	a	68.8	a	58.0	a	10.7	ab	1.10	a	9.88	a
2 X	51.4	a	65.6	a	51.3	b	10.5	b	1.35	a	8.55	a
p-value	0.454		0.7486		0.0466		0.0561		0.4384		0.3914	
lsd	2.7682		11.1		5.2762		0.2824		0.4063		2.0151	

Table 5.3.2.2. Fruit quality of tagged Nules Clementine mandarin fruit harvested on 7 May 2003 at Greendale.

Treatment	Fruit size		Fruit weight		Juice content		SSC		Acid		Ratio	
1/2 X	50.6	b	61.7	a	57.4	a	10.7	a	1.15	a	9.66	a
X	53.3	a	71.3	a	57.6	a	10.8	a	1.01	a	10.81	a
2 X	51.6	ab	66.4	a	57.5	a	10.6	a	1.09	a	9.77	a
p-value	0.008		0.2706		0.9912		0.7005		0.3849		0.2659	
lsd	1.7034		12.1		4.0308		0.5197		0.213		1.5936	

Table 5.3.2.3. Fruit size and internal quality of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Fruit size						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	46.2	b	49.9	b	53.1	a
X	46.9	ab	52.2	a	52.5	a
2 X	47.6	a	53.0	a	53.6	a
p-value	0.1451		0.0001		0.4618	
lsd	1.4145		1.4356		1.6778	
Fruit weight						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	46.8	b	58.1	b	67.9	a
X	49.5	ab	65.5	ab	66.0	a
2 X	52.1	a	69.3	a	72.2	a
p-value	0.072		0.029		0.457	
lsd	4.5		8.1		10.3	
Acidity						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	2.20	a	1.47	a	1.27	a
X	2.02	a	1.38	a	1.16	ab
2 X	1.90	a	1.34	a	1.05	b
p-value	0.2976		0.5235		0.0863	
lsd	0.3991		0.2462		0.1954	
Juice content						
Treatment	02/04/2004		16/04/2004		30/04/2004	

1/2 X	45.5	a	54.1	a	54.7	a
X	48.4	a	54.1	a	56.2	a
2 X	48.4	a	53.3	a	56.4	a
p-value	0.3784		0.8184		0.386	
lsd	5.1512		2.7704		2.9137	
SSC						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	11.1	a	10.8	a	11.2	a
X	11.2	a	10.8	a	11.0	a
2 X	10.8	a	10.6	a	10.8	a
p-value	0.4025		0.3155		0.3507	
lsd	0.5942		0.2917		0.5528	
Ratio						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	5.16	a	7.41	a	8.94	b
X	5.62	a	7.95	a	9.53	ab
2 X	5.75	a	7.95	a	10.44	a
p-value	0.4637		0.4837		0.1387	
lsd	1.0506		1.0893		1.4851	

Table 5.3.2.4. Fruit size and internal quality of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Fruit size						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	46.9	a	51.3	a	53.7	a
X	46.5	a	49.6	b	52.0	b
2 X	46.2	a	50.5	ab	53.1	ab
p-value	0.5909		0.1119		0.1291	
lsd	1.5039		1.5841		1.6756	
Fruit weight						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	523.4	a	657.2	a	754.2	a
X	499.5	a	593.3	a	679.0	a
2 X	491.8	a	641.7	a	726.5	a
p-value	0.6007		0.3865		0.2713	
lsd	72.633		98.671		95.051	
Acidity						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	1.81	a	1.28	a	1.00	a
X	1.91	a	1.40	a	1.10	a
2 X	1.94	a	1.26	a	0.97	a

p-value	0.5575		0.3789		0.233	
Lsd	0.2768		0.2226		0.149	
Juice content						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	48.7	a	56.3	a	58.0	a
X	51.2	a	56.7	a	58.6	a
2 X	49.2	a	56.7	a	59.4	a
p-value	0.5114		0.9667		0.4705	
lsd	4.6471		3.6063		2.5256	
SSC						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	10.4	a	10.5	a	10.9	a
X	10.6	a	10.6	a	11.0	a
2 X	10.4	a	10.4	a	10.9	a
p-value	0.6337		0.9089		0.9228	
lsd	0.6152		0.5439		0.7092	
Ratio						
Treatment	02/04/2004		16/04/2004		30/04/2004	
1/2 X	5.81	a	8.24	a	13.39	a
X	5.60	a	7.65	a	10.12	a
2 X	5.34	a	8.32	a	11.24	a
p-value	0.4146		0.5522		0.4672	
lsd	0.7834		1.3917		5.7065	

5.4 PROJECT: RIND CONDITION

Project Co-ordinator: John Bower (UKZNP)

5.4.1 Project summary

The project on rind condition has concentrated mainly on the pre and postharvest factors affecting rind disorders. The disorders were primarily limited to creasing and rind breakdown, although some work on premature aging was included.

In the case of creasing (5.4.2) various techniques were used to determine the mineral content of creased versus non-creased fruit. It was found that creased fruit from both the inland area of Ohrigstad as well as the more coastal region of Patensie, showed a negative correlation with Ca. It is suggested that this confirms other work, which implicates calcium pectate structure of cell walls. N₂, Mn and B also appeared to be deficient in creased fruits. The ratios of Mg/Ca and K/Ca may also be important. It was confirmed that small fruits are more likely to show creasing than large fruits and microscopy demonstrated that creasing is caused by cells detaching from each other. The use of GA decreased creasing which is again consistent with previous work. A gradient of mineral elements was found across the albedo, also indicating that the disorder starts on the outer side of the rind and progresses inwards. Future work needs to investigate field applications of calcium and other nutrients, and it is suggested that further work be conducted on the use of plant growth regulators.

A number of experiments considered aspects of rind breakdown. Experiment 758 (5.4.3) considered the effect of inside and outside fruit on later rind breakdown development of Clementine mandarins after storage

at 7.5°C and -0.6°C. Inside fruit was paler in colour, and showed more rind breakdown in storage. The lower temperature seemed to have some advantages. Attempts at anatomical work were not entirely successful, but techniques were developed which may be more successful in further work. Mineral analysis of inside and outside fruit showed some differences, but these will need to be confirmed in the coming season before a meaningful discussion is possible. Work on pigments, proline and rind sugars is still to be done. It is recommended that the work be repeated in the next season, but results thus far clearly indicate the complex nature of rind breakdown, with canopy position being of importance, but the effect may be modified by climate and postharvest conditions. Research by ExperiCo (5.4.4) did not show that fruit position in the canopy influenced rind breakdown, nor that mineral constituents, carotenoids or anti-oxidant capacity (which also did not seem useful as a rind breakdown predictor) were useful. Temperature, however, had an influence, with the higher storage temperature of 7.5°C being more conducive to rind breakdown (as was the case of Exp. 758). Where physiological parameters were compared between 'Nules' and 'Oroval' (5.4.5) most measured parameters were too variable to link to rind breakdown. Nevertheless, 'Nules' appeared to be more susceptible to rind breakdown than 'Oroval', while the latter were more susceptible to postharvest diseases and puffiness. Of note, is that physiological parameters were measured at harvest, and it is suggested that conditions during fruit development may be of more importance.

Further work on postharvest conditions which may relate to rind breakdown was conducted on Valencias from Limpopo (5.4.6). The effects of transport operations from the production area to Cape Town on fruit temperature was investigated, followed by the levels of CO₂ build-up during shipping to the USA and the UK. Temperature profiles during transport to the port indicated that although chilling injury temperatures did not occur (4°C) extremely high temperature (almost 40°C) did. There was considerable variation in temperature. This may have serious effects on rind condition. The CO₂ levels during shipping were found to be very high, particularly shipping to the USA, where levels reached 6500 ppm. However, it is believed that these are not high enough to cause rind damage. Further work on the effects of high postharvest CO₂ on puffiness and rind breakdown in Clementine mandarins (5.4.8) showed no negative effects. However, the CO₂ levels used in this laboratory-based trial were lower than those found in shipment, and it will be necessary to repeat the work using higher levels.

The effects of cooling rate were studied in Exp 766 (5.4.7). It was found that cooling to 3.5°C by forced air resulted in higher levels of rind damage than static cooling.

The work conducted throughout this project has indicated the complexity of rind disorders. These are clearly both pre and postharvest, which interact in a dynamic manner. Preharvest factors appear to interact throughout fruit development and solutions to the problems will not necessarily be found by measuring factors at harvest alone. While progress is being made in understanding some of the pre-disposing factors for rind disorders, the complexity makes it unlikely that rapid and simplistic solutions will be found.

Projekopsomming

Die projek het oorheersend op voor en na-oes faktore wat skilafwykings beïnvloed, gekonsentreer. Die afwykings is meestal op kraakskil en skilafbraak toegesptis, alhoewel van die werk op vroeër skil veroudering gedoen was.

In die geval van kraakskil (5.4.2) verskillende tegnieke was gebruik om die minerale inhoud van vrugte met of sonder kraakskil, te meet. Dit is gevind dat vrugte vanaf die binnelandse area van Ohrigstad, sowel as dié van Patensie, nader aan die kus, 'n negatiewe korrelasie met kalsium gehad het. Dit is voorgestel dat dit bevestig vorige resultate, wat aangedui het dat die pektaat struktuur van selwande belangrik is. N₂, Mn en B is ook as laag gevind in vrugte met kraakskil. Die verhoudings van Mg/Ca en K/Ca mag ook belangrik wees. Dit is ook bevestig dat klein vrugte meer kraakskil as groot vrugte wys, en mikroskopie het aangedui dat kraakskil gebeur as gevolg van selle wat los van mekaar kom. Die gebruik van GA het ook soos vorige werk resultate getoon, en 'n gradient van minerale inhoud vanaf buite tot binne die skil dui aan dat die kraakskil proses ontwikkel van buite tot binne. Toekomstige werk sal op boord aanwendings van kalsium en ander elemente wees, en dit is ook voorgestel dat groeireguleerders gebruik word.

Aantal eksperimente het op skilafbraak toegesptis. Eksperiment 758 (5.4.3) het na die effek van binne en buite vrugte op later skilafbraak by Clementines teen 7.5°C of -0.6°C opberging gebruik. Binne vrugte was ligter van kleur, en het meer skilafbraak gewys. Vrugte teen die laer temperatuur het beter gevaar. Werk op die anatomie was nie suksesvol nie, maar tegnieke was ontwikkel wat in die toekoms kan gebruik word. Analiese van mineraal inhoud het verskille getoon, maar meer werk is nodig voor enige gevolgtrekkings kan gemaak word. Werk op pigmente, prolien en suikers moet nog gedoen word, maar resultate dusver toon dat die probleem kompleks is, met ligging van vrugte binne die boom belangrik, maar klimaat en na-oes toestande ook van belang. Verdere werk deur ExperiCo (5.4.4) kon nie die effek van vrug posisie binne die

boom bewys nie, en mineral inhoud, karotenoïdes en anti-oksidente het ook geen betekenisvolle aanduidings bewys nie. Temperatuur was nietermin belangrik, met weereens die hoër temperatuur van 7.5°C meer genyg om skilafbraak te bevorder. Fisiologiese parameters is gebruik om die verskille tussen 'Nules' en 'Oroval' (5.4.5) wat skilafbraak betref, aan te dui. Die meeste syfers was te wisselvallig gewees, maar 'Nules' was meer vatbaar vir skilafbraak, en 'Oroval' meer genyg om na-oes siektes en pofferigheid te toon. Dit is merkbaar dat die fisiologie teen oes gemeet was, en dit is voorgestel dat toestande tydens vrugontwikkeling van meer belang kan wees.

Verdere werk op na-oes toestande wat skilafbraak kan beïnvloed was op Valencias vanaf Limpopo gedoen (Exp 759, 5.4.6). Die effek van die vervoer vanaf produksiegebied tot op Kaapstad is bekyk, Daarna, is die CO₂ vlakke tydens verskeping tot in die VSA en VK gemeet. Temperatuur tydens trok vervoer het gewissel vanaf 4°C (wat nie koueskade behoort te gee nie) tot amper 40°C. Hierdie wisselvallige temperature en die temperatuur wat so hoog gegaan het, kon ernstige gevolge gehad. Die CO₂ vlakke het tot 6500 dpm gegaan, maar dit is geglo dat al was dit hoog gewees, die vlakke nie hoog genoeg was om skade te doen nie. Nog werk op die effek van CO₂ op pofferigheid by Clementines (Exp 780, 5.4.8) het geen nadelige effek gehad nie, maar die vlakke van CO₂ was baie laer as wat in die praktyk gevind was, en verdere werk sal moet gedoen word. Die effek van verkoelingstempo was gemeet in exp. 766 (5.4.7). Dit is gevind dat verkoeling teen 3.5°C meer skade aan vrugte gedoen het as dit deur geforseerde verkoeling teenoor statiese verkoeling plaasgevind het.

Die werk van hierdie projek het bewys hoe kompleks skilafwykings kan wees, met 'n dinamiese interaksie van voor en na-oes faktore. Vooroes faktore kan 'n rol speel gedurende die hele vrugontwikkelings tydperk, en dus is dit onmoontlik dat vinnige en eenvoudige oplossings gevind sal word.

5.4.2 Citrus creasing

Experiment AH 565 by C Kaiser & E. de Vries (UP), J. Bower (UKZNP)

Opsomming

Kraakskil is een van die ergste fisiologiese afwykings van sitrus in Suid Afrika, met oes verliesse van tot 50%. Kraakskil is 'n afwyking van die wit gedeeltes van die skil. Mineraal elemente, sel struktuur en groot en klein vrugte met en sonder kraakskil vanaf Ohrigstad en Patensie was bekyk. Kalsium het 'n belangrike rol gespeel, met vermindering van kraakskil met hoër vlakke. Saam met kalsium, hou pektiensuur die selle meer vas aan mekaar. Die rol van Mg/Ca en K/Ca is ook belangrik gevind. Ander elemente soos N, Mn en B was laag in vrugte met kraakskil, en Zn en Fe in vrugte daarsonder. Die binnekant van die albedo het hoër element konsentrasies as die buitekant, en klein vrugte was meer geneig om kraakskil te wys as groot vrugte. Kleiner sel area was positief gekorreleer met kraakskil, en selle vanaf sulke vrugte word nie gerek nie.

Introduction

Citrus (*Citrus sinensis* L. Osbeck) is believed to be native to the subtropical and tropical regions of the Malaysian Archipelago (Webber, 1946) and South East Asia including South China, north-eastern India and Burma (Spiegel-Roy & Goldschmidt, 1996). Creasing ranks among the worst physiological disorders affecting citrus fruit in South Africa (also in other parts of the world) and in some years has accounted for yield losses of up to 50%. For instance, creasing posed a big problem from the early years especially in the Sundays River Valley in the Eastern Cape, South Africa. Normal fruit losses range between 5 and 10% early in the picking season and may be as much as 50% later in the season. Navels seem to be more prone to the disorder, but Valencias are not exempt.

Creasing is characterized by line fractures in the rind. These fractures develop across cell layers in a radial direction in the albedo, which is normal to the direction of stress and are expressed as creases in the flavedo. Due to the lack of mechanical strength of the fruit, postharvest splitting sometimes occurs (Gilfillan & Stevenson, 1977), which may cause postharvest rot (Anonymous, 1992). Creasing, which is a shearing in the albedo in more than one place seems to be positively correlated with fruit maturity (Anonymous, 1992). Factors such as rootstocks and nutrition also play a significant role in creasing. Sweet orange rootstocks accounted for the lowest incidence of creasing on 'Bellamy' Navels whereas 'Rangpur' lime accounted for the highest incidence (Treeby *et al.*, 1995). Experiments showed very little relationships between peel thickness and occurrence of creasing. The case with fruit size is controversial. In South Africa researchers observed a strong tendency for smaller fruit to show more creasing while Australian researches found an opposite result. Low levels of N have been shown to increase the amount of creasing, whereas, high P applications resulted in a higher incidence of creasing (Le Roux & Crous, 1938).

The role of calcium has received a particularly large amount of attention over the years and several studies have concluded that calcium plays a pivotal role in citrus rind creasing. Braccini *et al.* (1999) demonstrated clearly the role of calcium in complexing with uronic acids as did Debon & Tester (2001) and Norziah *et al.* (2001). Ratios of K/Ca and Mg/Ca have also been positively correlated with creasing of the fruit, whereas high values of Ca in the fruit were negatively correlated with creasing (Storey *et al.*, 2002). Fruit dipped in a calcium solution throughout the duration of the fruit growth cycle resulted in no creasing, whereas undipped fruit had a substantial amount of creasing (Treeby & Storey, 2002). There is also a wealth of literature on associations of pectin, its precursor uronic acid and microelements (Loaïc, *et al.*, 1996; Yamamoto *et al.*, 1997; Debon & Tester, 2001). Application of gibberellic acid under specific conditions has been shown to reduce creasing to some extent (Bevington, 1973; Embleton *et al.*, 1973). It is thought that this is due to gibberellic acid increasing the viability of the peel by rendering tissue more compact, thus delaying the conversion of amino acids into proteins later in the season.

In the different previous trials conducted, there have been controversies on nutrient levels and on nutrients crucial in creased vs. non-creased citrus fruits. This report attempted to resolve some of those controversies. Nutrient levels in creased and non-creased fruit, sampled from Patensie and Ohrigstad, were studied by four different methods to indicate nutrients crucial in creasing. Effort was made to select best method/s of nutrient analysis for this purpose. Cell size and structures of both creased and non-creased fruits are studied since there was no detailed previous study in this regard. A preliminary observation on the effects of spraying gibberellic acid on citrus creasing is also made.

Materials and methods

Area description

Navel orange fruit were sampled from two different sites *viz.*, Ohrigstad (Limpopo province, and Patensie (Eastern Cape province). Gibberellic-acid-sprayed Valencia oranges were obtained from Ohrigstad.

Plant materials

Twenty-one-year-old Valencia orange trees (for gibberellic acid treatment) and Navel orange trees (for all the other treatments) were selected from Ohrigstad for this study in the 2003/2004 season. Twenty-two-year-old Navel orange trees were also selected from Patensie at the same time. The trees from which the fruits were harvested are uniform in vigour and size and also were under similar tree management practises.

Technique

Fruit was sampled randomly within a block from trees of known creasing incidence and from trees bearing normal fruits. Two fruits per tree (either with or without creasing) were harvested at head height from the outer canopy of the respected trees within the block. In the case of gibberellic acid treatments, 10-ppm gibberellic acid was sprayed on the treatment trees 6 weeks before harvesting and fruit from sprayed trees were compared with creased fruit, which were not sprayed. The experiment was designed in a randomised block with three replications. Three trees were included per plot. In the case of EDX, Raman and FT-IR analyses, for fruits sampled from Ohrigstad, treatments were factorial combinations of two fruit sizes (Large and small) each at two levels for the presence of physiological problems (creased and non-creased) and two positions of the albedo (upper and lower side). On the other hand, for fruits sampled from Patensie, treatments were factorial combinations of two levels for the presence of physiological problems (creased and non-creased) each at two positions of the albedo (upper [outer] and lower [inner] side). The treatments for lab analysis consisted of single factor comparisons between creased and non-creased as well as large creased and small creased fruits sampled from Ohrigstad. In the case of fruits sampled from Patensie, the treatments were creased and non-creased albedos. With respect to the cell size study, in the case of fruits sampled from Ohrigstad, treatments were factorial combinations of two fruit sizes (large and small) each at two levels for the presence of physiological problems (creased and non-creased). Gibberellic acid spraying treatments consisted of single factor comparisons between sprayed and non-sprayed fruits. Treatments for fruits sampled from Patensie, for the cell size study, consisted of single factor comparisons of creased and non-creased fruits. Cell structures of large/small, sprayed/non-sprayed with gibberellic acid and creased/non-creased fruit peel segments were studied after observation under light microscopy.

Data recorded

Nutrient analysis using Scanning Electron Microscopy (SEM):

Creased and non-creased orange peel albedos were freeze dried at -80°C . Dried samples were mounted and sputter-coated with Gold in a Polaron E 5200 coating unit (Polaron Equipment, Watford, England) to a thickness of ± 10 nm. The albedos were mounted on aluminium stubs covered with double-sided carbon tape. Mounted orange peel albedos were sputter-coated as above. Prepared samples were then viewed in a JEOL JSM 840 Scanning Electron Microscope (JEOL, Tokyo, Japan) operated at an accelerating voltage of 20 kv.

Nutrient levels detected by laboratory chemical analysis of tissues:

Albedos of creased and non-creased citrus peels were excised, freeze dried at -80°C and ground. The ground samples were analysed for element mineral distribution by SGS laboratories, South Africa Limited.

Nutrient analysis using Raman spectroscopy:

For the series of Raman measurements, each sample was placed on the stage of an Olympus microscope and excited with the 514.5 nm line of a Coherent Innova 300 Argon ion laser. The scattered light was dispersed and recorded by means of a Dilor XY multichannel Raman Spectrometer equipped with a liquid nitrogen-cooled CCD detector.

Nutrient analysis using infrared spectroscopy:

Albedos of creased and non-creased citrus peels were excised, freeze dried at -80°C and ground. Only 2 mg of each ground sample was mixed with 100 mg of KBr and pressed under 6 tonnes for two minutes in making the disc. The FT-IR (Fourier Transform Infrared) spectroscopy was applied to all ground samples. The infrared spectra were recorded using an evacuated chamber of Bruker IFS 113v FT-IR (spectra resolution 2 cm^{-1}) using KBr discs as matrices.

Cell structure study using Light Microscopy:

Orange peel segments were prepared and fixed in a mixture of 2½% formaldehyde and 2½% glutaraldehyde in 0.075 M phosphate buffer. After about 24 hrs, the samples were dehydrated in an increasing ethanol concentration series (30%, 50%, 70%, 90%, 100%, 100%, 100% 10 min. each), immersed in 100% ethanol for 24 hrs and rinsed 3 times, 10 min. each, in 0.075 M phosphate buffer. The samples were then embedded by infiltrating with 50% LR white in ethanol for 1 h followed by infiltrating with pure LR white for 4 h and then polymerising the samples at 60°C for 24 h. Sections of 0.5-1.0 μm in thickness were made with an ultramicrotome (Reichert Ultracut E, Vienna, Austria) and transferred onto droplets of water on a specimen slide. The sections were dried on hot plate at 70°C and stained in Toluidine blue (0.2% Toluidine blue in 0.5% NaCO_3). The cell structures were then viewed with Nikon Optiphot microscope (Nikon Instech Co., Kanagawa, Japan). Pictures of the cell structures were taken with a Nikon DXM 1200 digital camera (Nikon Instech Co., Kanagawa, Japan) fitted on the microscope. The camera has Nikon ACT-1 ver. 2 software (Nikon Instech Co., Kanagawa, Japan)

Cell size determination:

The area and width of cells were determined from the pictures obtained by the cell structure study using Light Microscopy of different treatments. Measurements of cell size were made using image analyser (UTHSCSA Image Tool for Windows 3.00).

Statistical analysis:

Differences between treatments were determined with analysis of variance (ANOVA) using MSTATC statistical package (MSTATC, 1989). Whenever significant differences were detected, means were separated using Least Significant Difference (LSD) at 5% level of significance.

Results

Ohrigstad

Simple comparisons on nutrient levels were made between albedos of non-creased fruit vs. creased fruit (Table 5.4.2.1) and between large creased fruit vs. small creased fruit (Table 5.4.2.2). The aim of comparison between large and small creased fruit to see if there was any difference in the nutrient level between large and small fruit even if both are creased. The unit for N, P and Ca is g/100 g while the unit for the remaining nutrients analysed is ppm. Non-creased fruits (Table 5.4.2.1) had significantly higher Ca, B, N and Mn levels as compared with creased fruits. On the other hand, creased fruits had higher K, Mg, Fe and Zn levels as compared with large non-creased fruits. There was no significant difference for the other elements analysed between the albedos of creased and non-creased fruits.

Table 5.4.2.1. Nutrient level comparisons between albedos of non-creased and creased fruits from Ohrigstad (P=0,05).

Nutrients	Treatments	
	Non-creased albedo	Creased albedo
N	0.54a	0.47b
P	0.04a	0.03a
Ca	0.58a	0.52b
K	0.31b	0.39a
Mg	0.04b	0.05a
Na	0.02a	0.02a
Fe	20.55b	27.35a
Cu	2.30a	2.20a
Mn	3.05a	1.70b
Zn	12.80b	15.05a
B	23.30a	17.42b
Mo	0.07a	0.14a

A comparison was also made between albedos of two creased fruits (Table 5.4.1.2) varying only in size (large and small). It was observed that the contents of Ca and Mo were significantly higher in the albedos of large creased fruits and Na, Cu, Zn and B in the albedos of small creased fruits. There was no significant difference for the other elements analysed.

Table 5.4.2.2. Nutrient level comparisons between albedos of large creased and small creased fruits from Ohrigstad (P=0,05).

Nutrients	Treatments	
	Large creased albedo	Small creased albedo
N	0.47a	0.50a
P	0.03a	0.04a
Ca	0.52a	0.46b
K	0.39a	0.34a
Mg	0.05a	0.05a
Na	0.02b	0.04a
Fe	27.35a	32.25a
Cu	2.20b	3.60a
Mn	1.70a	2.65a
Zn	15.05b	20.70a
B	17.42b	26.06a
Mo	0.14a	0.03b

Patensie

Comparisons of nutrients have been made between non-creased vs. creased albedos (Table 5.4.2.3). As in the case of Ohrigstad, the units for N, P and Ca is g/100 g while the unit for the remaining nutrients analysed is ppm. The analysis results showed that N, Ca, Mn and B were significantly higher in the albedos of non-creased fruits while K, Mg, Fe, Zn, P and Cu were significantly higher in the albedos of creased fruits. There was no significant difference for the other elements analysed between the albedos of creased and non-creased fruits.

Comparisons between the two sites

The same trend of nutrient levels as in the case of creased and non-creased fruits sampled from Ohrigstad was observed in fruits sampled from Patensie. In both sites, albedos of non-creased fruits had higher levels of N, Ca, Mn and B. Similarly, albedos of creased fruits contained higher levels of K, Mg, Fe and Zn.

Table 5.4.2.3. Nutrient level comparisons between albedos of non-creased and creased fruits from Patensie (P=0,05).

Nutrients	Treatments	
	Non-creased albedo	Creased albedo
N	0.60a	0.50b
P	0.01b	0.02a
Ca	0.39a	0.25b
K	0.32b	0.42a
Mg	0.03b	0.05a
Na	0.05a	0.05a
Fe	27.60b	55.65a
Cu	1.55b	3.55a
Mn	4.70a	3.60b
Zn	11.90b	16.35a
B	28.06a	21.55b
Mo	0.02a	0.02a

Results from Nutrient levels detected by SEM analysis

This method didn't detect some of the nutrients that were analysed by laboratory methods and one-to-one comparison of the nutrients analysed by the two methods is not possible. The results for the different comparisons made are outlined below and the unit for all the nutrients is in Wt %.

Ohrigstad

In the analysis of fruits sampled from Ohrigstad, comparisons were made between large and small fruits of creased and non-creased albedos including observations for any nutrient variation between upper and lower parts of albedos. There was a significant difference for the interaction effects between size of fruits (large/small), presence of creasing and position of the albedo analysed (upper and lower side) with respect to levels of different nutrients analysed (Table 5.4.2.4).

The lower side albedo of large non-creased fruits had a significantly higher Ca level as compared with the other treatments. In large creased fruits, the lower side albedo had a significantly higher Na, Cu and Zn level than all the other treatments. The lower side albedo of large creased fruits had a significantly higher K content as compared with all other treatments except upper side albedo of small creased fruits. No clear trend was observed for the other elements analysed.

Table 5.4.2.4. Nutrient level comparisons after EDX analysis between large and small fruits of creased and non-creased albedos at different positions for fruits sampled from Ohrigstad. Means followed by different letters in the same row are significantly different by LSD test at $P < 0.05$.

Nutrients	Large fruit				Small fruit			
	Creased		Non-creased		Creased		Non-creased	
	Upper side	Lower side						
Na	0.00c	2.26a	0.02c	1.38b	0.00c	0.00c	0.01c	0.02c
Mg	2.22a	2.22a	2.09a	0.02b	0.03b	2.37a	0.00b	2.69a
P	0.86b	0.53bcd	0.00d	0.22bcd	0.91b	3.50a	0.02cd	0.01d
S	2.74b	2.59bc	0.03d	2.55bc	1.60c	6.50a	0.02d	0.02d
K	40.32b	47.36a	35.51c	26.30d	44.72ab	42.35b	29.62d	35.39c
Ca	29.46d	23.58e	43.26c	58.21a	21.58e	28.56d	49.57b	47.05bc
Cu	0.08c	3.08a	0.03c	0.00c	2.04b	1.98b	0.02c	0.00c
Zn	0.00d	8.00b	0.00d	0.00d	2.50c	13.40a	0.00d	0.00d

Patensie

Comparisons were made between creased and non-creased fruits including observations for any nutrient variation between upper and lower parts of albedos. There was a significant difference for the interaction effects between presence of creasing and position of the albedo (upper/lower sides) analysed (Table 5.4.2.5). The results showed that Na, P, K, Cu and Zn were constantly and significantly higher in the lower side albedo of creased fruits than the other treatments. The lower side of non-creased fruit albedos had a significantly higher Ca levels than the other treatments except compared with the result for upper side albedo of non-creased fruits.

The main treatment effects of presence of creasing and the position of the albedo analysed, but not the interaction effects, significantly affected the levels of Mg and S (Table 5.4.2.6). Creased fruit albedos had significantly higher Mg and S levels as compared with non-creased fruit albedos. The lower side of the albedo, similarly, had significantly higher Mg and S levels as compared with the upper side of the albedo.

Table 5.4.2.5. Nutrient level comparisons after EDX analysis between creased and non-creased fruits at different positions of the albedo for fruits sampled from Patensie. Means followed by different letters in the same row are significantly different by LSD test at $P < 0.05$.

Nutrients	Creased		Non-creased	
	Upper side	Lower side	Upper side	Lower side
Na	1.89b	30.05a	1.33b	0.00b
P	3.44b	19.16a	3.47b	3.51b
K	24.17b	40.20a	20.77b	13.65c
Ca	30.13b	11.38c	51.63a	59.41a
Cu	1.98b	6.63a	0.41c	0.21c
Zn	0.06c	8.99a	7.23b	0.29c

Table 5.4.2.6. Effects of presence of physiological problems (averaged across position of the albedo) and position of the albedo analysed (averaged across presence of physiological problems) on the nutrient levels of Mg and S. Means followed by different letters in the same column are significantly different by LSD test at $P < 0.05$.

Treatments	Nutrients	
	Mg	S
Presence of physiological problems		
Creased	3.05a	7.96a
Non-creased	1.55b	3.05b
Position of the albedo		
Upper side	1.55b	3.94b

Lower side	3.05a	7.09a
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Comparisons of the two sites

For fruits sampled from Patensie, there was a clear similar trend in that the lower side of creased fruit albedos had higher Na, Cu, K and Zn at both sites. On the other hand, the lower side of the albedos of non-creased fruits had a significantly higher Ca content.

Results from Nutrient levels detected by Raman

Nutrients levels from creased/non-creased, upper/lower position of the albedo, as well as large/small fruit albedos could not be detected by Raman.

Results from Nutrient levels detected by FT-IR

Unlike in the case of Raman, nutrient levels were detected from the different treatments. However, all the results for the various combinations of treatments and sites were the same. Data were thus not meaningful and are not presented.

Comparisons of nutrient analysis methods

Since no reliable data were obtained by Raman and FT-IR methods, comparison of nutrient analysis methods were made between laboratory analysis and nutrient analysis using EDX.

From nutrients analysed in both methods (of both sites), there is an exact equivalence in that Ca is significantly higher in the albedos of non-creased fruits, whereas the levels of Cu, Zn and K are significantly higher in creased fruit albedos, albeit no size consideration in fruits was sampled for laboratory analysis.

Cell structure study using light microscope

After the materials were prepared (as described in the materials and methods) and viewed under light microscope, various cell structures were observed for the different samples gathered from both sites. In general, intact and well-structured cells were observed for large/small non-creased fruits and for fruits sprayed with gibberellic acid (Fig. 5.4.2.1a, 5.4.2.1b & 5.4.2.1c respectively). Non-creased fruits sampled from Patensie, showed similar intact cell structures (Fig. 5.4.2.3a). On the other hand distorted and detached cells were observed for large/small creased fruits and non-sprayed (creased) fruits with gibberellic acid from Ohrigstad (Fig. 5.4.2.2a, 5.4.2.2b & 5.4.2.2c respectively) and for those sampled from Patensie (Fig. 3b). Oil glands can sometimes be confused with creased cells. However, oil glands have definite outer cell layers surrounding the internal gland constituents unlike the hole formed after the albedo is creased (Fig. 5.4.2.4).

The cell structures of creased fruit peels sampled from both sites were similar. The same holds true for non-creased fruit peel cell structures sampled from both sites.

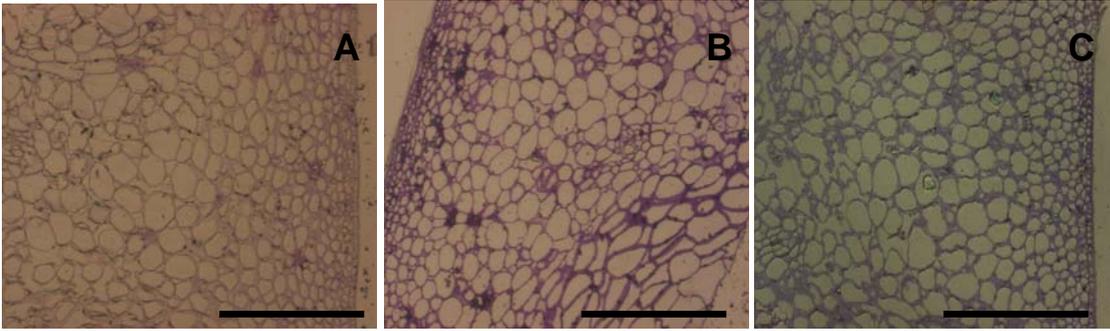


Fig. 5.4.2.1 Light micrographs for cross sections of citrus fruit peel showing intact and well-arranged structures for non-creased cells of large fruits (A), small fruits (B) and fruits sprayed with 10-ppm gibberellic acid (C) sampled from Ohrigstad. Scale bar $10^3\mu\text{m}$.

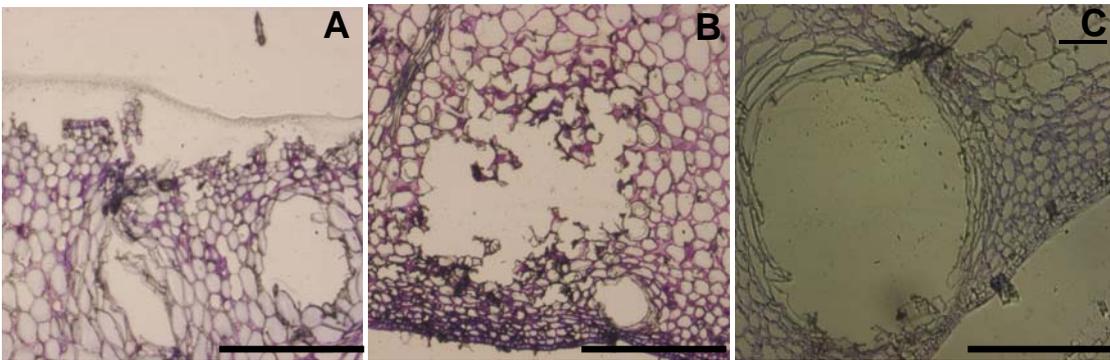


Fig. 5.4.2.2 Light micrographs for cross sections of citrus fruit peel showing dissociated and distorted structures for creased cells of large fruits (A), small fruits (B) and fruits not sprayed with 10-ppm gibberellic acid (C) sampled from Ohrigstad. Scale bar $10^3\mu\text{m}$.

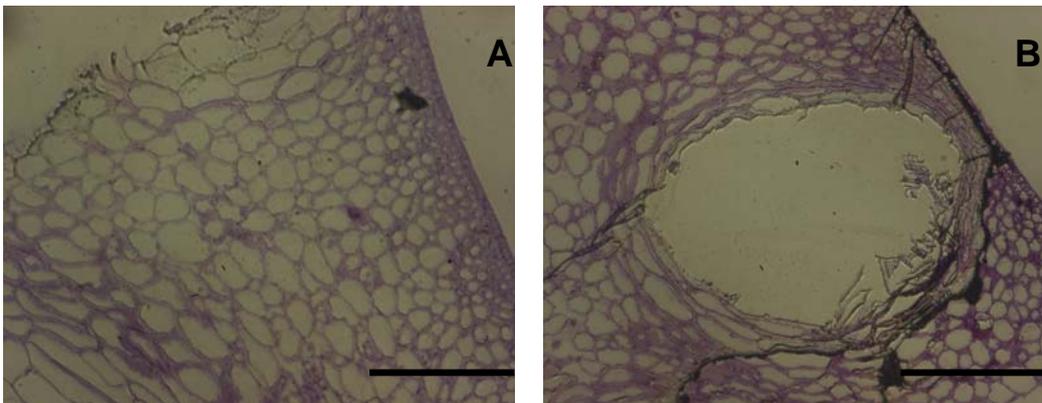


Fig. 5.4.2.3 Light micrographs for cross sections of citrus fruit peel showing cell structures of non-creased (A) and creased (B) fruits sampled from Patensie. Scale bar $10^3\mu\text{m}$.

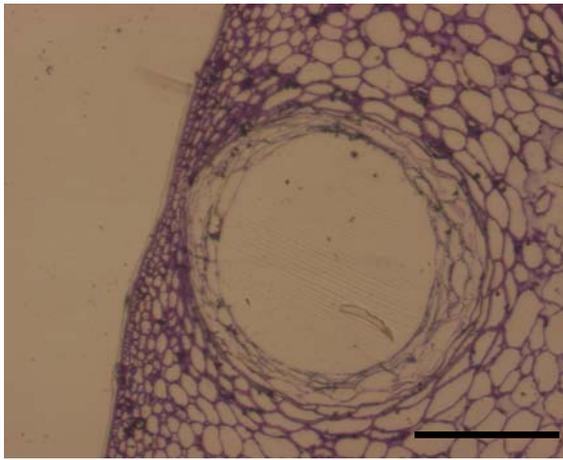


Fig. 5.4.2.4. Structure of a typical oil gland of citrus peel. Scale bar $10^3\mu\text{m}$.

Cell size determination

Area (μm^2) and width (μm) of cells from the different treatments were determined. The reason for determining cell width in addition of cell area was due to reduced width constantly observed on the pictures of creased fruits.

Ohrigstad

There was a significant difference for the interaction effects between size of fruits (small/large) and presence of physiological problem (creased/non-creased) for cell size parameters determined (Fig. 5.4.2.5). Cells of large non-creased fruit peels had significantly higher area as compared with the other treatments. The cell width of non-creased small fruit peels was significantly higher than the other treatments. In line with the above result, cell area and width of gibberellic-acid-sprayed fruits (at a rate of 10 ppm) were significantly higher than non-sprayed (creased) fruits (Fig. 5.4.2.6).

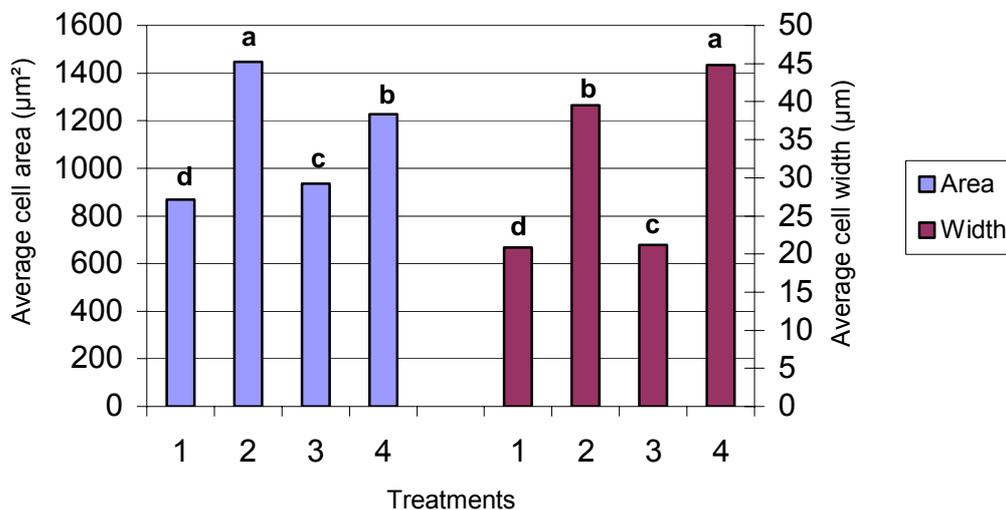


Fig. 5.4.2.5. Effects of fruit size and presence of physiological problem on cell area and width of fruits sampled from Ohrigstad. Treatments: 1, Large creased fruit; 2, Large non-creased fruit; 3, Small creased fruit; 4, Small non-creased fruit ($P=0,05$).

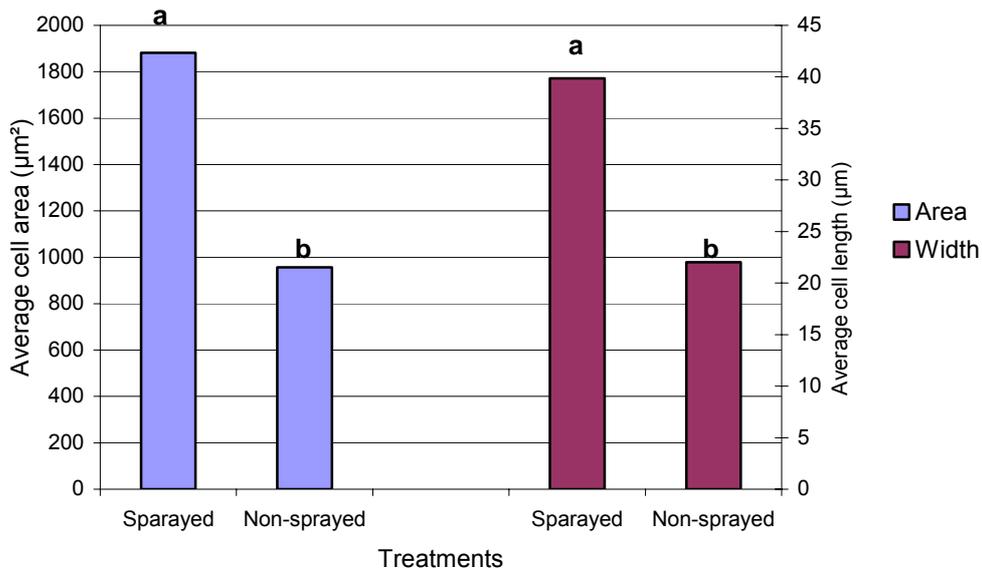


Fig. 5.4.2.6. Effects of spraying gibberellic acid (at a rate of 10-ppm) on cell area and width of fruits sampled from Ohrigstad ($P=0,05$).

Patensie

Area and width of non-creased fruit peel cells was significantly higher than creased fruit peel cells (Fig. 5.4.2.7).

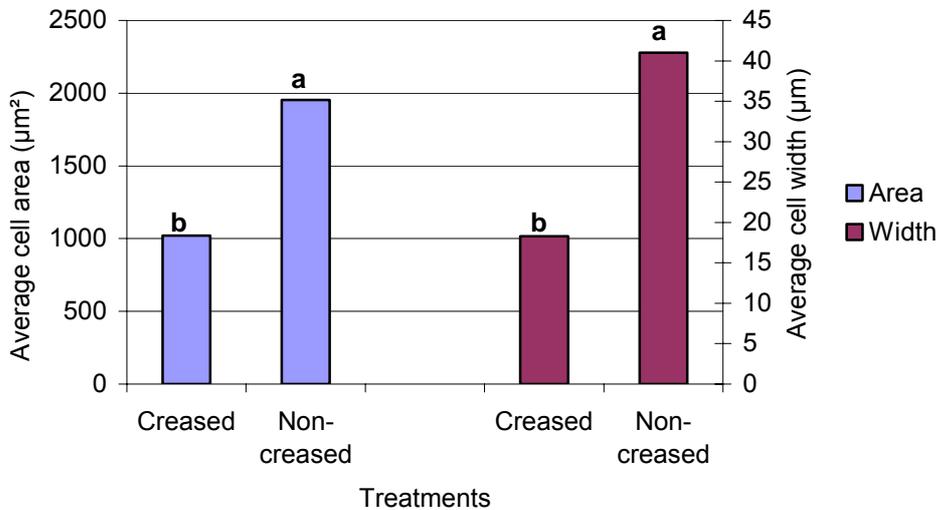


Fig. 5.4.2.7. Effects of presence and absence of physiological problem on cell area and width of fruits sampled from Patensie ($P=0,05$).

Comparisons of the two sites

Even though there was no size category for samples from Patensie, the general trend indicated that cells of non-creased fruit peels had a significantly higher area and width.

Discussion

The results from the lab analysis for nutrient levels obtained from both sites were similar. In both cases, significantly lower levels of Ca, N Mn and B were found to be linked to citrus creasing. The role of calcium has received a particularly large amount of attention over the years and several studies have concluded that calcium plays a pivotal role in citrus rind creasing. Braccini *et al.* (1999) demonstrated clearly the role of calcium in complexing with uronic acids as did Debon & Tester (2001) and Norziah *et al.* (2001). Storey *et al.* (2002) also showed that low levels of Ca during Stage 1 of fruit growth were positively correlated with creasing.

High Ca concentrations in the albedo were also negatively correlated with creasing. Treeby & Storey (2002) showed that by spraying navel oranges with a 1% calcium solution 24 times during the season the amount of creasing could be decreased by 50%. The effect of anions was in the order of $\text{Ca}(\text{NO}_3)_2$, CaCl_2 and CaCO_3 respectively. Repeated spraying of CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ reduced the amount of creasing by as much as 52%. Dubreucq *et al.* (1996) and Yamamoto *et al.* (1997) implicated the role of boron in complexing with uronic acid, which was also observed in the current study.

On the other hand, it is learnt from the current results that higher levels of especially K and Mg can be antagonistic to Ca levels and may cause creasing. Hence the ratio of Mg/Ca and K/Ca is crucial. As we can see from the results, the ratio of K/Ca and Mg/Ca was higher for creased fruits and lower for non-creased. The importance of these ratios has also been previously observed by Storey *et al.* (2002). Jones *et al.* (1967) also found higher K levels and lower Ca levels in the rind of creased Valencia fruit compared with unaffected fruit. On the contrary, Embelton *et al.* (1973) reported that K contributed in reduction of creasing. They also indicated that an increase in leaf N from 2.54 to 2.71% by soil application of urea reduced creasing and fruit size.

High Zn and Fe albedo levels, in the current study, were positively correlated with incidence of creasing. Contrary to this observation, the role of Zn in complexing with uronic acid was observed by Loaëc, *et al.* (1996); Yamamoto *et al.* (1997); Debon & Tester (2001) and that of Fe by Sipos *et al.* (1995) and Debon & Tester (2001). With respect to Mg, as in the current study, Kunken *et al.* (1997) found that magnesium did not play a role in complexing with uronic acid.

The comparison made between large and small creased fruits linked the impact of crop load to creasing. It is known that whenever there is a large crop on the tree, it has a negative effect on the size of fruits. All these fruits compete for nutrient reserves of the tree. This leads to the assumption that even if both large and small fruits compared had creased albedos the levels of Ca and Mo, of which their deficiencies are believed to be crucial in creasing, is higher in large as compared to small fruits. In line with this assumption, Le Roux & Crous (1938) observed that crop size had an effect on creasing, where large crops are associated with high incidences of creasing and small crops associated with low incidences.

The result obtained for nutrient levels from EDX analysis was similar to that of lab analysis. The most important result obtained from the EDX analysis was the variation in nutrient levels between the upper and lower parts of the albedo. In both creased and non-creased fruits, the lower side of the albedos had higher contents of a particular nutrient as compared to the upper side of the same sample. That may be the reason why creases are initially visible on the top part of the albedo and then progress downwards to lower parts of the albedo as well as upwards to the flavedo.

According to the current cell structure study using light microscopy, albedo cells of the non-creased fruits are intact and well structured. This could be due to the presence of higher Ca levels in those cells. With the presence of high levels of Ca salts in the pectic acid of the middle lamella, the cohesion of adjoining cells will be higher and protects cells from dissociation unlike in the case of creased cells. In creased cells, due to low levels of Ca salts, cells are liable for dissociation. Similarly, Holtzhausen (1982) and Storey & Treeby (1994) suggested that changes in cell wall cohesion of adjoining cells at the middle lamella to be the major cause of creasing. Storey *et al.* (2002) described that the cell walls of albedo cells have high pectin content and the middle lamella is composed mainly of calcium salts of pectic acid. The possible role of Ca in cell adhesion may be fundamental to the normal development of albedo tissue with the very long cell protuberances (Storey & Treeby, 1994). In creased fruit, an increase in pectin methylesterase activity and water-soluble pectins is observed which suggests an increase in the degradation of pectins (Monselise *et al.* 1976).

On the other hand, Spraying of gibberellic acid reduced the incidence of creasing and cell dissociation, compared to the non-sprayed (creased) fruits. This beneficial effect of gibberellic acid has also previously been reported by Embelton *et al.* (1973) and Agusti *et al.* (2002). Gibberellic acid applied alone or combined with KNO_3 or $\text{NH}_4\text{H}_2\text{PO}_4$ was also reported to reduce creasing (Gambetta *et al.* 2002). According to Jona *et*

al. (1989), two major phenomena seem to be related to the creasing disorder: cell wall lysis and decrease in cell wall polysaccharide content, particularly pectic substances. According to them, when trees were sprayed with gibberellic acid early in the season, the level of pectins later in the season was much higher and there was no significant decrease (only fluctuations) throughout the period considered.

The cell size determination study revealed that, in the case of creased fruits, the cells are detached from one another and scattered singly. This means that the cells lost their ability of being stretched. This led to the flaccid cell structure, reduced width and consequently reduced cell area. The outer peripheries of normal (non-creased) cells are bound to other surrounding cells and hence are stretched with wider width and cell area. Spraying of gibberellic acid was found to be beneficial in reducing the incidence of creasing and increasing cell area as compared to non-sprayed (creased) fruits.

Conclusion

The information obtained from the current observations will render additional information to the existing knowledge relevant to nutrients involved in creasing. The observation regarding microscopic investigation of creased and non-creased fruits is the first in its nature and gave us a clear picture as to what is happening to the cells. The pictures from the cell structure study enabled determination of creased and non-creased cell area. All in all, it is concluded that nutrient levels in tissues are important in citrus creasing.

Future Research

As observed from the current, as well as previous observations, calcium is found to be very crucial in citrus creasing. Therefore, a detailed field experiment on application of different Ca sources is essential, which is underway in the same project. There is also a future plan on investigating the effect of gibberellic acid and plant growth regulators under field conditions, which are found to be promising in alleviating the problem of citrus creasing from the current and previous observations.

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5.4.3 The effect of light and competition on the development of rind disorders

Experiment 758 by Paul Cronje, Graham Barry (CRI) Marius Huysamer (SU)

Opsomming

Die verhoogde voorkoms van skildefekte soos skilafbraak van Clementine mandaryne vereis die kritiese evaluering van faktore wat die kwaliteit van die skil beïnvloed asook die praktyke in die boord wat die uitvoer potensiaal negatief kan beïnvloed. Die posisie van die vrug gedurende ontwikkeling, soos onderskei as binne en buite vrugte, is geëvalueer. Die evaluasies sluit in die voorkoms van skilafbraak asook minerale element, pigmente en die hoeveelheid prolien ontledings. Die eksperiment was gedoen op 'Nules Clementine' mandaryne in die Stellenbosch omgewing. Die vrugte is na oes opgeberg vir 3 maande by 7.5°C of -0.6°C. Die binne vrugte het onder albei die temperatuur regimes meer skilafbraak vertoon. Die mineraal element ontledings het getoon dat daar geen betekenisvolle verskille tussen die mikro elemente van binne en buite vrugte nie. Daarteen oor was daar betekenisvolle verskille in kalium, kalsium en magnesium. Die data moet egter in die volgende seisoen se data geverifieer word voor afleidings gemaak kan word. Die negatiewe effek wat na-oes praktyke soos ontgroening en gebrekkige temperatuur beheer op skilafbraak het was voorheen getoon. As die vooroes praktyke wat tot skilafbraak bydrae uitgewys kan word sal dit kan lei na 'n beter begrip vir die meganisme wat die afwyking kan veroorsaak.

Introduction

The increased incidence of rind disorders, such as rind breakdown (RB), necessitates the critical evaluation of horticultural practices that could negate the export potential of citrus fruit. Specific cultural practices such as pruning, planting density, and nitrogen application are such horticultural aspects that could directly influence the development of postharvest disorders. The objective of this experiment is to quantify and compare the physiological developments of low and high-risk fruit in relation to rind breakdown. Previous research at CRI identified low light levels within the tree canopy as a major factor determining the quality of the fruit in relation to RB (van Rensburg, 2003). This corresponds with reports for the export market that identified pale yellow fruit to be more prone to RB development than well-coloured orange fruit.

The aim of this ongoing experiment is to study the rind of 'Nules Clementine' mandarin developing under low and high light conditions. The study consists of measuring changes on three different levels in the fruit:

- a) Anatomical (Agusti *et al.*, 2001; Maia *et al.*, 2003)
- b) Biochemical (Agusti *et al.*, 2001)
- c) Physiological reactions (Yehoshua *et al.*, 2001)

This ongoing experiment is planned to test the hypothesis that fruit position within the canopy is the main factor in the development of RB.

Materials and method

The fruit were harvested from Welgevallen Experimental Farm, University of Stellenbosch, on 16 May 2004 according to their position during their development, e.g. inside or outside of the canopy. The fruit receiving full sunlight during development were classified as outside fruit and those situated in the canopy away from most of the leaves and with no direct sunlight were classified as inside fruit. The inside fruit were somewhat smaller and less well coloured compared with the outside fruit. The fruit were picked in wooden bins and put in a 3-day degreening treatment whereafter they were separately packed according to either the inside or outside classification. These fruit were divided into two storage treatments and were stored at either 7.5°C or -0.6°C for the duration of the experiment.

On each evaluation date 8 cartons with 25 fruit each were removed from storage and evaluated for occurrence of rind breakdown (RB). After the evaluation date the flavedo of the fruit was removed, frozen in liquid nitrogen, freeze-dried and ground, whereafter it was stored at -80°C. From this pooled material, mineral analyses as well as proline, sugar and pigment analyses were done. A full mineral analysis of fruit sampled from February until harvest as well as during storage was done at Bemlab (Somerset West). From these fruit, oil glands that showed RB symptoms or were unaffected were fixed for anatomical evaluation.

Results

Rind Breakdown evaluation

The occurrence of RB was lower than the previous year (CRI annual report 2003) but a steady rise in the disorder could be seen as the storage period progressed (Fig 5.4.3.1). The unexpected decrease in RB, especially in the fruit stored at 7.5°C at the 11 Aug evaluation could be a result of higher rate of decay, masking the RB symptoms. The inside fruit at both storage temperatures had higher incidence of RB. The fruit stored at -0.6°C did have lower incidence during the first period of the postharvest storage. After the fifth evaluation (9 August) the differences between RB incidences was less evident.

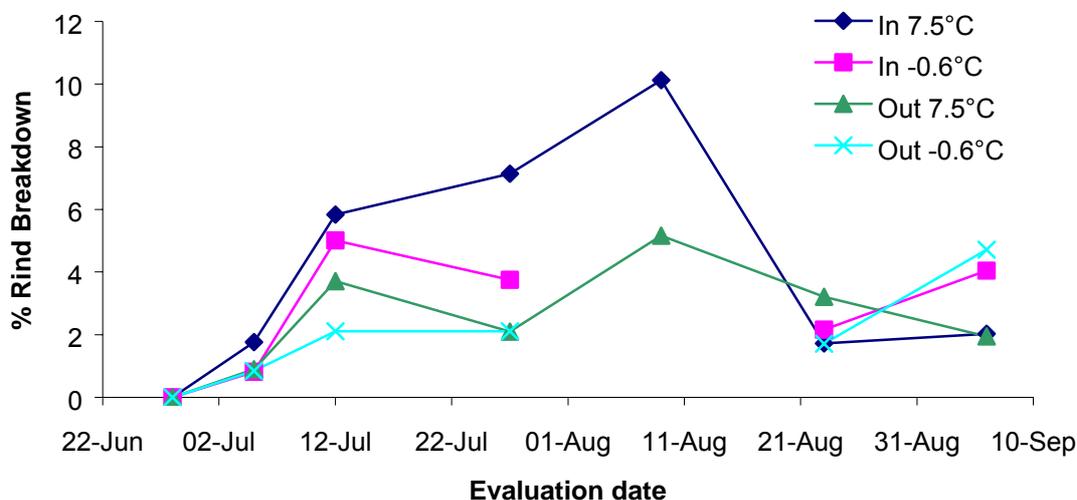


Figure 5.4.3.1. Occurrence of Rind Breakdown on inside and outside 'Nules Clementine' mandarins during storage at either 7.5 or -0.6°C.

Anatomical studies

During the 2004 season, rind samples of yellow and orange fruit were prepared for microscopic analysis. The first fixing was done with 2.5% glutaraldehyde and 2% formaldehyde in a 0.075 M phosphate buffer (pH=7.4) followed by osmium tetroxide and dehydration and storage in alcohol. At the Laboratory for Microanalysis, Univ. of Pretoria, the samples for the SEM (Scanning Electron Microscope) were dried with critical point CO₂ equipment before fixing on a stub and coating with gold. The samples selected for the light microscope, were imbedded in LR white and polymerised at 60°C whereafter the slides were prepared with an ultramicrotome and stained with 1% aniline blue.

Anatomical studies of citrus oil glands proved to be more difficult than anticipated. The oil in the gland reacted with the LR white epoxy resulting in soft gel-like areas in the rind making the cutting of the material for the light microscope very difficult. A strategy for the 2005 season sample's fixing was decided on and will focus on the correct dissection of the oil gland. One side of the gland will be cut away in order for the oil to be "washed out" during fixing and allow the fixing of the rest of the gland without causing distortion.

The fixing procedure for the SEM was effective and more samples will be taken during 2005 to do more comparative studies. Evidence of damage to the area between the oil gland and the epidermal cells was found. If this could be corroborated in next year's studies, it could answer the question as to which part of the gland starts to leak causing damage (Fig. 5.4.3.2) as opposed to non leaking cells where no damage was seen (Fig. 5.4.3.3).

Differences between the natural wax occurring on the surface (cuticle) of inside vs. outside fruit (sampled after fruit set) were seen. This will not be the main focus of the microscopic study but will be documented.

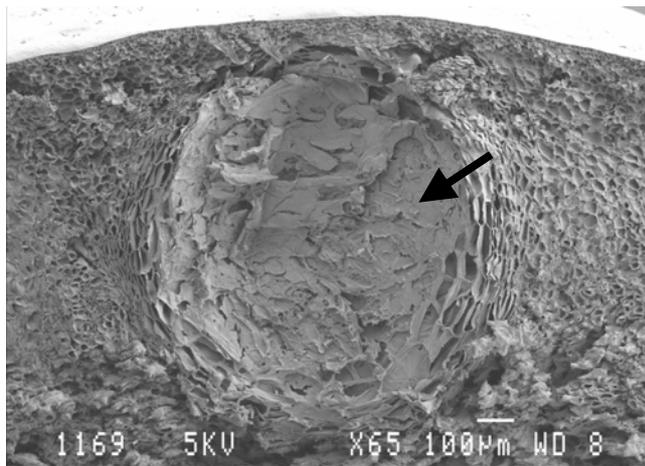


Figure 5.4.3.2. Damage of the flavedo between the oil gland and the epidermal cells of 'Nules Clementine' mandarin probably due to leakage of oil after rind breakdown.



Figure 5.4.3.3. No damage of the flavedo cells surrounding the oil gland or the epidermal cells of 'Nules Clementine'

Mineral analysis

There were differences between inside and outside fruit in most of the nutrients analysed (Fig. 5.4.3.4 and 5.4.3.5). The results show differences in mineral content between fruit developing in a high and low light environment. These results will be confirmed in the next season's data.

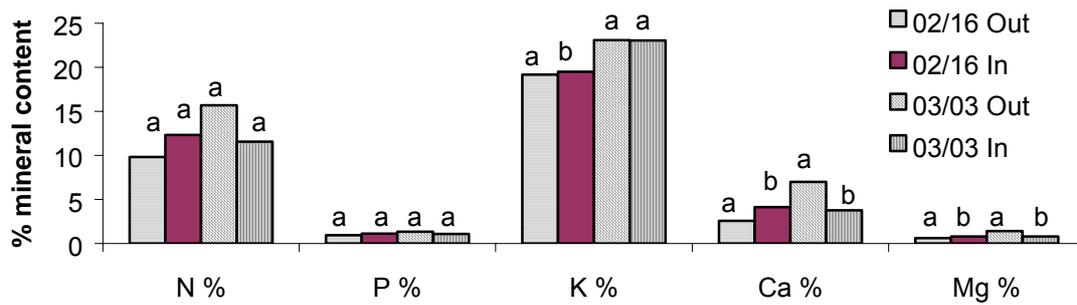


Figure 5.4.3.4. Macro-nutrient mineral content (%) of the flavedo from 'Nules Clementine' mandarins from inside and outside the canopy. Significant differences between treatments are shown with different lettering ($P > 0.1$). Each individual date was analysed separately.

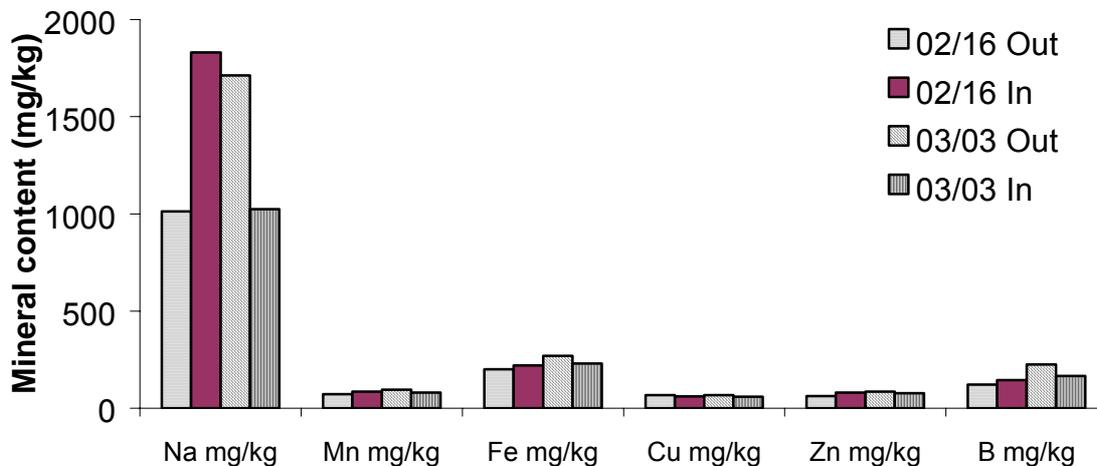


Figure 5.4.3.5. Micro-nutrient mineral content (mg/kg) of the flavedo from 'Nules Clementine' mandarins from inside and outside the canopy. There were no significant differences between treatments ($P > 0.1$).

Proline

A methodology for analysis of proline has been worked out and analysis will commence during March 2005.

Discussion

The positional development and storage conditions have been shown to directly influence the incidence of RB. The differences found between the mineral nutrient content supports the theory that the positional effect during the development of the fruit remains foremost as a factor in RB development. Two possible factors could be influenced by the position within the tree canopy: access to direct sunlight as the primary source of photosynthates. The inside fruit are normally not surrounded by photosynthetically active leaves and would therefore be far removed from the leaves actively fixing carbon and exporting it to the strongest sink. The effect of direct sunlight on the rind is also thought to increase the rind strength. This situation could lead to the reduced levels of minerals seen. It is known that the outside fruit have more carotenoids (better colour development) but the mechanism by which they could influence rind strength is not known.

The effect of postharvest handling, e.g. degreening and temperature management, have been shown to influence RB significantly (Cronje and Van Rensburg, 2003). The higher storage temperature resulted in an increase of RB and could indicate that RB is the result of a too rapid respiration rate and thus early senescence. The lower incidence of RB reported from some areas in the Western and Eastern Cape compared with 2003 could be the result of more favourable climatic conditions during growth and development phases of the fruit and support the complex nature of this disorder.

Conclusion

The causative factors and their reactions on the mechanism responsible for collapse of the oil glands of 'Nules Clementine' mandarins, remains to be elucidated. However, progress has been made into the effect of postharvest practises on rind breakdown development. These factors, of which temperature management remains the most critical, could probably lead to early and rapid senescence of the rind. If the rind has developed in sub optimal conditions such postharvest stresses could lead to the triggering of the rind breakdown mechanism.

Future research

The experiment is part of an ongoing study researching the physiological changes that occur in the rind during the postharvest period. During the next season the effect of water balance of the rind as influenced by various postharvest practices will be studied. Samples will be taken for mineral, nutrient, pigment and proline analysis to document these changes during the storage period

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Acknowledgements

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5.4.4 Comparison between inside (shaded) and outside (non-shaded) 'Nules' Clementine mandarin fruit regarding physiological and biological properties of the rind as well as on the occurrence of chilling injury and rind breakdown after storage

Experiment CL01/04 by P. Khumalo, A. de Kock and J. Davids (ExperiCo)

Opsomming

'Nules' Clementine vrugte is vanaf verskillende posisies binne en buite die bome gepluk, en teen -0.5°C of 7.5°C vir 12 weke opgeberg. Vrugte was almal omtrent die selfde wat kleur, TOV and suur betref. Verskille is by minerale, P en K, sowel as anti-oksidente en totale karotenoied vlakke betref, maar geen korrelasie met skilafbraak kon gevind word nie, as gevolg van die feit dat vrugte vanaf die verskillende posisies op die boom die selfde skilafbraak gehad het. By hierdie werk, net temperatuur het'n verskil gemaak, met skilafbraak by 7.5°C en koueskade by -0.5°C . Hierdie werk behoort by twee areas in die 2005 seisoen te gedoen word.

Introduction

It is generally considered in the industry that fruit from the inside of the tree is more susceptible to rind breakdown than fruit from the outside of the tree. Inside fruit is also said to have a paler colour than outside fruit, implying that there is a difference in pigment content. This also raises the question of anti-oxidant capacity, which could be linked to the ability of the fruit to withstand low temperature storage. The physiological properties of fruit from both shaded and sunlit positions (inside and outside) in the canopy were therefore investigated in order to determine the role that shading may play in the development of rind breakdown. Specific objectives were as follows:

- To establish differences in sensitivity to chilling injury and rind breakdown, after storage plus shelf life, between shaded and non-shaded fruit on a tree.
- To compare, at harvest, some biochemical and physiological properties of the rind from shaded and non-shaded fruit.

- To establish whether the biochemical and physiological rind properties measured, on fruit from different canopy positions, influence sensitivity to chilling injury and rind breakdown.

Materials and methods

Cultivar

'Nule Clementine' mandarin

Trial site

Paarl

Treatments

1. *Canopy position*
Inside (shaded) fruit
Outside (non-shaded) fruit
2. *Storage temperature*
-0.5 °C
7.5 °C
3. *Sampling detail*

'Nules Clementine' mandarin fruit originating from either the inside or outside of the tree canopy were harvested on 13 May 2004 from an orchard in Paarl. Fruit was immediately transported to Stellenpack in Simondium for degreening and packing thereafter.

4. *Fruit degreening*

Prior to degreening fruit was drenched in the following fungicide mixture: Benlate (1 kg/1000 ℓ), Deccomone (5 ℓ/1000 ℓ) and Guazatine (Deccotine) (2.5 ℓ/1000 ℓ). The fungicides were mixed in water with a wetting agent, Cittowet, added at a concentration of 100 ml/1000 ℓ. After drenching, the fruit was held at ambient ($\pm 20^\circ\text{C}$) for 8-12 hours, and thereafter put into a degreening chamber. Degreening was conducted using 1-2 ppm ethylene at 19-21°C and at an RH of 90-95%. Fruit from both populations were degreened for a total period of five days. After degreening, fruit was held at ambient ($\pm 20^\circ\text{C}$) for 12-24 hours before packing.

5. *Fruit packing*

Fruit was packed at a commercial packhouse (Stellenpack) in Simondium. The procedure followed in fruit packing involved moving fruit through a warm water (35-40°C) bath containing Imazalil at 500 ppm. The fruit was then dried in a hot (40-45°C) tunnel, after which a light wax with Decowax was applied. After waxing the fruit was again dried in a hot (40-50°C) air tunnel and then sorted and packed into the MO5I open display plum carton, which contained ± 50 fruit.

6. *Fruit Storage*

Equal samples of fruit from the different canopy positions were stored for a maximum period of 12 weeks at the respective temperatures (-0.5 and 7.5°C), and then subjected to a shelf life period of 1 week at 20°C before evaluations were conducted.

Experimental layout and statistical detail

The trial was analysed as a completely randomised design. Data collected at harvest were analysed using a Student t-test whereas data collected after cold storage and shelf life were analysed using a two-way ANOVA on STATISTICA, factor A being the fruit canopy position and Factor B being the storage temperature. Treatment means were compared using the LSD method. Each treatment was replicated seven times, with a carton of fruit comprising a replicate.

Examination stages and parameters

At Harvest

- Rind colour rating – measured on 10 fruit per replicate using the Outspan skin colour chart set number 36. Where 8 = green immature fruit with no colour break, 1 = deep orange and fully coloured fruit.
- Hue of the rind – measured on 10 fruit per replicate using the Minolta colour reader. Where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).
- Equatorial diameter (mm) – the equatorial diameter of 10 fruit per replicate was measured using a caliper.
- Juice content (%) – the mass of 10 fruit per replicate was measured before juice was extracted from these fruit. After the juice was extracted from the fruit, the rind and pulp that remained were again weighed. The difference between the two masses expressed as a percentage indicated juice percentage.
- Brix (°) – determined on a pooled juice sample from 10 fruit per replicate using the ATAGO DBX-30 digital refractometer
- Titratable citric acid content (%) – was determined on a pooled juice sample from 10 fruit per replicate. The titratable citric acid content was measured by titrating 25 ml of juice with 0.1 N sodium hydroxide to an end point pH of 8.2. The result was converted to citric acid by the equation: titratable citric acid content = (ml NaOH/ 25 ml) x (0.1 N NaOH/0.1562)

Antioxidant Capacity

The antioxidant capacity was determined from the rinds of five fruit per replicate from the different canopy positions using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications. Trolox (6- 2,5,7,8- tetramethylchroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical CO.) was used as an antioxidant standard. Trolox (2 mM) was prepared in methanol. From the Trolox stock solution a series of dilutions (in methanol) were prepared: 0, 0.2, 0.6, 1, 1.4, 1.8 and 2 mM.

ABTS (2,2' – Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich chemical CO. was dissolved in distilled deionised water to a concentration of 8 mM. The ABTS radical cation ABTS^{•+} was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (± 20°C) for 12 hours. After this time period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2900 µl of the diluted ABTS radical cation to 100 µl of each of the serial Trolox dilution. The mixture was shaken, allowed to react for six minutes and an absorbance reading measured at 734 nm on a spectrophotometer (Cary 50 conc UV- visible by Varian). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The strips were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Before analysis, the frozen strips were ground in a Molinix coffee grinder to a fine powder. Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. A sample of 0.2 g was extracted using 1% HCl in 95% methanol. Methanol or acidified methanol has been used, as a solvent, by other investigators to extract antioxidants from fruit pulp (Bocco et.al. 1998; Siddhuraju et.al. 2002; Gorinstein et al. 2003 and Gorinstein et al. 2004). The extraction of antioxidants, in this experiment, was done in three phases. The sample was first extracted with 10 ml of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged, (Sorvall RC-58 refrigerated centrifuge) for 5 minutes at 1000 rpm and 4.5°C after which the supernatant was retained. The residue was again extracted with 10 ml of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 100 µl of the antioxidant extract from the citrus rind was drawn and 2900 µl of the ABTS radical cation was added. The mixture was allowed to react for six minutes, after which the absorbance was measured at 734 nm on the Cary 50 conc spectrophotometer.

Calculation of the antioxidant capacity

Antioxidant capacity (mM Trolox equivalents/g sample) = (slope x abs_{734nm} + C)/(g sample used in analysis)

Where: Slope = slope of the standard curve
 $abs_{734\text{ nm}}$ = absorbance at 734 nm
 C = y intercept.

Rind pigments

The total chlorophylls and carotenoid content were determined from the rinds of five fruit per replicate. The pigments were measured using the spectrophotometer as described by Lichtenthaler (1987).

Sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. These strips were immediately frozen in liquid nitrogen and stored at -80°C . The frozen strips were ground in a Molinix coffee grinder to a fine powder. Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. The fine powder was then freeze dried and again stored at -80°C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL of cold 95% ethanol + BHT (100 mg/L) + DDC (200 mg/L). The sample was then vigorously stirred (Votex-2Gennie from Scientific Industries) twice, for one minute in each case. After which the sample was placed in a dark fridge and allowed to extract for 1 hour and 30 minutes. The sample was then filtered through an ash-less filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649, and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and the total carotenoids (C_{x+c}) were calculated using the following equations:

$$\begin{aligned} C_a &= 13.36A_{664} - 5.19A_{649} \\ C_b &= 27.43A_{649} - 8.12A_{664} \\ C_{a+b} &= 5.24A_{664} + 22.24A_{649} \\ C_{x+c} &= \frac{1000A_{470} - 2.13 C_a - 97.64 C_b}{209} \end{aligned}$$

Rind moisture

Ten fresh rind discs were removed from the five fruit per replicate (two discs per fruit), using a cork borer size 12. These discs were weighed fresh then oven dried at 70°C for 72 hours and re-weighed after drying. The difference between the fresh weight and dry weight expressed as a percentage indicated the rind moisture content.

Mineral nutrient status

Rind samples obtained from the equatorial region of five fruit per replicate were taken to BremLab in Somerset West, South Africa, for mineral nutrient status determination.

After cold storage plus a shelf life

- Rind colour rating – measured on 10 fruit per replicate, using the Outspan skin colour chart set No. 36. Where 8 = green immature fruit with no colour break, 1 = deep orange and fully coloured fruit.
- Hue of the rind – measured on 10 fruit per replicate, using the Minolta colour reader CR-10. Where 0° = red, 90° = yellow, 180° = green and 270° = blue. (McGuire, 1992).
- Rind breakdown (%) – fruit was classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ± 40 fruit per replicate by determining the percentage of fruit with the spots regardless of size.
- Chilling injury (%) – fruit was classified as chilling injury when discoloured blemishes were seen on the rind. The incidence of this disorder was measured on ± 40 fruit per replicate by determining the percentage of fruit with the blemishes regardless of size.
- Decay incidence measured on ± 40 fruit per replicate.
- TSS ($^{\circ}\text{Brix}$) – determined on a pooled juice sample of 10 fruit per replicate. However in the 2002 season this variable was determined on a pooled juice sample of 20 fruit from each harvest date. The brix (total soluble solids) were measured on the ATAGO DBX-30 digital refractometer.
- Titratable citric acid content (%) – determined on a pooled juice sample of the 10 fruit per replicate. However in the 2002 season this variable was determined on a pooled juice sample of 20 fruit from each harvest date. This variable was measured by titrating 25 mL of juice with 0.1 N sodium hydroxide to an end point pH of 8.2. The result was converted to citric acid by the equation: titratable citric acid content = (mL NaOH/ 25 mL) x (0.1 N NaOH/0.1562)

Results and discussion

Table 5.4.4.1. Characteristics at harvest of inside (shaded) and outside (non-shaded) 'Nules' fruit sampled in Paarl.

Response variable	Canopy position		P-value
	Inside fruit	Outside fruit	
Rind colour rating	5.3 (0.2) ¹	5.4 (0.1)	0.426
Hue of the rind	89.5 (3.0)	83.5 (2.4)	0.009
TSS (°Brix)	11.3 (0.3)	11.4 (0.2)	0.369
Titrateable citric acid (%)	1.1 (0.06)	1.0 (0.08)	0.048
Equatorial diameter (mm)	59.9 (1.8)	63.7 (1.7)	0.009
Mass (g)	98.7 (7.1)	115.5 (8.6)	0.009
Juice content (%)	53.0 (0.9)	54.5 (1.5)	0.091
N (mg/100 g fresh mass)	272.8 (34.2)	238.8 (31.6)	0.141
P (mg/100 g fresh mass)	31.2 (4.4)	25.1 (3.5)	0.034
K (mg/100 g fresh mass)	338.6 (44.3)	285.2 (22.9)	0.043
Ca (mg/100 g fresh mass)	199.7 (45.6)	241.0 (35.1)	0.148
B (mg/kg fresh mass)	7.9 (0.6)	7.6 (0.8)	0.506
Rind water content (%)	76.1 (1.4)	76.8 (0.3)	0.304
Antioxidant capacity (mM Trolox equivalents/g sample)	5.4 (0.2)	6.3 (0.8)	0.001
Total chlorophylls (µg/g dry weight)	1.1 (1.1)	1.4 (0.8)	0.616
Total carotenoids (µg/g dry weight)	11.3 (1.4)	18.7 (1.0)	0.001

¹Figure in parenthesis is the standard deviation

Table 5.4.4.2. Quality of inside (shaded) and outside (non-shaded) 'Nules' fruit after storage for 12 weeks at -0.5°C or at 7.5°C plus one week at 20°C.

	Response variable				
	Rind breakdown (%)	Chilling injury (%)	Decay (%)	Hue of rind	Rind colour rating
Canopy position (A)					
Inside fruit	3.2	10.4	12.9	59.4	2.0
Outside fruit	2.0	14.5	12.7	58.6	2.0
Storage temperature (B)					
-0.5 °C	0.0a ¹	24.9b	14.3	64.5b	2.8b
7.5 °C	5.2b	0.0a	11.4	53.4a	1.1a
P-values					
A	0.256	0.268	0.945	0.261	1.000
B	0.000	0.000	0.317	0.000	0.000
A x B	0.256	0.268	0.388	0.422	0.255

¹Value in the same column followed by different letters indicate significant differences according to the LSD test.

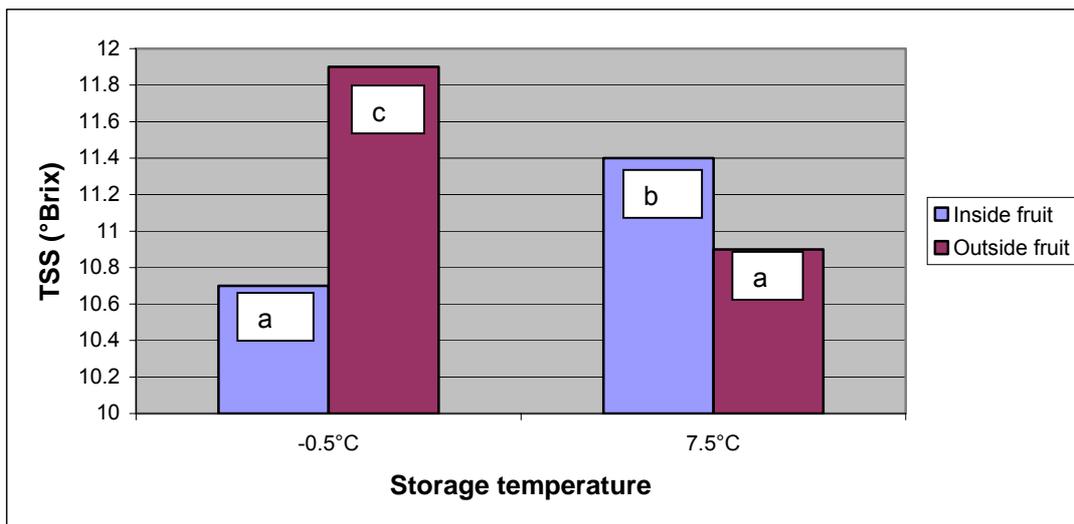


Figure 5.4.4.1. TSS after storage plus shelf life on hules' fruit harvested at different canopy positions and stored at -0.5°C or at 7.5°C . Significant interaction occurred between storage temperature and canopy position ($P = 0.001$). Bars with different letters differed significantly.

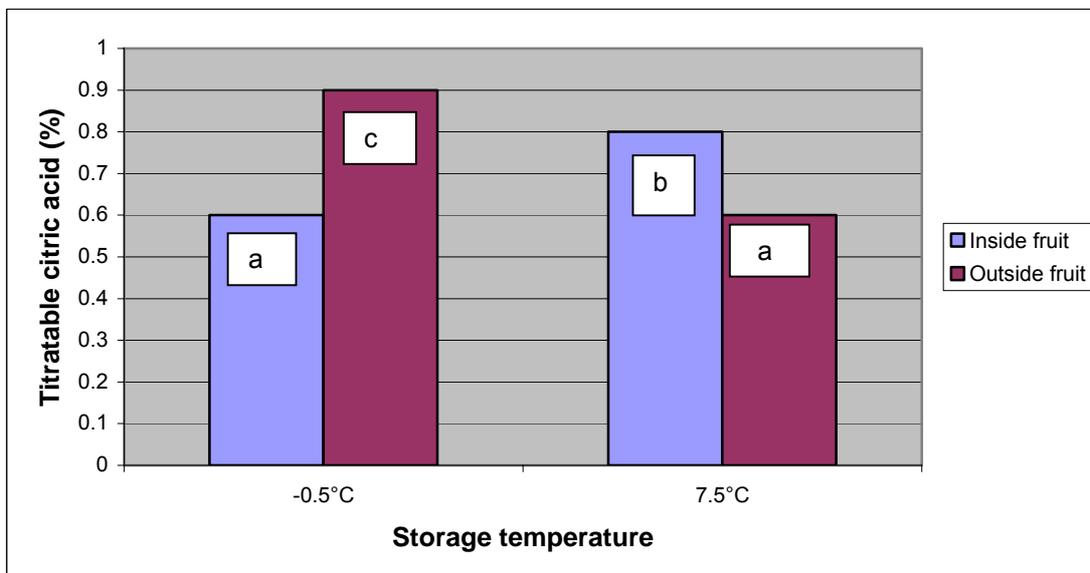


Figure 5.4.4.2. Titratable citric acid content after storage plus shelf life on hules' fruit harvested at different canopy positions and stored at -0.5°C or at 7.5°C . Significant interaction occurred between storage temperature and canopy position ($P = 0.001$). Bars with different letters differ significantly.

It can thus be stated that the rind colour rating was similar between fruit originating from the inside and outside of the tree (Table 5.4.4.1). However, colour measured with the colour meter showed that the hue of the rind was lower in fruit originating from the outside of the tree canopy, suggesting a more advanced colour compared to fruit originating from the inside of the tree canopy. Internal quality measured in terms of TSS and titratable citric acid was similar in fruit originating from the different canopy positions. Fruit originating from the outside of the tree canopy were larger and heavier than fruit originating from the inside of the tree canopy. Juice content was not significantly different between fruit from the different canopy positions. Most of the mineral nutrients, with the exception of calcium, were higher in fruit originating from the inside of the tree canopy than outside fruit. This response was statistically significant for P and K, but not for N and B. The rind water content and total chlorophylls in the rind were similar between fruit from both canopy positions. The antioxidant capacity and total carotenoids were significantly higher in fruit originating from the outside of the tree compared to inside fruit.

After cold storage plus shelf life, rind breakdown and chilling injury were not significantly affected by canopy position. Only storage temperature was a significant factor in the occurrence of these disorders (Table

5.4.4.2). Rind breakdown was only observed in fruit stored at 7.5°C whereas chilling injury was only observed in fruit stored at -0.5°C. Decay development was not significantly influenced by canopy position or storage temperature. A significant interaction was observed between canopy position and storage temperature on the TSS (Figure 5.4.4.1) and titratable citric acid (Figure 5.4.4.2) content of fruit. At the storage temperature of -0.5°C, inside fruit had a lower TSS and titratable citric acid content than outside fruit. However, in fruit stored at 7.5°C the reverse occurred. Inside fruit had a higher TSS and titratable citric acid content than outside fruit. Only storage temperature was a significant factor on colour development of fruit from the different canopy positions. Fruit stored at 7.5°C were more orange in colour than fruit stored at -0.5°C, a lower hue angle and lower colour rating, in fruit stored at 7.5°C, indicated this trend.

Conclusions

In this work, only storage temperature appeared to have influenced the presence of rind disorders. Further, the higher temperature appeared to predispose fruit to more rind disorders than lower shipping temperature. The antioxidant results were not useful in predicting the likelihood of rind breakdown, and within the population of fruit tested, canopy position made no difference. Fruit appeared to have had a low rind breakdown potential, making interpretation of data difficult. Further work will clearly be needed.

Future research

The experiment should be repeated in order to confirm results obtained in the 2004 season. Furthermore it is recommended that the trial be done in two areas, Paarl and Saron.

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5.4.5 Comparison between 'Nules' and 'Oroval' Clementine mandarins on selected physiological and biochemical rind properties as well as on the occurrence of rind breakdown after storage Experiment CLO2/04 by P. Khumalo, A. de Kock and J. Davids (ExperiCo)

Opsomming

'Nules' en 'Oroval' Clementine mandaryne was geoes vanaf boorde in Paarl, Saron en Robertson. Vrugte is ontgroen, verpak en opgeberg teen 7.5°C vir 10 weke. Oor die algemeen was 'Nules' en 'Oroval' vanaf die Paarl omtrent dieselfde met net klein verskille in TOV en suur. 'Nules' vanaf Saron en Robertson het hoër TOV en laer suur as 'Oroval' gehad. Variasie in mineraal elemente van 'Nules' en 'Oroval' het tussen die areas gewissel, met geen verskille by die Paarl, en sekere verskille by Saron en Robertson. Die anti-oksidente en pigmente het nie verskil nie. Na opberging plus rakleefyd, 'Nules' vrugte is uitgewys as meer vatbaar vir skilafbraak, en 'Oroval' meer vatbaar vir bederf en pofferigheid. Verskille in skilafbraak kon nie aan anti-oksidente of pigmente gekorreleer word nie, omdat hierdie fisiologiese parameters dieselfde was, maar vlakke van skilafbraak verskillend was. Die eksperiment behoort in 2005 weer te gedoen word, en die biologiese en fisiologiese faktore minstens twee keer gedurende die opbergings tydperk gemeet te wees.

Introduction

It is uncertain as to the degree of difference in susceptibilities to rind breakdown between different cultivars, and to what extent any such differences can be attributed to specific physiological parameters. The objectives of the work were therefore:

- To establish the difference in sensitivity to rind breakdown between 'Nules' and 'Oroval Clementine' mandarins.
- To compare, at harvest, selected biochemical and physiological rind properties of the two 'Clementine' mandarin selections.
- To establish whether the biochemical and physiological rind properties measures, on 'Nules' and 'Oroval' fruit influence sensitivity to rind breakdown.

Material and methods

Cultivar

'Nules Clementine' mandarin

'Oroval Clementine' mandarin

Trial sites

Paarl

Saron

Robertson

Sampling detail

'Nules' and 'Oroval Clementine' mandarins were harvested from Paarl on 13 May 2004, Saron on 18 May 2004, and from Robertson on 31 May 2004. Both cultivars were harvested from the inside of the tree canopy. After each harvest, fruit was immediately transported to Stellenpack in Simondium for degreening and packing.

1. Fruit degreening

Prior to degreening fruit was drenched in the following fungicide mixture: Benlate (1 kg/1000 ℓ), Deccomone (5 ℓ/1000 ℓ) and Guazatine (Deccotine) (2.5 ℓ/1000 ℓ). The fungicides were mixed in water with a wetting agent, Sitowet, added at a concentration of 100 ml/1000 ℓ. After drenching the fruit was held at ambient ($\pm 20^{\circ}\text{C}$) for 8-12 hours. Thereafter put into a degreening chamber. Degreening was conducted using 1-2 ppm ethylene at 19-21°C and at an RH of 90-95%. Fruit from both populations were degreened for a total period of five days. After degreening, fruit was held at ambient ($\pm 20^{\circ}\text{C}$) for 12-24 hours before packing.

2. Fruit packing

Fruit was packed at a commercial pack house (Stellenpack) in Simondium. The procedure followed in fruit packing involved moving fruit through a warm water (35-40°C) bath containing Imazalil at 500 ppm. The fruit was then dried in a hot (40-45°C) tunnel, after which a light wax with Decowax was applied. After waxing the fruit was again dried in a hot (40-50°C) air tunnel and then sorted and packed into the MO51 open-display plum carton, which contained ± 50 fruit.

3. Fruit Storage

Fruit samples were stored for 10 weeks at 7.5°C, and then subjected to a shelf life period of 1 week at 20°C before evaluations were conducted.

Experimental layout and statistical detail

The trial was analysed as a completely randomised design. All data collected in this experiment were analysed using a Student t-test on STATISTICA. Each treatment was replicated eight times in each area with one carton constituting a replicate. Fruit from each area was analysed separately.

Examination stages and parameters

At Harvest

- Rind colour rating – measured on 10 fruit per replicate using the Outspan skin colour chart set number 36. Where 8 = green immature fruit with no colour break, 1 = deep orange and fully coloured fruit.
- Hue of the rind – measured on 10 fruit per replicate using the Minolta colour reader. Where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).
- Equatorial diameter (mm) – the equatorial diameter of 10 fruit per replicate was measured using a calliper.
- Juice content (%) – the mass of 10 fruit per replicate was measured before juice was extracted from these fruit. After the juice was extracted from the fruit, the rind and pulp that remained was re-weighed. The difference between the two masses expressed as a percentage indicated juice percentage.
- TSS (°Brix) – determined on a pooled juice sample from 30 fruit at each harvest date. This sample was considered sufficient to indicate maturity of the fruit at harvest. The brix were measured on the ATAGO DBX-30 digital refractometer.
- Titratable citric acid content (%) – was determined on a pooled juice sample from 10 fruit per replicate. The titratable citric acid content was measured by titrating 25 mL of juice with 0.1 N sodium hydroxide to an end point pH of 8.2. The result was converted to citric acid by the equation: titratable citric acid content = (mL NaOH/ 25 mL) x (0.1 N NaOH/0.1562).

Also measured at harvest, with more detail provided, were:

Antioxidant Capacity

The antioxidant capacity was determined from the rinds of five fruit per replicate from the different 'Clementine' selections using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications. Trolox (6- 2,5,7,8- tetramethylchroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical CO.) was used as an antioxidant standard. Trolox (2 mM) was prepared in methanol. From the Trolox stock solution a series of dilutions (in methanol) were prepared: 0, 0.2, 0.6, 1, 1.4, 1.8 and 2 mM.

ABTS (2,2' – Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich chemical CO. was dissolved in distilled deionised water to a concentration of 8 mM. The ABTS radical cation ABTS^{•+} was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (± 20°C) for 12 hours. After this time period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2900 µL of the diluted ABTS radical cation to 100 µL of each of the serial Trolox dilution. The mixture was shaken, allowed to react for six minutes and an absorbance reading measured at 734 nm on a spectrophotometer (Cary 50 conc UV- visible by Varian). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The strips were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Before analysis, the frozen strips were ground in a Molinix coffee grinder to a fine powder. Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. 0.2 g of the sample was extracted using 1% HCl in 95% methanol. Methanol or acidified methanol has been used, as a solvent, by other investigators to extract antioxidants from fruit pulp (Bocco et.al. 1998; Siddhuraju et.al. 2002; Gorinstein et.al. 2003 and Gorinstein et.al. 2004). The extraction of antioxidants, in this experiment, was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged, (Sorvall RC-58 refrigerated centrifuge) for 5 minutes at 1000 rpm and 4.5°C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 100 µL of the antioxidant extract from the citrus rind was drawn and 2900 µL of the ABTS radical cation was added. The mixture was allowed to react for six minutes, after which the absorbance was measured at 734 nm on the Cary 50 conc spectrophotometer.

Calculation of the antioxidant capacity

Antioxidant capacity (mM Trolox equivalents/g sample) = (slope x $\text{abs}_{734\text{nm}}$ + C)/(g sample used in analysis)

Where: Slope = slope of the standard curve
 $\text{abs}_{734\text{nm}}$ = absorbance at 734 nm
C = y intercept.

Rind pigments

The total chlorophylls and carotenoid content were determined from the rinds of five fruit per replicate. The pigments were measured using the spectrophotometer as described by Litchenthaler (1987). Sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. These strips were immediately frozen in liquid nitrogen and stored at -80°C . The frozen strips were ground in a Molinix coffee grinder to a fine powder. Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. The fine powder was then freeze dried and again stored at -80°C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 ml of cold 95% ethanol + BHT (100 mg/l) + DDC (200 mg/l). The sample was then vigorously stirred (Votex-2Gennie from Scientific Industries) twice, for one minute in each case. After which the sample was placed in a dark fridge and allowed to extract for 1 hour and 30 minutes. The sample was then filtered through an ash less filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649, and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and the total carotenoids (C_{x+c}) were calculated using the following equations:

$$\begin{aligned}C_a &= 13.36A_{664} - 5.19A_{649} \\C_b &= 27.43A_{649} - 8.12A_{664} \\C_{a+b} &= 5.24A_{664} + 22.24A_{649} \\C_{x+c} &= \frac{1000A_{470} - 2.13 C_a - 97.64 C_b}{209}\end{aligned}$$

Rind moisture

Ten fresh rind discs were removed from the five fruit per replicate (two discs per fruit), using a cork borer size 12. These discs were weighed fresh then oven dried at 70°C for 72 hours and re-weighed after drying. The difference between the fresh weight and dry weight expressed as a percentage indicated the rind moisture content.

Mineral nutrient status

Rind samples obtained from the equatorial region of five fruit per replicate were taken to BremLab in Somerset West, South Africa, for mineral nutrient status determination.

After cold storage plus a shelf life

- Rind colour rating – measured on 10 fruit per replicate, using the Outspan skin colour chart set No. 36. Where 8 = green immature fruit with no colour break, 1 = deep orange and fully coloured fruit.
- Hue of the rind – measured on 10 fruit per replicate, using the Minolta colour reader CR-10. Where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).
- Rind breakdown (%) – fruit was classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ± 40 fruit per replicate by determining the percentage of fruit with the spots regardless of size.
- Decay incidence measure on ± 40 fruit per replicate.
- Puffiness – fruit was classified as puffy when there was disintegration of the albedo resulting in separation of the flavedo from the pulp (Kuraoka et al., 1977). The disorder was measured using 10 fruit per replicate.
- TSS ($^{\circ}\text{Brix}$) – determined on a pooled juice sample of the 10 fruit per replicate. However, in the 2002 season this variable was determined on a pooled juice sample of 20 fruit from each harvest date. The brix (total soluble solids) were measured on the ATAGO DBX-30 digital refractometer.
- Titratable citric acid content (%) – determined on a pooled juice sample of the 10 fruit per replicate. However, in the 2002 season this variable was determined on a pooled juice sample of 20 fruit from each harvest date. This variable was measured by titrating 25 ml juice with 0.1 N sodium hydroxide

to an end point pH of 8.2. The result was converted to citric acid by the equation: titratable citric acid content = (mℓ NaOH/25 mℓ) x (0.1 N NaOH/0.1562).

Results and discussion

Table 5.4.5.1. Characteristics at harvest of 'Nules' and 'Oroval Clementine' mandarins sampled from Paarl.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind colour rating	5.2 (0.2) ¹	5.3 (0.2)	0.744
Hue of the rind	90.8 (2.7)	84.1 (3.0)	0.006
TSS (°Brix)	11.0 (0.1)	11.9 (0.2)	0.001
Titratable citric acid (%)	1.0 (0.02)	1.1 (0.06)	0.016
Equatorial diameter (mm)	61.9 (1.3)	62.4 (1.6)	0.633
Mass (g)	109.9 (7.1)	111.6 (9.2)	0.754
Juice content (%)	51.9 (3.3)	50.9 (4.1)	0.691
N (mg/100 g fresh mass)	266 (27.1)	255.8 (22.4)	0.535
P (mg/100 g fresh mass)	31.0 (1.7)	28.5 (2.7)	0.131
K (mg/100 g fresh mass)	323.4 (20.6)	305.6 (36.2)	0.368
Ca (mg/100 g fresh mass)	217.7 (31.8)	207.4 (33.4)	0.631
B (mg/kg fresh mass)	8.2 (0.6)	8.3 (0.7)	0.779
Rind water content (%)	75.4 (1.1)	73.7 (1.5)	0.079
Antioxidant capacity (mM Trolox equivalents/g sample)	3.2 (1.0)	4.2 (0.3)	0.055
Total chlorophylls (µg/g dry weight)	3.4 (1.3)	2.0 (1.7)	0.174
Total carotenoids(µg/g dry weight)	13.0 (2.5)	14.0 (1.1)	0.441

¹ Value in parentheses indicates standard deviation

Table 5.4.5.2. Post storage quality of 'Nules' and 'Oroval' Clementine mandarins sampled from Paarl.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind breakdown (%)	3.7 (4.0) ¹	1.4 (2.0)	0.160
Decay (%)	6.1 (3.9)	3.4 (1.3)	0.077
Puffiness(%)	0.2 (0.6)	2.0 (2.5)	0.073
TSS (°Brix)	10.9 (0.2)	11.8 (0.3)	0.001
Titratable citric acid (%)	0.72 (0.09)	0.81 (0.06)	0.127
Rind colour rating	1.2 (0.1)	1.0 (0.1)	0.067
Hue of the rind	53.9 (1.5)	54.7 (0.7)	0.337
Rind moisture (%)	68.2 (1.9)	69.2 (2.1)	0.489

¹ Value in parentheses indicates standard deviation

Table 5.4.5.3. Characteristics at harvest of 'Nules' and 'Oroval' Clementine mandarins sampled from Saron.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind colour rating	5.4 (0.3) ¹	5.4 (0.2)	0.526
Hue of the rind	87.6 (0.8)	88.1 (2.1)	0.624
TSS (°Brix)	12.2 (0.4)	11.0C (0.1)	0.001
Titrateable citric acid (%)	1.05 (0.03)	1.27 (0.05)	0.001
Equatorial diameter (mm)	61.4 (1.8)	60.6 (1.7)	0.530
Mass (g)	101.2 (7.3)	101.2 (7.4)	0.997
Juice content (%)	52.4 (1.6)	56.8 (0.9)	0.001
N (mg/100 g fresh mass)	230.8 (29.1)	264.8 (21.8)	0.034
P (mg/100 g fresh mass)	29.9 (3.8)	30.0 (2.7)	0.983
K (mg/100 g fresh mass)	356.0 (32.0)	318.4 (44.8)	0.165
Ca (mg/100 g fresh mass)	140.4 (26.6)	128.4 (26.1)	0.492
B (mg/kg fresh mass)	5.92 (0.3)	4.84 (0.2)	0.001
Rind water content (%)	78.2 (1.0)	80.3 (1.2)	0.017
Antioxidant capacity (mM Trolox equivalents/g sample)	2.0 (0.4)	1.8 (0.6)	0.577
Total chlorophylls (µg/g dry weight)	0.8 (0.6)	0.2 (0.5)	0.131
Total carotenoids (µg/g dry weight)	15.2 (0.9)	17.0 (2.5)	0.181

¹ Value in parentheses indicates standard deviation

Table 5.4.5.4. Post storage quality of 'Nules' and 'Oroval' Clementine mandarins harvested from Saron.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind breakdown (%)	14.9 (5.1) ¹	0.0 (0.0)	0.001
Decay (%)	21.1 (6.3)	34.0 (7.9)	0.003
Puffiness(%)	0.0 (0.0)	7.1 (3.7)	0.001
TSS (°Brix)	11.5 (0.5)	11.2 (0.3)	0.154
Titrateable citric acid (%)	0.72 (0.06)	1.01 (0.05)	0.001
Rind colour rating	1.1 (0.2)	1.1 (0.1)	0.681
Hue of the rind	54.8 (0.9)	53.7 (1.2)	0.127
Rind moisture (%)	74.0 (0.4)	77.8 (0.4)	0.001

¹ Value in parentheses indicates standard deviation

Table 5.4.5.5. Characteristics at harvest of 'Nules' and 'Oroval' Clementine mandarins harvested from Robertson.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind colour rating	5.2 (0.1) ¹	4.8 (0.2)	0.015
Hue of the rind	80.3 (3.9)	75.2 (1.9)	0.032
TSS (°Brix)	12.2 (0.1)	10.3 (0.2)	0.001
Titrateable citric acid (%)	1.05 (0.05)	1.41 (0.09)	0.001
Equatorial diameter (mm)	64.3 (1.2)	61.8 (1.6)	0.022
Mass (g)	120.5 (9.4)	99.8 (6.9)	0.004
Juice content (%)	57.1 (2.7)	51.7 (1.6)	0.005
N (mg/100 g fresh mass)	230.8 (6.22)	264.8 (29.1)	0.033
P (mg/100 g fresh mass)	29.9 (3.9)	29.9 (2.66)	0.983
K (mg/100 g fresh mass)	288.0 (41.3)	316.2 (41.0)	0.310
Ca (mg/100 g fresh mass)	195.0 (31.4)	128.9 (26.4)	0.007
B (mg/kg fresh mass)	6.7 (0.4)	4.8 (0.5)	0.001
Rind water content (%)	74.7 (1.9)	79.5 (1.4)	0.002
Antioxidant capacity (mM Trolox equivalents/g sample)	2.7 (0.5)	2.1 (0.4)	0.092
Total chlorophylls (µg/g dry weight)	2.6 (1.9)	0.8 (0.5)	0.075
Total carotenoids (µg/g dry weight)	19.8 (1.7)	17.9 (2.4)	0.185

¹ Value in parentheses indicates standard deviation

Table 5.4.5.6. Post storage quality of 'Nules' and 'Oroval' Clementine mandarins harvested from Robertson.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind breakdown (%)	17.6 (3.9) ¹	0.5 (0.8)	0.001
Decay (%)	11.1 (5.4)	36.1 (5.3)	0.001
TSS (°Brix)	11.5 (0.3)	10.0 (0.3)	0.001
Titrateable citric acid (%)	0.74 (0.03)	0.96 (0.08)	0.001
Rind colour rating	1.2 (0.1)	1.2 (0.2)	0.693
Hue of the rind	55.6 (0.7)	55.0 (1.7)	0.475
Rind moisture (%)	69.9 (1.2)	73.8 (1.1)	0.001

¹ Value in parentheses indicates standard deviation

At harvest:

Paarl

'Nules' and 'Oroval' fruit harvested from Paarl had similar colour ratings, even though the hue angle of the rind showed that 'Nules' was slightly greener than 'Oroval', a result which contradicts the rind colour rating (Table 5.4.5.1). 'Nules' had lower TSS and acidity compared to 'Oroval', but the differences were marginal. The fruit were of similar size and weight and also had similar juice contents. All the mineral nutrients (N,P,K,Ca and B) measured were of a similar concentration in both cultivars. The rind water content at harvest was not significantly different between the two cultivars. The antioxidant capacity was slightly higher in 'Oroval' than in 'Nules', but this observation was not statistically significant at 5%. The rind pigments, chlorophylls and carotenoids, were similar between the two 'Clementine' selections.

Saron

The rind colour, determined with the Outspan skin colour chart or the Minolta colour reader, was similar for both 'Clementines' harvested in Saron (Table 5.4.5.3). However, 'Nules' seemed to be slightly more mature as this 'Clementine' selection had higher TSS and lower acidity compared to 'Oroval'. The fruit size and weight were similar in both cultivars, however, the juice content was slightly higher in 'Oroval'. The concentration of P, K and Ca was similar in both 'Clementine' selections. The concentrations of N and B were significantly different between the two selections, with 'Oroval' having a higher N concentration whereas 'Nules' had a higher B concentration. The antioxidant capacity and rind pigments were not significantly different between the 'Clementine' selections.

Robertson

'Oroval' fruit had a lower rind colour rating and hue of the rind compared to 'Nules', indicating that this fruit was less green (Table 5.4.5.5). However, 'Nules' fruit had higher TSS and lower acidity compared to 'Oroval', suggesting that this fruit may have been slightly more mature, despite having a greener rind. 'Nules' fruit was larger, heavier and had a higher juice content compared to 'Oroval'. The N concentration was higher in 'Oroval' than in 'Nules', while Ca and B were higher in 'Nules' than in 'Oroval'. The other mineral nutrients, P and K were of a similar concentration in both 'Clementine' selections. 'Oroval' had higher rind moisture content than 'Nules'. The antioxidant capacity and rind pigment concentration was not significantly different between the two 'Clementine' selections.

After cold storage plus shelf life:

Paarl

Generally low levels of rind breakdown were recorded in fruit from this area. The tendency was for 'Nules' to develop higher levels of the disorder than 'Oroval'. However, this difference was not statistically significant (Table 5.4.5.2). The levels of decay and puffiness were not significantly different between the two 'Clementine' selections. After storage 'Oroval' had a higher TSS content whereas the acidity of the two selections was similar. Rind colour, rated using the Outspan skin colour chart or the measured Minolta colour reader, and rind moisture were similar between the two 'Clementine' selections.

Saron

'Nules' had higher levels of rind breakdown compared to 'Oroval' (Table 5.4.5.4). However, 'Oroval' tended to be more susceptible to decay and puffiness compared to 'Nules'. The TSS of the two selections was similar after storage but the acidity of 'Oroval' remained high compared to that of 'Nules'. Rind colour of 'Oroval' and 'Nules' was similar after storage. The rind moisture content of 'Oroval' was higher than that of 'Nules'.

Robertson

The rind breakdown incidence was higher in 'Nules' fruit compared to 'Oroval' (Table 5.4.5.6). Decay levels were higher in 'Oroval' than in 'Nules'. The TSS was significantly higher in 'Nules' fruit while acidity of this fruit was significantly lower than of 'Oroval'. Rind colour after storage, measured with the colour chart or colour meter, was similar for both 'Clementine' selections.

Conclusions

'Nules' appeared to be more susceptible to rind breakdown than 'Oroval', although the latter showed more waste susceptibility. The data did not show any clear links to the anti-oxidant capacity or pigment levels. Further work should however, be conducted, to clarify these aspects.

Future research

The experiment should be repeated in order to confirm results obtained in the 2004 season. Furthermore, changes in the antioxidant capacity and rind pigments should be monitored during cold storage.

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5.4.6 Postharvest conditions possibly influencing rind disorders

Experiment 759 by Paul Cronje, Graham Barry (CRI) and Marius Huysamer (SU)

Opsomming

Die omgewingstoestande, waaronder temperatuur en gas samestelling die belangrikste is, verander tydens die na-oes hantering van sitrus. Stresvolle toestande wat kan ontstaan tydens die praktyke sal negatief inwerk op die skil kwaliteit van die vrug en kan aanleiding gee tot die ontwikkeling van skil defekte. Die doel van die projek is om vas te stel wat die toestande tydens vervoer na die hawe asook tydens verskeping is. Die toestande en die effek op vrug kwaliteit sal gevolglik tydens eksperimente onder gekontroleerde toestande getoets kan word.

Gedurende die 2003 seisoen is Valencias van die Limpopo produksie area na die Kaap vervoer. Die vrugte het sirkelvormige pok merke tydens aankoms getoon wat oor die vrug versprei is. Die olie kliere was nie beskadig nie en slegs die oppervlak was uitgedroog en versonke. Geen verdere letsels het ontwikkel nie. Tydens 2004 is temperatuur profiele van 'n vrag Valencia leroen van Letsitele, verpak in karton kratte, wat na Stellenbosch met 'n trek vervoer is gemeet. Die temperatuur het nie onder 4°C gedaal, waar potensiele koue skade gewoonlik voorkom, nie maar daar was 'n drastiese verskil in temperatuur vanaf die minimum nag temperatuur van 4°C tot 40°C gedurende die dag wat die trek in Bloemfontein gestaan het. Die toestande kan vrug kwaliteit negatief beïnvloed deur verhoogde vog verlies asook 'n verhoogde respirasie tempo. Beide sal lei tot 'n verlies aan rakleefyd.

Die gas toestande en veral CO₂ tydens verskeping van sitrus na die VSA en VK is nie bekend nie. Daar was vermoed sekere skil defekte soos skilafbraak, powwerigheid en kleurverlies het 'n verband met hoë CO₂ vlakke tydens verskeping. Die VSA verskeping, waar sterilisasie toestande van -0.6°C vir 22 dae gehandhaaf moet word, kan problematies wees agv onvoldoende ventilasie. Om die waardes te bepaal is 'n CO₂ moniterings sisteem gebou wat in die vragdek tussen die vrugte geïnstalleer kan word. Die sisteem is na die VSA en VK gestuur en die data is suksesvol ingewin. Die maksimum vlak van CO₂ na die VK was 1600 dpm en na die VSA 6500 dpm. In vergelyk met gewone lug (≈ 350 dpm), is die vlakke baie hoog maar geen bewyse is in die literatuur gevind wat dui daarop dat dit skil kwaliteit negatief kan beïnvloed nie.

General

Environmental changes that occur around the harvested fruit must be seen as primary factors influencing the development of rind disorders such as rind breakdown, pitting, and chilling injuries. The objective of this project was to gather information on the operational environment during fruit transport in order to keep laboratory trials within realistic boundaries. The first part of the project focused on measuring the temperature changes during the transport of Valencias from Letsitele, Limpopo province to Stellenbosch with a truck. The second part focused on the gaseous conditions and temperature changes during shipment of citrus to the USA and the UK.

5.4.6.1 - Truck transport

Introduction

During 2003, reports of Valencia orange type fruit from the Limpopo province showing round, pock-like marks were received from a Stellenbosch packhouse. The fruit did not show any of these scars upon harvest and were placed in bins and transported to a packhouse in Stellenbosch wherafter they showed the marks. The marks were round-shaped and occurred randomly on the fruit. There was no visible oil damage and all

the oil glands were found to be intact and containing oil. The mark looked as if it was due to damage to the epidermal part of the flavedo due to a drying effect. Normally, chilling injuries or desiccation could result in such symptoms.

Materials and methods

Thermocouples, connected to two temperature data loggers (Grant, Squirrel XQ1600), were installed during loading of the carton bins of fruit on a truck. The dimensions of the truck were L =13 m, W = 2.5 m H = 2.2 m. Two rows of 8 bins were placed alongside each other with two rows on top (Fig 5.4.6.1.1). The thermocouples were distributed in order to cover all dimensions of the load. At each sampling point two thermocouples measured the pulp and air temperature. The whole load was covered with a blue plastic tarpaulin at five o'clock in the afternoon on 12 August 2004. The Valencias were harvested in week 33 on the Constantia Products farm in Letsitele. The truck left at 5 pm and arrived in Bloemfontein at 6 am the following morning, where it spent the day before leaving for the Cape around 5 pm and arriving in Stellenbosch at Cape Citrus packhouse at 6 am. The thermocouples were removed and the data successfully downloaded.

Results

The trip consisted of three stages: Letsitele to Bloemfontein, stopover in Bloemfontein and trip from there to Stellenbosch. A steady decline of the load temperature occurred during stage 1 (Figs. 5.4.6.1.2, 5.4.6.1.3 & 5.4.6.1.4). A slight temperature difference in the pulp of fruit at the front and middle compared with the back were recorded with fruit from the back cooling down at a slower rate (Fig. 5.4.6.1.5).

During the second stage the truck was parked in Bloemfontein for service and maintenance during the day. The first increase in temperatures could be due to repositioning of the truck whereafter it stayed static for the rest of the day (Figs. 5.4.6.1.2 - 5.4.6.1.4). The temperatures increased dramatically during this period and maximum temperatures recorded were between 45-50°C under the tarpaulin at the back of the truck. The higher temperatures at the back of the truck could indicate that the truck was parked with the back towards direct sunlight. In the graphs (Figs. 5.4.6.1.2 – 5.4.6.1.4) the thermocouples at the top of the truck recorded the highest readings except at the back of the truck where the bottom recordings were higher; this could be due to the heat being reflected from the tarmac surface.

The last stage commenced at around 5 pm and a quick drop in temperatures can be seen (Figs. 5.4.6.1.2 – 5.4.6.1.4). The temperatures decreased at a rate of about 10°C in an hour whereafter it decreased less rapidly. The minimum temperatures recorded were between 5-10°C around 3 a.m.

Discussion

Citrus, as a subtropical crop can be safely stored between 4-8°C (Kader, 1987) thus the temperatures recorded on this trip could not be responsible for chilling injury. The study was, unfortunately, not done during the coldest months (June-July) but will be repeated in 2005 during these periods.

The most valuable information from this experiment was the exaggerated and sudden changes in the temperatures recorded during the 36-hour trip. Whereas the temperatures did not reach chilling injury levels, the high temperatures during the stop would certainly impact negatively on the postharvest quality. The most obvious factor affected will be an increase in water loss from the fruit. Increasing the product's temperature increases the free energy of the water molecules, which increase their movement and potential for exchange between the fruit and environment. Temperature also affects the amount of moisture that can be held in the air surrounding the product and as the temperature decreases, the maximum amount of moisture that can be held by the air also decreases (Kays and Paull, 2004). It is therefore critical for maintaining quality of the citrus fruit that such temperature differences should be avoided.

References cited

- Kader, A.A., 2002. Postharvest technology of horticultural crops. University of California, Agriculture and Natural Resources Publication 3311.
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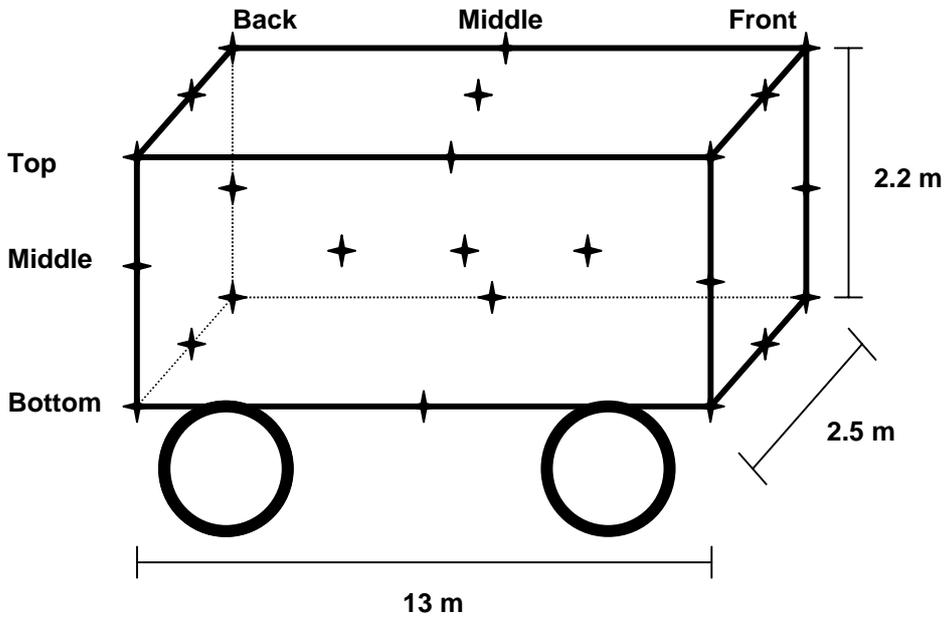


Figure 5.4.6.1.1. Schematic representation of the truck dimensions and the placement of thermocouples in the load of 'Valencia' oranges.

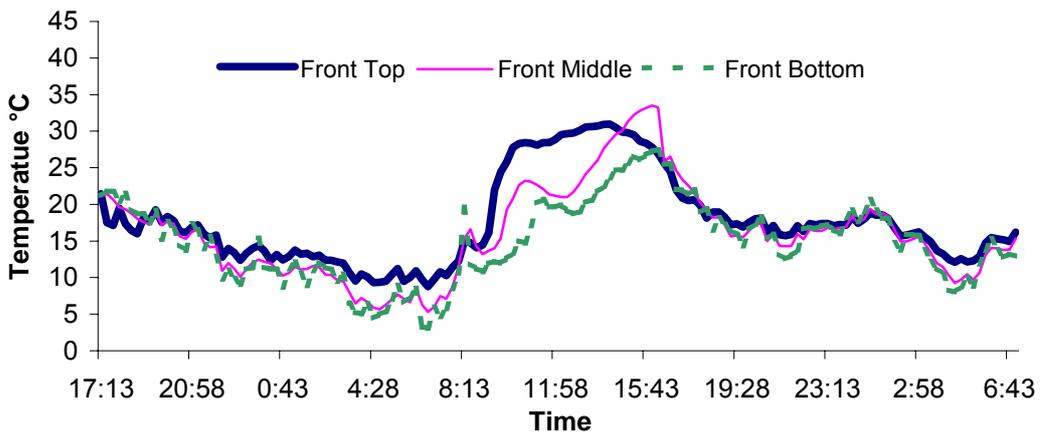


Figure 5.4.6.1.2. Air temperature at the front of the load.

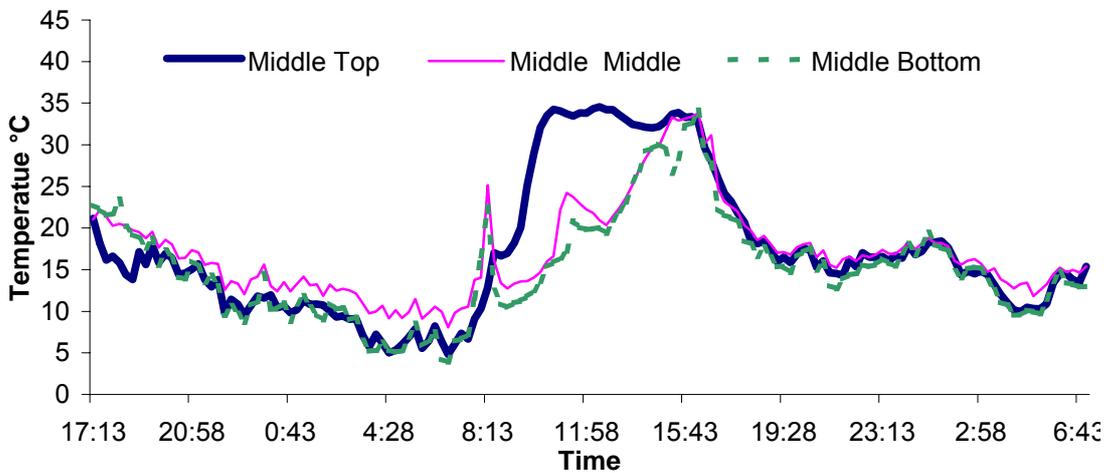


Figure 5.4.6.1.3. Air temperature at the middle of the load.

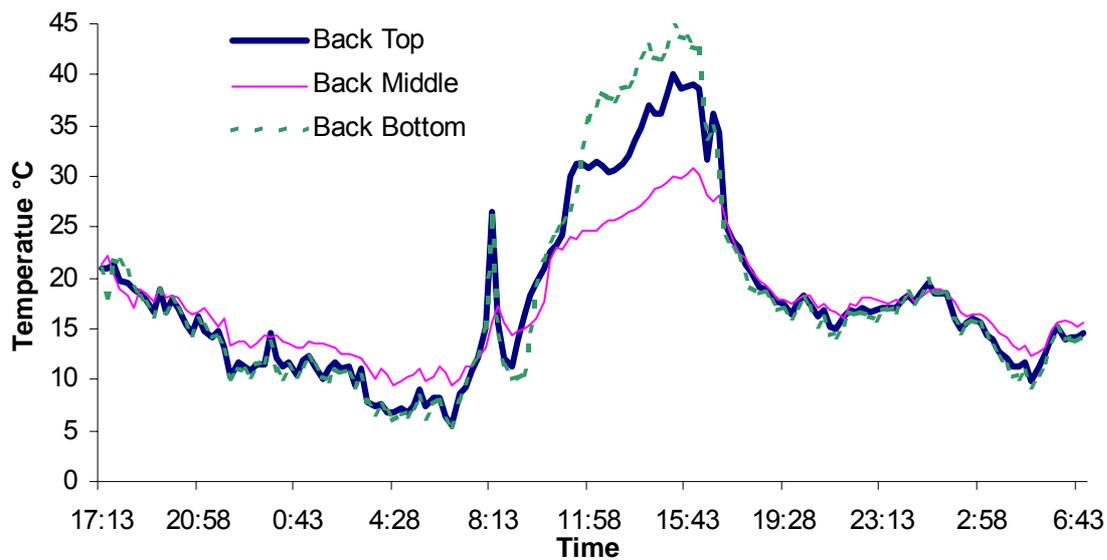


Figure 5.4.6.1.4. Air temperature at the back of the load.

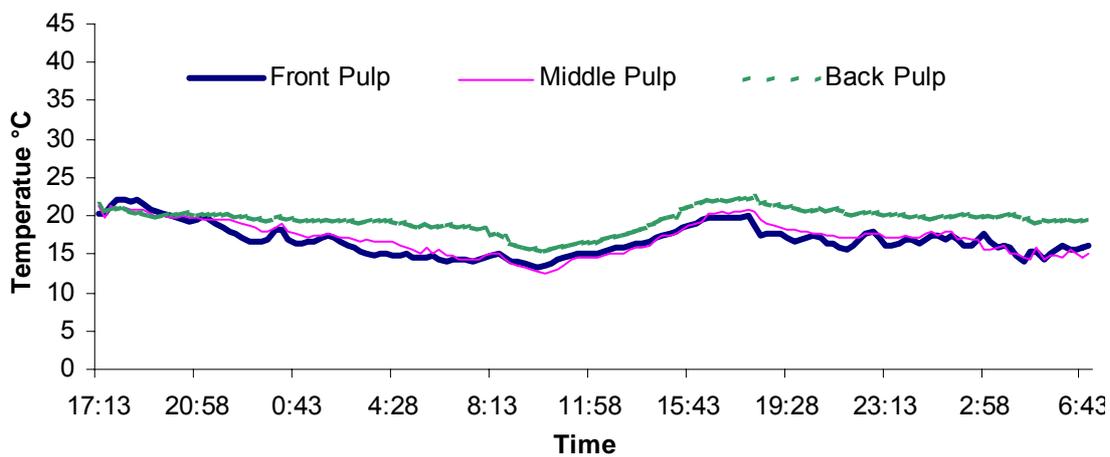


Figure 5.4.6.1.5. Pulp temperature during truck transport from Letsitele to Stellenbosch.

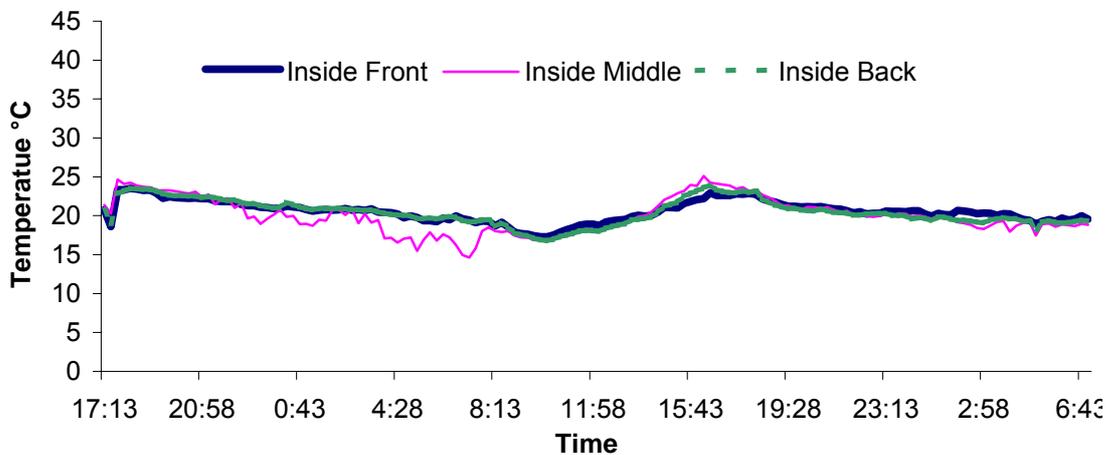


Figure 5.4.6.1.6. Air temperature inside load of fruit during truck transport from Letsitele to Stellenbosch.

5.4.6.2 - Shipping conditions

Introduction

This part of the experiment was done in order to obtain information regarding the atmospheric conditions during shipment of citrus fruit to the USA and UK markets. Shipments to these markets differ not only in regard to length of shipment but also as to the temperatures required by the USDA for sterilisation purposes. This requirement states that citrus must be shipped for a minimum of 22 days at -0.6°C in order to kill False Codling moth and fruit fly larvae in and on the fruit. To obtain these low temperatures the holds of the ship cannot be ventilated as regularly as shipments to the EU and UK. Due to serious losses every year in these special markets as a result of rind disorders such as rind breakdown, puffiness and colour loss, the question was asked if there could be build-up of CO_2 within the hold that could cause these physiological disorders. Currently the only CO_2 values available are those measurements by the ship to comply with hazardous conditions at workplace laws that prohibit anyone from working in an environment with more than 0.05% CO_2 . This information from the ship was found to be inadequate and unreliable. The aim of this experiment was to measure CO_2 levels within the open hold during shipment (not containers).

Materials and methods

A CO_2 sampling and logging system was built from various components by Campbell Scientific Africa in Technopark, Stellenbosch. The system consists of a Li-Cor 840 $\text{CO}_2/\text{H}_2\text{O}$ gas analyser (Li-Cor Bioscience, Nebraska, USA), a CR10X datalogger (Campbell Scientific, Inc. Logan, USA) and a VICI Valve system (Valco Instruments Co. Inc) and is powered by 220V obtained from the ship's power supply. The system (Fig.5.4.6.2.1) is built into a plastic box and can easily be installed in the hold, taken off and taken on board as normal luggage on an aeroplane. The system samples at seven points in the hold via 0.5 cm diameter tubing connected to a small pump. The datalogger was programmed to sample air four times per day from each of these points. The CR10X datalogger also doubles as a temperature logger by connecting thermocouples to it. These thermocouples are placed alongside the tubes to measure temperature at the same site where CO_2 is measured.

The first run was to the USA on board the Zembra Nova, loaded with mandarins from Cape Town to Philadelphia, USA. The CO_2 analyser can analyse at two settings, 3000 ppm or 20 000 ppm but at the 20 000 ppm setting the ability to measure the H_2O levels is lost. For the first run and due to inadequate information, the sampling level of 3000 ppm was chosen, which proved to be much too low, as this value was reached within 3 days at sea. Thereafter the new setting of 20 000 ppm was chosen for the rest of the work.

The second run was on board the Snowflower loaded with Valencias from Port Elizabeth to Sheerness UK. With the help of Dewald Millard from Capespan, the system was installed in a hold and retrieved 14 days later in Sheerness. The last run was once again to the USA in a hold loaded with late Valencias.



Figure 5.4.6.2.1. The CO₂ data logger system being installed behind the cooling wall.

Results

The aim of gathering information on the environment within a hold of citrus fruit was successfully executed with the CO₂ logger system. The first run to the USA helped the researcher in understanding the complications of installing the system on a ship. Addressing issues such as power supply on the ship as well as coordination with PPECB and the ship's agent and captain are vital for the successful execution of such a project. The system was found to be very reliable and robust and did not lose any data during all three of the shipments.

The results from the first shipments are shown in Fig. 5.4.6.2.2 and the maximum detection level of 3000 ppm was measured within 5 days. This concentration was higher than expected. Fig. 5.4.6.2.3 plots the data from the shipment to Sheerness and to Philadelphia with a period of calibration between these two shipments. The calibration check of the equipment was done at ambient CO₂ levels of around 350 ppm. The temperature recorder was also checked with a water bath and a mercury thermometer. The sensors were well within accepted boundaries. The difference between the USA and UK shipments is the result of the enforced sterilization protocol for any citrus export from RSA to the USA. To obtain this protocol of 22 days at -0.6°C the hold will not be supplied with fresh air on a regular basis, as the ships cooling equipment cannot adequately regulate the temperature.

During the shipment to the UK, there was an initial build-up of CO₂ as well as a rise in the temperature, which decreased to the set temperature of 4°C and a CO₂ concentration of 1500 ppm. This decrease started once the ship had sailed and air circulated through the holds. These levels stayed constant and are not thought to be detrimental to the rind of Valencias.

The second shipment to the USA was also very successful and showed a build-up of CO₂ during the voyage even though the hold was supplied with fresh air. Each drop in CO₂ levels coincides with a small, and presumably insignificant increase in temperature illustrating the complexity of maintaining sterilization temperature while keeping CO₂ levels low. The maximum level of 6200 ppm is very high compared to ambient CO₂ concentrations of 350 ppm but there is no literature that indicates a negative effect of this level of CO₂ on rind quality. During the season 'Nules Clementine' mandarins were used in controlled atmosphere experiments that will be reported on in Experiment 780 (CRI annual report 2005). This experiment looked at the effect of 3000, 5000 and 10 000 ppm CO₂ compared with ambient air on the rind quality of 'Nules Clementine' mandarin.

Discussion

Oranges are reported to be able to be stored at 3 to 8°C for up to 3 months depending on the cultivar, maturity at harvest as well as production area (Arpaia and Kader, 2004). Davis and Albrigo (2003) found that oranges and mandarins in particular might be stored for 2 months or more at 0 to 4°C with little loss of fruit quality. Therefore, the temperature conditions to the USA are thought to be potentially very detrimental to the rind quality of the fruit if the temperature was reduced below the set temperature during the shipment.

The CO₂ levels measured were within accepted boundaries as reported in the literature. Gas levels of 5 to 10% CO₂ and 0 to 5% O₂ have been used to retain firmness and delay senescence of oranges and mandarins, but at higher fungistatic levels (10 to 15% CO₂) negative effects such as off-flavours due to the accumulation of fermentative metabolic substances occur (Arpaia & Kader, 2004).

To conclude, the levels of CO₂ found in the holds was higher than the researcher anticipated and following from this information an experiment studying the higher levels of CO₂ on citrus during the shipment period will be undertaken during 2005.

Conclusions

The atmospheric conditions surrounding the citrus fruit are known to have a negative influence on the fruit quality. The temperature changes during truck transport from the northern provinces could impact on water loss and quality of the rind. The huge variation in the temperatures recorded is of concern, as these fluctuations will cause loss of quality and a reduction in the marketability of the fruit.

The gaseous environment during shipment of fruit to the UK and USA was different. The higher levels of CO₂ measured during shipment to the USA are the result of inadequate ventilation due to the sterilisation protocol. These values are not thought to be high enough to cause reduction of the rind quality and lead to physiological disorders.

Future research

During 2005 both these experiments will be repeated. It will be necessary to confirm the 2004 findings and to gather more data under various conditions such as different ships and citrus types.

Acknowledgments

The author thanks the following persons involved with the project:

The ships agents: Mark Ackland, Oscar Jasman in Cape Town and Tony Costello in the UK. Dewald Millard (Capespan) at the harbour in PE for help with the installation. Bernard Henning (PPECB) and Staal du Plesies (Capespan) for help in Cape Town during installation. The USA Alliance and especially Piet Smit, Gerrit van der Merwe and Steve Turner for bringing the system back from Holt. Johan Visagie and Charl le Roux from Campbell Scientific Africa for building the equipment and making the experiment viable.

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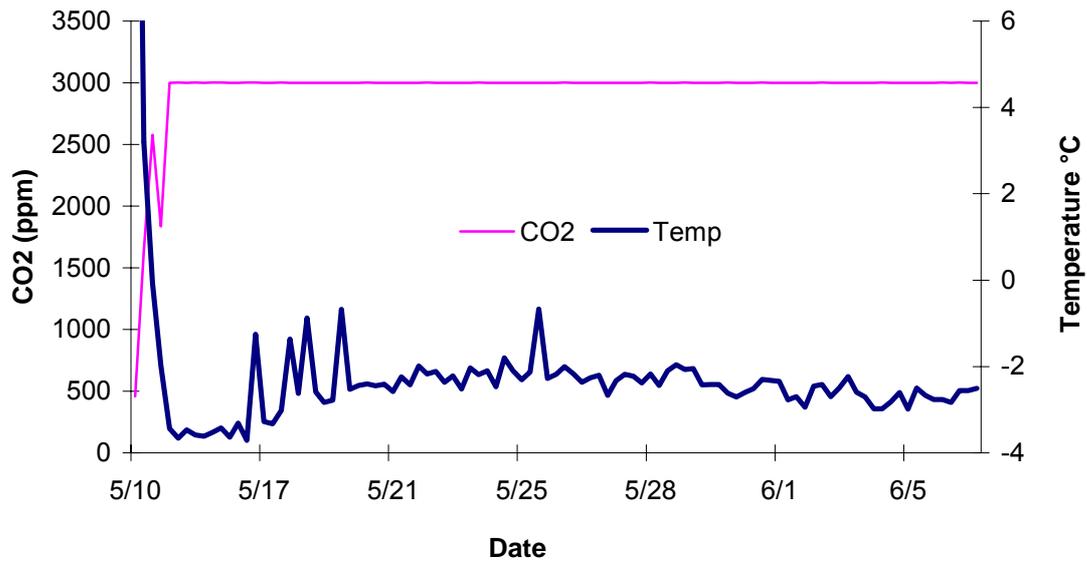


Figure 5.4.6.2.2. Measurements of CO₂ levels and temperature during Clementine mandarin shipment to the USA.

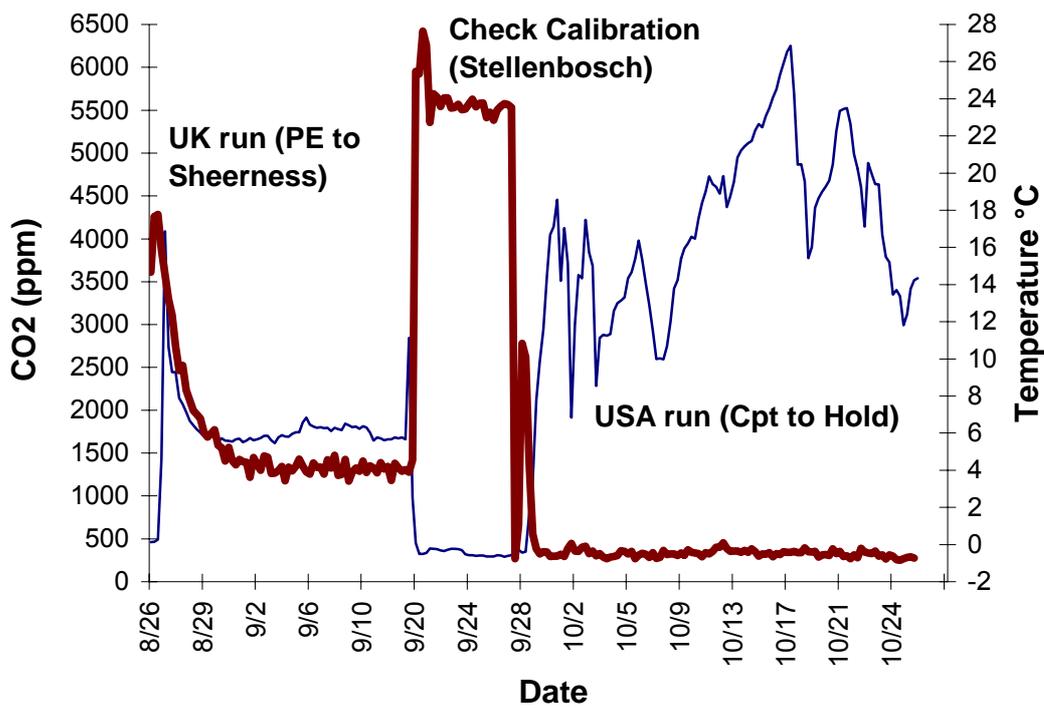


Figure 5.4.6.2.3. Measurements of CO₂ levels and temperature during Valencia oranges shipment to the UK and USA.

5.4.7 Chilling injury to Valencia type oranges

Experiment 766 by Paul Cronje (CRI) Marius Huysamer (SU)

Opsomming

Na geforseerde verkoeling van Valencia lemoene van Citrusdal in 2003 is daar ligbruin aaneenlopende letsels op sommige vrugte gevind. Dit het die vraag laat ontstaan of dit agv die verkoeling is of agv meer sensitiewe vrugte. Vir die proef is Valencias (verpak in teleskopiese kartonne) gedurende September opgeberg in a kouekamer wat gestel was op 3.5°C. Die vrugte was in twee pallette verdeel, een pallet is onderwerp aan geforseerde verkoeling en die ander aan statiese verkoeling. Na 3 dae is die verkoeling gestaak en die vrugte opgeberg by kamer temperatuur en weekliks geëvalueer. Daar was 5 kartonne uit 18 van die geforseerde verkoeling wat dieselfde simptoom gewys het na 3 weke. Die statiese verkoeling het geen van die simptoom getoon nie. Dit wil dus blyk of 'n te vinnige afkoeling tempo by die laat Valencia lemoene kan lei tot simptome van koue skade.

Introduction

Most plant products contain substantially more heat at harvest than normally acceptable for subsequent handling and storage. The heat contained at harvest (field heat) largely comprises thermal energy from the environment surrounding the product. To maintain maximum storage potential of the fruit it is desirable to remove field heat as quickly as possible after harvest: the longer it is postponed the shorter the shelf life of the fruit. Postharvest cooling happens in two stages; firstly bringing down the fruit's temperature to, or approaching, the desired storage temperature and secondly maintaining the desired temperature through continued removal of respiratory heat and heat moving into the storage environment. The two techniques to reduce field heat used in the South African citrus industry are room pre-cooling and forced-air cooling. Room pre-cooling is where products are placed in a room with refrigerated air being blown horizontally and circulated through the room. The disadvantages are the long time fruit have to stay in the rooms due to the slow rate of cooling and therefore an increase in water loss. Forced air cooling takes place in a cold room where packed fruit are cooled by creating a negative pressure across opposite faces of stacks. The cold air is forced to move around individual fruit rather than merely around the exterior of the container, as in cold room or static cooling. The danger of forced air cooling is a too sudden temperature loss that could manifest as chilling related injuries (CI), as well as increased water loss due to the increase in air velocity over the fruit. The dominant means of heat transfer is via forced convection due to the movement of air around the fruit. Because of this, air movement into the cartons is essential for rapid cooling. This is achieved using containers with ventilation holes or slots in the sides. Removal of approximately 5% of the surface area of the container side decreases the cooling time by approximately 25% (Kays and Paull, 2004).

During the 2002 and 2003 seasons brown discolorations of the rind developed on Midnight Valencia oranges that were harvested in Citrusdal area and forced-air cooled in Cape Town harbour to a temperature of 3.5°C. The discolorations were dark brown and occurred in continuous areas normally on one side of the fruit, irrespective of position on the fruit. The dark brown markings become lighter when fruit were removed from the cold room and kept at ambient temperature. After a few days the markings became lighter and developed a grey/silver colour. Observations with a dissection microscope showed that damaged occurred as sunken areas between the oil glands. These sunken areas were probably a result of damage and desiccation to the epidermal cell layers. This disorder poses a problem to the marketability of the fruit as the external quality of the fruit is negatively affected. The objective of this experiment was to see if forced air-cooling could be responsible for this rind injury.

Materials and methods

Late season Valencia oranges were packed at Goede Hoop Sitrus in Citrusdal on 23 September 2004 in telescopic cartons (A15C) of which every second layer of fruit was wrapped. Three different count sizes were chosen (T105, T72 and T48) of which 13 cartons of each were transported directly after packaging to Hortec cold rooms in Stellenbosch where they were divided into two groups. The cartons were packed on a wooden pallet with 3 layers of 9 cartons each and placed in the cold room set at 3.5°C. Thermocouples were inserted at 16 points in the stack and distributed in all dimensions of the stack. At each measuring point the air and pulp temperature was taken. On the one stack of fruit a forced air cooler was fixed and set at 20 mm static pressure difference. The other stack of fruit was static cooled in the same cold room. After 3 days of cooling the fruit were removed from the cold store and placed at room temperature (20°C) and evaluated for any chilling injury (CI).

Results

After one week of room temperature the development of the disorder became evident and fruit was evaluated weekly for 3 weeks before the final evaluation. The cooling rates of the air and pulp in the forced air-cooled fruit were much higher than in static cooling (Fig 5.4.7.1). The air temperature in the forced air stack reached 3.5°C within 12 hours and pulp temperatures of 3.5°C in 26 hours whereas the static air temperature approached 3.5°C only after 72 hours and the pulp temperature did not reach the desired temperature (min 4.5°C). As expected there was a temperature gradient across the pallets, especially the forced air cooling, but the occurrence of the CI did not correspond with one area in the pallet.

The results of cold damage incidence are shown in Table 5.4.7.2. Even though the occurrence of CI was low in the forced air treatment, max 3 fruit per carton, when compared to the static cooling (no CI symptoms) it could be concluded that the forced air cooling resulted in the CI symptoms. The wrappings around every second layer of fruit could have resulted in a lower rate of cooling, thereby reducing the opportunity for cold damage in the forced air treatment.

The necessity and advantages of forced air-cooling make the practice irreplaceable in the fruit industry, but must be used within product specifications. During the continuation of this experiment strategies that could be less detrimental to the fruit will be tested.

Conclusions

The damage caused by the forced air cooling could be due to damage of the epidermal cells and resulting water loss. The reason for the damage is most probably the too rapid cooling tempo and the inability of the fruit to adapt to these conditions. It is therefore necessary to implement the forced air cooling with more care and not to use too rapid a cooling rate.

Future research

During 2005 the experiment will be repeated to confirm the 2004 findings.

Reference cited

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Acknowledgment

I would like to thank Jan Coetzee from Goede Hoop Sitrus Bpk. for supplying the fruit and assistance with the experiment.

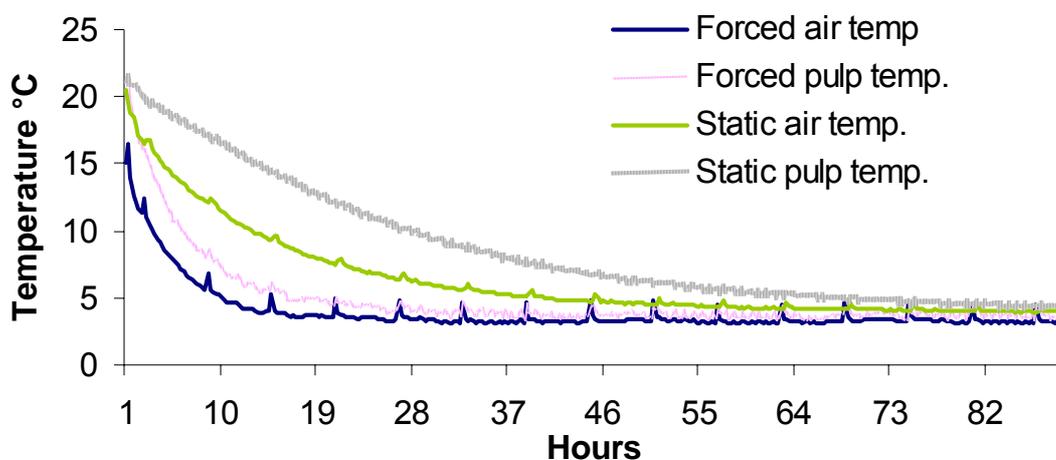


Figure 5.4.7.1. Difference in rate of cooling between static and forced air-cooling, in cold room at 3.5°C. Data represent the average of 16-point readings throughout each pallet.

Table 5.4.7.2. Incidence of Valencia oranges showing Chilling Injury (CI) after 89 hours of either forced air or static cooling.

Forced air			Static air		
Carton	Size	CI (Fruit per ctn)	carton	Size	CI (Fruit per ctn)
1	105	0	1	72	0
2	105	0	2	105	0
3	72	1	3	48	0
4	72	0	4	105	0
5	48	1	5	72	0
6	48	2	6	105	0
7	48	0	7	105	0
8	48	0	8	48	0
9	105	0	9	72	0
10	48	2	10	48	0
11	48	0	11	72	0
12	105	0	12	72	0
13	72	0	13	48	0
14	48	0	14	105	0
15	48	3	15	105	0
16	105	0	16	72	0
17	72	0	17	48	0
18	72	0	18	48	0

5.4.8 Effect of CO₂ on rind condition of Clementine mandarin

Experiment 780 by Paul Cronje, Graham Barry (CRI) and Marius Huysamer (SU)

Opsomming

Gedurende 2003 is proef gedoen waar 20% CO₂ aanleiding gegee het tot 'n verhoogde powwerigheid van 'Nules Clementine' mandaryne, is 'n daaropvolgende proef by lae meer realisties vlakke gedoen. 'Nules Clementine' is tydens opberging by -0.6°C aan vyf CO₂ konsentrasies 0, 0.03 (lug), 0.3, 0.5 en 1% blootgestel vir 32 dae. Die vrugte is ontleed vir kleurverlies asook skilafbraak en powwerigheid. Geen skil afbraak het voorgekom nie slegs een behandeling (0.5% CO₂) het 'n betekenisvolle verhoging in powwerigheid teweeg gebring. Daar was egter 'n betekenisvolle kleur verlies in die pulp van die vrugte. Die verskynsel is nog nie voorheen gedokumenteer vir sitrus vrugte nie, en kan 'n gevolg wees van die hoër suur toestand in die pulp wat negatief inwerk op karotene. Die proef sal herhaal word gedurende 2005 om die resultate te bevestig.

Introduction

During export to the USA, fruit are shipped at -0.6°C for 22 days in order to comply with the sterilisation protocol. Exporters of citrus to the USA report a change in quality of these fruit of which a loss of colour during the shipment period is the most obvious. Coupled with the colour loss are symptoms of physiological breakdown of the rind that are seen as puffiness and rind breakdown.

Puffiness: an expansion of the rind that causes the rind to pull away from the pulp and in an advanced stage of the disorder the segments separate from each other.

Fruit yellowing: fruit that comply to colour specifications prior to shipping are perceived in the USA as yellow fruit below colour specifications. Could be as result of loss of orange pigmentation.

Rind Breakdown (RB): Subepidermal collapse of oil glands that occur 3 to 4 weeks after harvest.

During experiments to determine the effect of high CO₂/low O₂ concentrations on False Codling Moth, a negative reaction resembling rind breakdown was seen on Valencia oranges. Treatments of 5% O₂ and 20% CO₂ resulted in 7-14% RB whereas 10% O₂ and 60% CO₂ resulted in 7-22% RB (CRI annual report 2001).

From these results as well as a perception in the market that high CO₂ levels could be detrimental to the rind quality, an experiment studying the effect of high CO₂ was conducted in 2003. The effect of three levels of CO₂ (5%, 10% and 20%) was studied as it affects rind condition of 'Nules Clementine' mandarins. The 20% CO₂ treatment resulted in puffiness and concurs with previous research done on controlled atmosphere.

From this information and the fact that the gas composition in the ships hold during the voyage is unknown, CO₂ levels in the ships hold were thought to be detrimental to fruit quality. CO₂ is a product of respiration of fruit necessary to supply energy for maintenance and synthetic reactions after harvest. Together with O₂ and ethylene, CO₂ is an important gas influencing respiration. In citrus fruit high CO₂ levels increase the respiration rate that may be partly due to the mechanism of fixation of carbon dioxide by malic enzyme and phosphoenolpyruvate carboxylase (Kay & Paull, 2004). It was therefore hypothesised that the high CO₂ concentration could increase respiration and lead to detrimental rind conditions. The object of this experiment was to study the effect of elevated CO₂ levels on rind condition of 'Nules Clementine' mandarin citrus fruit.

Material and methods

Premixed gas from Afrox was used as part of five CO₂ treatments: 0%, 0.03% (normal air) and 0.3, 0.5 and 1% CO₂. In all treatments 21% O₂ and N₂ made up the balance. 'Nules Clementine' mandarins harvested on 16 May 2004 from the University of Stellenbosch experimental farm Welgevallen, were degreened and packed according to normal commercial practices. Thereafter for each of the 5 replications per treatment, 10 fruit were selected, weighed and placed in a bucket with a connection to a flow board and an outlet from the cold room. The buckets were kept in a cold room at -0.5°C for 32 days (to mimic the maximum commercial period at these conditions). The flow rate of the treatment gases was high enough to prevent a build-up of ethylene and CO₂ inside the bucket.

After the storage period the fruit were kept at ambient temperature (20°C) with the bucket open to prevent CO₂ and ethylene build-up. Thereafter fruit were evaluated for colour differences with a chromameter (Minolta NR 4000, Osaka, Japan). The symptoms of rind disorders were scored and the fruit were cut open to evaluate the degree of puffiness as well as the internal colour change of the pulp. The degree of puffiness was measured with a calliper according to the distance between the pulp and the peel and the separation of the centre of the fruit as the segments separated (Figure 5.4.8.1). The data were analysed with GLM procedures of SAS 2002.



Figure 5.4.8.1. Measurement of degree of puffiness.

Results

During the evaluation for RB no symptoms of the disorder were found on any fruit. Only the 0.5% CO₂ treatment gave significantly higher levels of puffiness as measured by segment separation (Fig. 5.4.8.2). Loss of rind colour was observed in all of the treatments and not specifically in the higher CO₂. The control fruit (normal air, 0.03% CO₂) was less well coloured than the higher CO₂ treatments as measured by the higher lightness and Hue° values (where higher lightness values denotes lighter fruit and high Hue° more

yellowness) (Fig 5.4.8.3). The fruit were cut open for puffiness evaluation and the pulp of the high CO₂ treatments was visibly lighter. This was documented with the chromameter and a significant loss of colour of the pulp was found as the CO₂ levels increased (higher lightness and Hue° values) (Figure 5.4.8.4).

Discussion

The suspected effect of higher CO₂ levels on rind quality (loss of colour and development of disorders) was not seen during this experiment. These data will be validated during the 2005 season and higher CO₂ concentrations such as 1% and 5% will be used. These levels are thought to be realistic after the data collected during experiment 759 (5.4.5) showed that the maximum CO₂ level that was measured in the hold of a ship was 6200 ppm (0.6%).

The probability of low temperature being responsible for colour loss was shown by Van Wyk (2004), where colour loss was documented in Navel oranges. The lack of rind breakdown development was unexpected and no explanation can be given at this time. The loss of colour of the pulp has not been documented in citrus, but in a report by Perkins-Veazie and Collins (2004), a reduction of orange colour (and lycopene) of fresh cut watermelon pieces was recorded. They argued that the probable causes were due to the high CO₂ levels or the physical injury of cutting resulting in juice leaking from vacuoles that could result in lower pH that are detrimental to carotenes. This result concurs with Wright and Kader (1997) who found an increased degradation of carotenes during storage of peaches and persimmons >12% CO₂.

Conclusions

The influence of higher CO₂ during the postharvest period did not cause the rind disorders puffiness and rind breakdown to develop. There was also no loss of colour, as expected. These levels tested could be too low to cause these disorders to develop and will be increased during the next set of experiments.

Future research

The experiment will be repeated to confirm the data obtained in 2004. The levels of CO₂ will be increased as a result of the data obtained in experiment 759.

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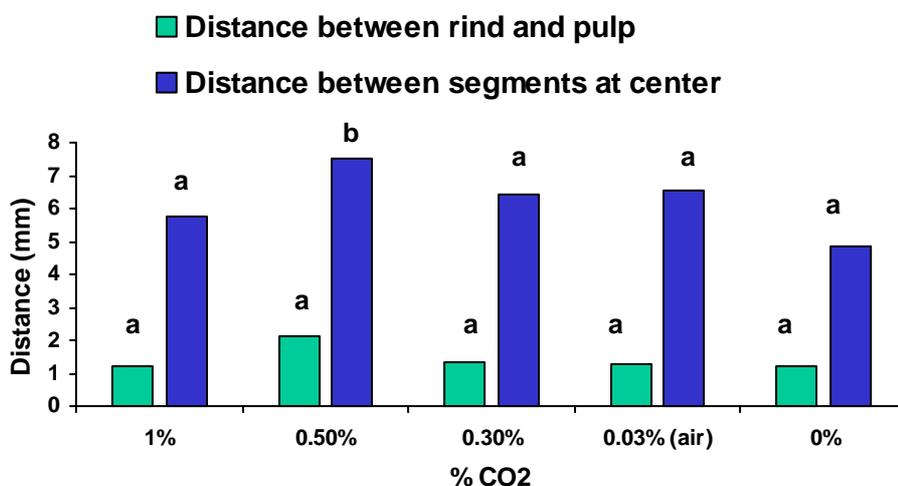


Figure 5.4.8.2. Measurement of puffiness severity in 'Nules Clementine' mandarins as influenced by elevated CO₂ concentrations.

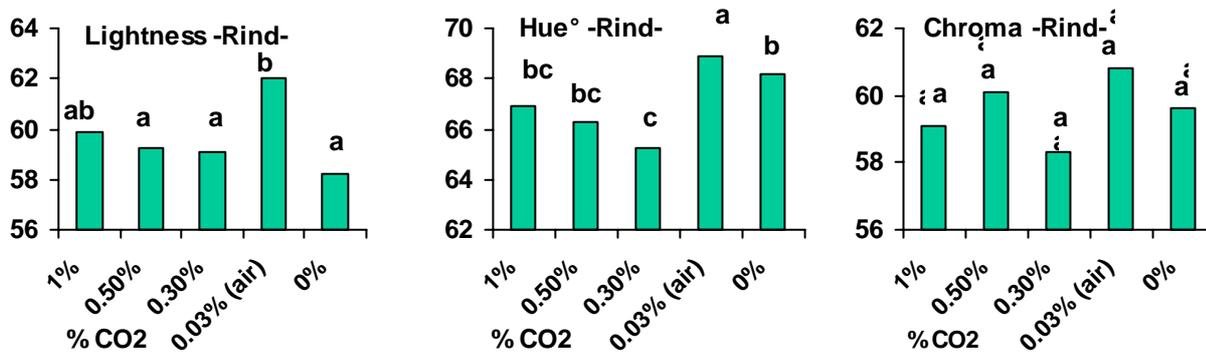


Figure 5.4.8.3. Effect of elevated CO₂ levels on rind colour of 'Nules Clementine' mandarins.

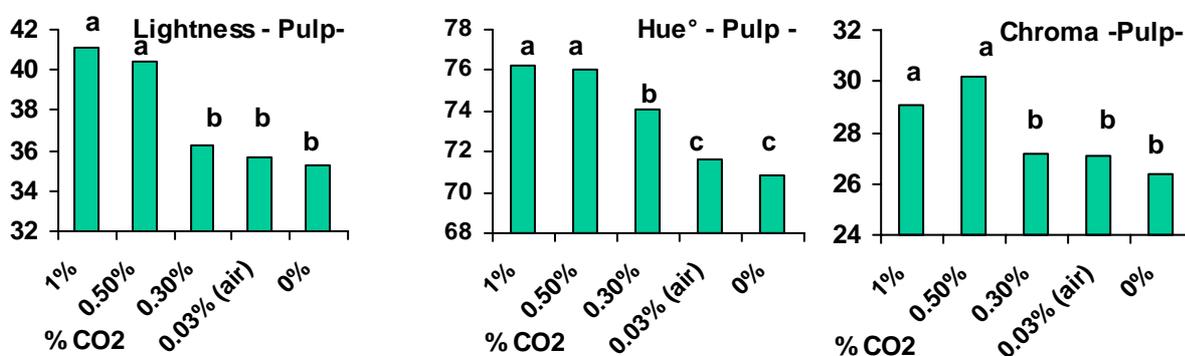


Figure 5.4.8.4. Effect of elevated CO₂ levels on pulp colour of 'Nules Clementine' mandarins.

6 PROGRAMME: CULTIVAR AND ROOTSTOCK DEVELOPMENT

6.1 PROGRAMME SUMMARY

By Tim G. Grout (Research & Technical Manager: CRI)

Once again there is no report on the Breeding project conducted by the ARC due to their failure to reach agreement with the CGA/CRI on the sharing of intellectual property. The project summary below therefore serves as the programme summary as it covers all research funded in the programme.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsings en Tegnieuse Bestuurder: CRI)

Daar is weereens geen verslag oor die telingsprojek wat by die LNR uitgevoer is nie, omdat geen ooreenkoms met die CGA/CRI bereik kon word op die deling van die intellektuele eiendom nie. Die projek opsomming hieronder dien dus as die program opsomming aangesien dit al die navorsing wat in die program uitgevoer is, dek.

6.2 PROJEK: EVALUASIES

Projek Koördineerder: Thys du Toit (CRI)

6.2.1 Projekopsomming

Die doel van die Kultivar en Onderstam- evaluasieprojek is om nuwe bostam- en onderstam kultivars te vestig en te evalueer en te vergelyk met bestaande kommersiële kultivars en aanbevelings te maak aan die Suid-Afrikaanse Sitrusbedryf.

Verdere onderhandeling deur die CGA / CRI met die LNR oor mede eienaarskap van kultivars uit die ITSG se telingsprogram het plaasgevind, maar geen oplossing is gevind nie. Befondsing deur die bedryf van projekte wat deur die LNR hanteer word, is gestaak en daarom is evalueringsverslae van projekte wat deur hulle hanteer word nie aan ons verskaf nie. Vir die CRI hanteer Johan Joubert al die Noordelike en binnelandse evaluasie projekte en Chris Alexander die Wes- en Oos – Kaap evaluasies op kontrak. Om die belange van die sitrus produsente te beskerm in die proses van kultivar kommersialisering het die CGA die CRI versoek om 'n bedryfs Kultivar Bestuurder pos te skep en dr Graham Barry is vanaf 1 Januarie 2005 in hierdie pos aangestel. Van die kern funksies van hierdie pos sluit in om te onderhandel vir nuwe kultivar regte internasionaal en plaaslik en om 'n veldtog te loots vir die soek na plaaslike belowende mutasies. Hierdie kultivars sal dan deeglik deur die CRI se Kultivarevalueerders in verskillende geskikte produksie areas geëvalueer word. Terselfdertyd sal groeipuntenting en pre-immunisering deur die CRI se laboratorium op Nelspruit op hierdie kultivars gedoen word waarna materiaal aan die Sitrus Grondvesblok op Uitenhage beskikbaar gestel sal word vir vermeerdering en voorsiening aan die bedryf via geakkrediteerde kwekerye. Die eienaars van hierdie nuwe kultivars sal ook gehelp word met die patentering en kommersialisering daarvan.

Vir produsente om kompetend te bly in 'n groeiende Internasionale vrugtebedryf moet vrugte geproduseer word van hoë gehalte en met 'n hoë opbrengs. Nuwe kultivars met beter eienskappe is nodig om sekere bestaande seleksies te vervang en kultivargroepe aan te vul om solank as moontlik op die mark beskikbaar te wees asook eksotiese kultivars om 'n nis mark te vul.

Opsomming per kultivar groep om te bepaal of ons aan die behoefte voldoen:

Satsumas: Die soek na vroeër en later Satsuma seleksies wat die huidige Miho Wase en Owri kan aanvul, was nog nie suksesvol nie en gaan voort.

Clementine: Om die Nules piek af te plat moet vroeër en later seleksies gevind word. Die Esbal is vroeër, maar het 'n kleinvrug probleem.

Mandaryne: 'n Groot aantal belowende seleksies is geëvalueer, maar die bome is nog jonk met onbetroubare resultate. Die Murcott x Clem kruising lyk belowend.

Nawels: Simptome van moontlike onverenigbaarheid van die Fukumoto nawel op citrange onderstamme is in Suid- Afrika waargeneem en alhoewel dit nie tipies dieselfde is as in Kalifornië nie is produsente gewaarsku deur middel van 'n artikel in die Vrugte Joernaal van Okt / Nov 2004. Die soektog na 'n vroeër nawel as die huidige Lina en Newhall gaan dus voort. Die Cara Cara lewer nog steeds goeie resultate, maar het

produksie probleme met kraakskil, sonbrand en windmerke en word swak bemark. Die huidige laat nawels moet nog goed met mekaar vergelyk word met duidelike aanbevelings.

Midseisoeners: 'n Behoeftebepaling moet gemaak word om te bepaal of daar 'n plek is vir hierdie kultivar groep. Rooi gepigmenteerde seleksies het koue nodig vir interne kleur ontwikkeling en het 'n beperkte mark.

Valencias: Geen nuwe seleksies met beter en uitstaande kenmerke is tot dusver gevind om die bestaande kommersiële seleksies te vervang of aan te vul nie. Die Ruby Valencia met 'n rooi interne kleur se potensiaal sal in die mark getoets moet word. Mouton Early het in die Wes-Kaap evaluasies belowende resultate gelewer.

Suurlemoene: Die Villafranca met gemiddeld 0.3 sade vaar die beste as 'n amper saadlose suurlemoen, maar sal teenoor die Eureka SL geëvalueer moet word. Die mark vra vir vroeër suurlemoene asook langwerpige vrugte.

Onderstamme: Die evaluering van onderstamme is 'n langtermyn projek en vanjaar het die droogte en uiterste koue in die Vaalharts gebied die proefpersele benadeel en dit is moeilik om gevolgtrekkings te maak. C- 35 is reeds bewys as 'n goeie onderstam wat uitstekende interne gehalte bostam vrugte lewer, maar het vanjaar gewys dat dit baie gevoelig is vir uiterste koue. Turkey Valencia op Rangpur lime en Growweskiisuurlemoen wys onverenigbaarheid en word dus nie aanbeveel nie.

Project summary

The objective of the Cultivar and Rootstock Evaluation project is to establish and evaluate new scion and rootstock cultivars, compare their performance to existing commercial cultivars and make recommendations to the South African Citrus Industry.

Further negotiations took place between CGA / CRI and the ARC in respect of joint ownership of ITSC cultivars emanating from their breeding programme, but the outcome was not successful. Funding by the industry of projects undertaken by the ARC was stopped and therefore no reports were received. Johan Joubert of the CRI is responsible for the northern and inland evaluation projects, while Chris Alexander is contracted to undertake evaluations in the Western and Eastern Cape.

In order to protect the interests of citrus producers in the process of cultivar commercialisation, the CGA requested CRI to establish the position of Industry Cultivar Manager. Dr Graham Barry was appointed to this post as from 1 January 2005. Some of the key functions of this post will include negotiating for rights to new cultivars both locally and internationally, and launching a campaign to search for promising local cultivar mutations. These cultivars will then be thoroughly evaluated in the various production areas by CRI Cultivar Evaluators. The cultivars will simultaneously be put through shoot tip grafting and pre-immunisation at CRI in Nelspruit, after which material will be released to the Citrus Foundation Block in Uitenhage, for increase and supply to the Industry via accredited nurseries. These cultivar owners can be assisted in patenting and commercialisation.

In order for South African citrus growers to remain competitive in the growing international fruit industry priority must be given to the production of high quality fruit with increased yields. New cultivars with improved characteristics are required to; replace certain existing selections, to spread the marketing season and supply exotic cultivars for niche markets.

Summary per cultivar group to assess if the objectives have been achieved:

Satsumas: The search for earlier and later Satsuma selections to complement the Miho Wase and Owari has not been successful and will continue.

Clementine: The objective here is to flatten the Nules peak with earlier and later selections. The Esbal is earlier, but has a small fruit size problem.

Mandarins: A large number of promising selections were evaluated, but the results are still unreliable due to the young trees. The Murcott x Clem hybrid appears promising.

Navels: Symptoms of possible incompatibility of the Fukumoto navel on citrange rootstocks have been observed in South Africa. This is not typical of Californian observations. Citrus growers were warned of this danger in an article published in the SA Fruit Journal of Oct/Nov 2004. The search for navels maturing earlier than the Lina and Newhall is therefore continuing. The Cara Cara is performing well but there are

some production problems including creasing, sunburn and wind damage. The fruit is poorly marketed. The current late navels must be thoroughly compared to each other so that clear recommendations can be made.

Midseasons: An assessment must be made to determine if there is a place in the market for the cultivar group. Red pigmented selections require a cold climate for the development of the internal colour, thereby limiting production areas.

Valencias: No new selection showing improved and outstanding characteristics has been found to replace or complement existing commercial selections. The potential of the Ruby Valencia with its red internal colour must be tested in the market. The Mouton Early delivered promising results in the Western Cape.

Lemons: The Villafranca with an average 0.3 seeds per fruit is the most promising in terms of being a nearly seedless lemon, but will have to be evaluated and measured against the Eureka SL. The market requires an earlier lemon as well as an elongated fruit.

Rootstocks: The evaluation of rootstocks is a long term project and the drought in general and the excessive cold in the Vaalharts area affected trial sites negatively, making it difficult to arrive at any conclusions. C- 35 has already proved to be a very good rootstock which delivers excellent internal scion fruit quality, but this year it became evident that this rootstock is sensitive to extremely cold temperatures. Turkey Valencia on Rangpur lime and Rough lemon showed incompatibility and is therefore not recommended.

6.2.2 Sub-Project: Evaluation of cultivars in the Cape region

By C.J. Alexander (Private)

6.2.2.1 Subprojekopsomming

Die verslag behels werk wat deur Chris Alexander, gehelp deur Zongezile Zondi, in die suiderlike sitrusproduksiegebiede van die Oos en Weskaap gedoen is. Dankie aan al die kwekers wat proefbome op hulle plase aanhou wat geevalueer kon word.

Sub-Project summary

This report comprises work conducted by Chris Alexander and assisted by Zongezile Zondi in the southern citrus production areas of the Eastern and Western Cape. Thank you to all growers who co-operated by having trees available for evaluation on their farms.

6.2.2.2 Evaluation of Satsuma mandarins in the Cape areas

Experiment 57 by C J Alexander (Private)

Opsomming

Die doel van hierdie projek is om geskikte, hoë gehalte seleksies te vind wat vroeg in die seisoen opkleur en die vroeë mark kan binnedring, asook om produksie pieke te voorkom. Nie een van die satsuma seleksies het aan die doelstelling van 'n vervroegde seisoen beantwoord nie. Daar is egter alternatiewe vir die huidige kommersiële reeks seleksies wat die seisoen van die Miho Wase tot na die Owari-seleksie kan verleng. Die Ohtsu, Aoshima en Ueno is laat, maar gehalte swak en die kommersiële Dobashi Beni nog nie in produksie nie. Evaluasies van Dobashi Beni en die later seleksies moet nog voortgaan. Die ITSG Satsuma x Nova seleksie moet in ander areas gevestig word sodra materiaal vrygestel word.

Introduction

The objective of the Satsuma project is to provide high quality, well coloured fruit early in the southern hemisphere marketing season, to capitalise on market opportunities between the late northern hemisphere season and early southern African citrus season and to overcome anticipated production peaks by extending the harvest season earlier. The Satsuma X Nova, Ohtsu, Aoshima, Ueno and Dobashi Beni were evaluated.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi-commercial scale, with Miho Wase control where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting.

Fruit quality was compared with the following standards previously considered most acceptable by the market place, based on visual and organoleptic tests: 48% juice; 9.0% TSS; 0.7 – 1.5% acid; 7.5:1 ratio; colour T3 of set 36 of CRI Blemish standards. Zero seeds/fruit. A list of the selections and sites evaluated is given in Table 6.2.2.2.1 and internal quality tests in Table 6.2.2.2.2.

Table 6.2.2.2.1. Satsuma trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Satsuma X Nova	Addo	ITSC	2000	CC	3
Dobashi Beni	Uitenhage	CFB, Block 7	1994	TC	2
Ohtsu	Uitenhage	CFB, Block 8	1997	SC	6
Ohtsu	Grabouw	Whitehall	1997	SC	5
Aoshima	Uitenhage	CFB, Block 7	1994	TC	2
Aoshima	Rheenendal	Candlewood	2002	TC	5
Aoshima	Grabouw	Whitehall	1997	SC	5
Ueno	Uitenhage	CFB, Block 8	1997	TC/SC	3/3
Ueno	Karatara	Lancewood	1998	TC	2 topwork
Ueno	Stellenbosch	Slaley	1995	CC	10
Miho Wase (control)	Karatara	Lancewood	1996	TC	commercial
Miyagawa Wase (control)	Uitenhage	CFB, Block 7	1994	TC	2
Miyagawa Wase (control)	Stellenbosch	Slaley	1995	CC	commercial
Okitsu Wase (Control)	Grabouw	Whitehall	1997	SC	5
Owari (control)	Uitenhage	CFB	1991	SC	semi comm

Results and Discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.2.2. which need to be referred to when reading the text. The overall quality of the satsumas at Grabouw this season was poor (low sugars and acid), which is reflected in the selections evaluated.

ITSC Satsuma X Nova

It was reported that the SE x Nova performed similar to previous seasons, i.e. similar to slightly earlier than Miho Wase. The fruit degreens well and does not go puffy like Satsumas. Budwood is not available.

Dobashi Beni

The CFB trees had a fair crop of medium-large fruit size, mainly T7 at the end of April, T4-5 on 15 May. Older Owaris had a better crop, smaller size and T5 in May. The fruit was puffy and not good eating quality, Owari slightly better. Both open cores, Dobashi a deeper colour. Both met export standards, Owari higher sugars, but lower acid. Dobashi sugars were slightly lower than 2002. The fruit has a slightly flat shape and smooth rind with odd seed in both selections (cross pollinated blocks). No comparison was done of the rind colour between the selections.

The semi commercial orchard in the Western Cape should produce some fruit next season.

Ohtsu

The trees at the CFB (next to a windbreak) had a poor crop of large fruit size, T7-8 on 28 April and T6-7 on 15 May (Ueno T4-5, Aoshima T5, and older Owari T5). The quality in May was poor and puffy with an unacceptable test (large fruit).

Trees at Grabouw had a good to excellent crop of medium-large to large fruit size, T5-6 on 5 May (Aoshima mainly T6, Okitsu T1-4). Fruit quality was poor with slightly puffy fruit and the test had unacceptably low sugars (poor quality season). The Aoshima also had poor quality and Okitsu slightly better but past peak by about two weeks.

Fruit shape is flat with a smooth rind, a good orange flesh colour and easily peeled. There was odd sunburn at Grabouw and trees have medium vigour.

Aoshima

The CFB trees had a poor crop of large fruit size, T7 on 28 April, T5 on 15 May (older Owari on Swingle T5). Eating quality was poor with low sugars and acid, fruit puffy and the test was unacceptable due to low acid and low juice (large fruit).

The young trees (first crop) at Rheenendal had a good crop of medium-large to large fruit size, T3 on 22 June, still green when the older Owaris were pickled three weeks earlier. The fruit was puffy and overmature with poor quality.

Trees at Grabouw had a fair to good crop of medium-large to large fruit size, mainly T6 on 5 May (Ohtsu T5-6 and Okitsu T1-4). Fruit quality was poor with low sugars, fair acid and puffy. The test had unacceptably low sugars (poor quality season). The Ohtsu also had poor quality and Okitsu slightly better but past peak by about two weeks.

Fruit shape is flat with a smooth rind, a good orange flesh colour and easily peeled. There was slight sunburn at Grabouw and odd seed at the CFB (mixed block). The trees have medium vigour.

Ueno

The CFB trees had a poor crop of large fruit size, colour T7-8 on 28 April and T4-5 on 15 May the Owari on Swingle T5, Miyagawa Wase T2-3. The fruit had poor quality with open cores and oily. The test on Troyer was poor.

Trees at Stellenbosch are stressed by the adjacent windbreak, the crop poor to fair with medium to medium small fruit size, T 6 on 16 April T4-6 on 5 May. Adjacent non stressed Miyagawa Wases were partly picked in April, remaining fruit T3-5. The fruit was not yet mature in April, slightly open cores with high sugars and excessive acid in May but this is probably stress related.

The tree at Karatara had a good crop of medium fruit size, T7 on 26 April, T2-4 on 22 June. Adjacent Miho Wase were partially picked in April, past peak and starting to puff, similar crop and fruit size, colour T1-4. Ueno taste was poor in April, lacking any flavour, easily peeled and open cores. There was little difference in quality between the selections except the Ueno is later with a tighter peel, firmer and not overmature. The orchard had a lot of irrigation/ rain since January. The fruit was still poor quality in June and overmature, the test unacceptable.

The fruit is flat and smooth with a deep orange flesh colour. In all cases there was some seed, cross pollination at the CFB and Karatara, and Clementines nearby at Stellenbosch. It is difficult to estimate maturity due to the unacceptable quality, but definitely later than Miho Wase, later than Miyagawa Wase and inferior quality to older Owari at the CFB although similar colour. The trees are vigorous except at Lancewood.

Conclusion

Satsuma X Nova

This selection apparently performed the same as in previous seasons, with early maturity, firm fruit and degreening well. It needs to be established in other areas as a potential early Satsuma.

Dobashi Beni

Production was fair with good fruit size and a quite acceptable test. Maturity about late April. Wait until semi commercial orchard comes into production.

Ohtsu

The yields varied between sites from poor to good to excellent and good to large fruit size. Internal quality was poor. Due to the poor quality it is difficult to estimate maturity but it is later than the Okitsu Wase and later colour than Owari. There is too little information available to make recommendations.

Aoshima

The yield was variable between the sites with good to large fruit size and poor quality. Due to the poor quality it is difficult to estimate maturity, but it is later than Okitsu Wase and later colour than Owari. Odd seed in a mixed block. There is too little information available to make recommendations.

Ueno

The yields were poor to good, with medium to large fruit size. Fruit quality was poor and some seed where cross pollinated. Due to the poor sites, recommendations cannot be made.

Table 6.2.2.2. Internal fruit quality data for Satsuma mandarins for the Eastern and Western Cape areas tested during the 2004 season.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave Seed
Dobashi Beni	TC	CFB	15/05	4-5	1X	51.4	9.5	0.94	10.1	0.9
Ohtsu	SC	CFB	15/05	6-7	1XXX	43.2	8.2	0.77	10.6	0
Ohtsu	SC	Whitehall	05/05	5	1X	50.5	7.6	0.94	8.1	0
Aoshima	TC	CFB	15/05	4-5	1XXX	43.2	9.2	0.65	14.2	1.2
Aoshima	SC	Whitehall	05/05	5-6	1X	50.2	6.9	0.94	7.3	0
Ueno	TC	CFB	15/05	4-5	1XXX	48.1	7.8	0.73	10.7	1.6
Ueno	CC	Slaley	05/05	4	4	57.2	15.8	1.70	9.3	0.2
Ueno	TC	Lancewood	22/06	3-4	2	46.7	8.2	0.68	12.1	1.5
Miyagawa Wase	TC	CFB	15/05	2-3	1XXX	43.9	7.8	0.59	13.2	0
Okitsu Wase	SC	Whitehall	05/05	3-4	1	54.4	8.1	0.91	8.9	0
Owari	SC	CFB	15/05	4-5	1X	58.0	10.3	0.88	11.7	0.3

Future research

Establish the ITSC Satsuma X Nova as an early maturing selection in other areas as soon as possible. Evaluate Dobashi Beni when semi commercial block comes into production. As there is growing interest in the planting of late maturing Satsumas, establish new sites including all these selections. In the interim re-evaluate the old discarded sites that included Ohtsu, Aoshima and Ueno.

6.2.2.3 Evaluation of Clementine mandarins in the Cape areas

Experiment 63 by C J Alexander (Private)

Opsomming

Die doel van die projek is om 'n breë verskeidenheid kultivars met hoër gehalte, goeie skilkleur en met goeie vruggrootte te verskaf om die pieke in die middel Clementine seisoen af te plat deur die oestyd beide vroeër en veral later te verleng. Early Marisol het verbeter maar lyk op die stadium nie baie belowend as 'n goeie vroeër Clementine nie en moet vir nog een jaar geëvalueer word. Esbal produksie en gehalte was baie goed met kleiner vruggrootte as Nules wat dalk probleme veral in koeler gebiede kan skep. Cadoux het goeie produksie maar klein vruggrootte gehad en word nie verder geëvalueer nie. Daar was te min inligting oor die Tardif de Janvier I en II. Die Sidi Aissa en Ain Toujdate is midseisoen seleksies met goeie produksie, maar vruggrootte aan die kleiner kant en is nie besonders nie. Die CELL lyk baie soos die Clemlate met groen blomente en vertraagde vrugkleur. Clementarde het relatief klein vrugte gehad met te veel variasie tussen persele om aanbevelings te maak. Die Hernandina is soortgelyk aan die Clemlate met relatief klein vruggrootte. Die Tardivo het goeie produksie met relatief klein vruggrootte en relatief lae suur gehad met vrugkleur soos die Clemlate. Nour bome is dig met swak produksie en relatief klein vruggrootte, dalk vertraagde vrugkleur en groen blomente. Verder evaluasies is nodig, veral die laat seleksies.

Introduction

The objective of the project is to find suitable superior Clementine selections to help flatten the existing midseason production peaks by extending the harvest season both earlier and particularly later in accordance with market needs.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi commercial scale, using Marisol, Nules or Clemlate as controls where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the standards previously considered most acceptable in the market place: 48% juice; 9.5% TSS; 0.7 – 1.5% acid; 8.0:1 ratio; colour T3 of set 36. Seed maximum average 3.0 seeds per fruit. Oleocellosis evaluations were done on some samples – the percentages given are rated for oleo present but exportable - class I, or not exportable - class 2 fruit. A list of selections and sites evaluated is given in Table 6.2.2.3.1.

Table 6.2.2.3.1. Clementine trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Early Marisol	Stellenbosch	L'Avenir	1999	CC	3 topwork
Esbal	Uitenhage	CFB Block 7	1995	TC	1
Esbal	Clanwilliam	Twaktuin	1998	CC	com topw
Cadoux	Addo	ITSC Addo	1998	CC	4
Tardif de Janvier I	Uitenhage	CFB Psylla house	1999	TC	1
Tardif de Janvier I	Buffeljagsrivier	Sovereign	1999	SC	4 topwork
Tardif de Janvier II	Uitenhage	CFB Block 4	2001	CC	4
Sidi Aissa	Uitenhage	CFB Block 8	1997	TC	6
Sidi Aissa	Buffeljagsrivier	Sovereign	1999	SC	3 topwork
AinToujdate	Uitenhage	CFB Block 8	1997	CC	6
AinToujdate	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
CELL	Uitenhage	CFB Block 7	1995	TC	2
CELL	Buffeljagsrivier	Sovereign	1999	SC	5 topwork
CELL	Citrusdal	Brakfontein	1997	SC	3
CELL	Citrusdal	Brakfontein	1998	CC	5 topwork
Clementarde	Uitenhage	CFB Block 8	1997	CC	6
Clementarde	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Hernandina	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Tardivo	Buffeljagsrivier	Sovereign	1999	SC	10 topwork
Tardivo	Citrusdal	Brakfontein	1998	CC	5 topwork
Nour	Uitenhage	CFB Block 8	1999	SC	2
Nour	Addo	Summersby	1999	CC	commercial
Nour	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
Nour	Stellenbosch	L'Avenir	1999	CC	4 topwork
Nour	Citrusdal	Brakfontein	1988	CC	5 topwork
Marisol (control)	Stellenbosch	L'Avenir	1992	CC	commercial
Nules (control)	Uitenhage	CFB Block 4	2001	SC	3
Nules (control)	Buffeljagsrivier	Sovereign	1990	TC	commercial
Nules (control)	Clanwilliam	Twaktuin	1992	TC	commercial
Nules (control)	Citrusdal	Brakfontein	1995	SC	commercial
Nules (control)	Citrusdal	Brakfontein	1996	CC	commercial
Clemlate (control)	Buffeljagsrivier	Sovereign	1999	SC	3 topwork
Clemlate (control)	Citrusdal	Brakfontein	1997	SC	3

Results and discussion

A discussion of each selection follows with rootstock, production, fruit size and fruit colour presented in Table 6.2.2.3.2 and internal quality results in Table 6.2.2.3.3 which need to be referred to when reading the text.

Early Marisol

The trees had a good to excellent crop of medium to medium-large fruit size (counts 1-4), (Marisol - excellent crop, small to medium fruit size, counts 2-5), both colour T4-5 on 16 April. The fruit had fair to good eating quality, the Marisol sweeter. The Early had an acceptable test, the Marisol much higher sugars and acid but failing on ratio. The fruit picked easily. Fruit shape was round with a slight nipple and slightly pebbly rind, fairly easily peeled with a thin, oily and brittle rind. and cores mostly closed. The flesh colour was orange to pale, cores mostly closed and both seedless. The Marisol peeled easier with a slightly thicker rind, slightly open cores and juicy. The Early was either at peak or one week short of full maturity in April, Marisol similar to one week later but higher acid. There was slight sunburn while Marisol had some split.

Esbal

The eating quality at the CFB in mid May was good but not quite ready, the test excellent. There were some seed in a mixed block. Younger Nules on Swingle had a better crop of larger fruit size, similar to slightly later colour and good quality but juice test slightly too low and not quite ready.

The eating quality of both Esbal and Nules at Clanwilliam were good on 19 April with acid evident in Esbal, Nules acid a bit low. Both about 1-2 weeks short of full maturity. Both Esbal and Nules tests were excellent

and the fruit seedless. Both selections picked fairly easily. The fruit reportedly degreened well. The fruit colour is given below:

Percentage per fruit colour transparency (100 fruit) on 19 April				
	T5	T6	T7	T8
Esbal	5	45	47	3
Nules		9	77	14

The average fruit size and count distribution is given below:

Selection	Average fruit size (mm)	Percentage count distribution (100 fruit) on 19 April						
		1x	1	2	3	4	5	<5
Esbal	54.3		3	17	28	32	11	9
Nules	58.6	3	12	34	29	19	2	1

Oleo on 21 April: Esbal 99% class1, 1% class 2. Nules 100% class 1.

The fruit shape is round with a slightly pebbly rind, fairly easily peeled and oily. The flesh colour was orange at the CFB otherwise pale orange to deeper orange.

Cadoux. Most fruit were picked by 14 May and those remaining were medium small, fruit colour T1. Fruit shape is flattish, with a smooth rind and odd seed. The quality was good and fruit mature with an excellent test.

Table 6.2.2.3.2. Comparison of the production, fruit size and rind colour of various Clementine selections at the different trial sites on different dates in the Cape areas during 2004.

Area	Selection	Root-stock	Production	Fruit Size	Colour transparency - date						
					19 April	14/15 May	19/20 May	1 June	17 June	23 June	10 July
Uitenhage	Esbal	CC	Poor	Medium		4					
	Tardif de Janvier I	TC	Fair	Medium							1
	Tardif de Janvier II		Fair	Medium				3-5			
	Sidi Aissa	TC	Good	Medium small		4-5		3			1
	Ain Toujdate	CC	Poor	Medium small		5		2-3			1
	CELL	TC	Poor	Medium				5-6			1-2
	Clementarde	CC	Poor	Small				7-8			2-3
	Nour	CC	Poor	Small				6-7		5	2-3
Nules	SC	Good	Medium-large		4-5						
Buffeljagsrivier	Tardif de Janvier I	SC	Poor - fair	Medium small - small			4-5			3-5	
	Sidi Aissa	SC	Good	Medium			2-3			1	
	Ain Toujdate	SC	Poor - fair	Medium - medium+			2-4			1	
	CELL	SC	Poor - fair	Medium - med small			6			3	
	Clementarde	SC	Good -excell	Medium - med small			6-7			3-5	
	Hernandina	SC	Poor -good	Medium - med small			6-7			4-5	
	Nour	SC	Fair	Medium - med small			5-6			1-2	
	Clemlate	SC	Fair	Medium			5-6			4	
	Tardivo	SC	Good-excell	Medium - small			5-7			3-5	
Nules		Fair -good	Medium - med small			1-3			1		
Clanwilliam	Esbal	TC	Excellent,	Some medium small, mainly medium - medium-large (mainly 51 - 58 mm)	5-8, mainly 6-7						
	Nules	TC	Excellent	Medium - medium-large - large (mainly 55 - 63 mm)	7-8						
Citrusdal (older trees)	CELL	SC	Poor - fair	Medium - medium-large - large			6-7	6-7	4-5		

Area	Selection	Root-stock	Production	Fruit Size	Colour transparency - date						
					19 April	14/15 May	19/20 May	1 June	17 June	23 June	10 July
	Clemlate	SC	Poor	Medium - medium-large			6-7	6-7 later than CELL	5		
	Nules	SC	Picked	Medium - medium-large fruit left			4-6				
Citrusdal (younger trees)	Marisol	CC					picked				
	Sidi Aissa	CC					picked				
	Ain Toujdate	CC					picked				
	CELL	CC	Partly picked	Medium - medium-large fruit left			6-7	5-6	4-5		
	Clementarde	CC					picked				
	Nour	CC	Odd - poor	Medium - medium-large			6-7	5-6	3-5		
	Tardivo	CC							4-5		
	Nules	CC	Picked	Medium-large fruit left			4-5				

The Buffeljagsrivier trees may have been subjected to stress at some stage. Brackets = 60% or more fruit in this range in mm.

Tardif de Janvier I and II

The T de J I at the CFB had good eating quality and a very good test, but overmature on 10 July.

Buffeljagsrivier fruit were not mature on 20 May, but with good quality and test on 23 June and the most tart of all the selections at Buffeljagsrivier. At or one week short of full maturity in June. Some of the fruit had green stylar ends. These trees may have at some stage been subjected to some stress.

Fruit shape is round, a slight nipple at Buffeljagsrivier, fairly smooth to smooth rind, peelable and oily, odd seed in a mixed block. Trees have medium vigour with a dark brown to black stem.

The T de J II at the CFB had good eating quality with fair sugars and acid. The test was good although the acid borderline (low) on 1 June. At or close to peak maturity. The fruit was seedy (mixed block).

The T de J I had flatter and coarser fruit than Tde J II, both peelable but oily and seedless. T de J II had slightly flat fruit with a smooth rind, open cores, peelable and oily with a dark stem.

Sidi Aissa and Ain Toujdate

Both the Sidi Aissa and Ain Toujdate at the CFB had good eating quality on 15 May, the latter higher sugars and slightly more acid. Tests were very good. Larger, younger Nules had much lower acid and were granulating in May. Maturity probably June, pickable from mid May, overmature in July.

Both selections at Buffeljagsrivier were past their peak by 20 May, sweet but lacking acid, also on 23 June, although little change over this period. The taste does not correspond to the tests where the acid levels were good. Both developing a deep red colour in May with greenish stylar ends, completely coloured in June. The Sidi Aissa starting to become puffy in June. Nules were at or just past peak in May with good quality and tests.

Both selections had round fruit shape with a smooth to finely pebbled rind, fairly easily peeled and oily with good orange flesh and open cores. There was some seed (mixed block).

CELL

The CFB fruit had a fair taste on 1 June, mature by 10 July with good tests although the acid had dropped by nearly 0,2% over this period.

The Buffeljagsrivier trees had a slightly poorer crop and smaller fruit size than Clemlate, similar to earlier colour in June. The fruit had good eating quality, raggy and very oily, peak maturity around end May/early June. The test was good, acid the same as Nules in June, Clemlate lower. Much poorer colour than Nules fairly green stylar ends, the Clemlate also.

The younger Citrusdal trees had good eating quality on 19 May, maturing about a week later, overmature and slightly puffy on 17 June. Slightly superior quality than Nour. The tests were good, acid lower than Nour. The fruit was slightly raggy. There were a lot of green stylar ends in early June and the fruit was easily picked with minor oleo, also slightly green stylar ends and minor oleo on 17 June.

The older Citrusdal trees on Swingle had a slightly better crop and fruit size than Clemlate. Eating quality was good on 19 May (Nules past peak), acid could be higher, almost insipid on 1 June. Peak maturity around third week of May, similar to fractionally earlier than Clemlate. CELL better quality but more raggy. The tests were good, Clemlate acid dropping faster in later June. Both overmature by 17 June. Clemlate had more seed, but is planted next to Fortuna. Both had some green stylar ends and minor oleo on 1 June. On 17 June the Clemlate had quite a bit of oleo, the CELL very little and some slightly green stylar ends.

Fruit shape is round to flat, sometimes a slight nipple and pebbly rind and sometimes a ring on the stylar end. Fairly easily peeled but can be oily with a brittle rind. There were some green stylar ends. The trees are dense with a black stem. The fruit is seedless with no cross pollination.

Clementarde

The CFB fruit had very high acid on 1 June, fair eating quality on 10 July. The test in July was very good, acid 1.16% and mature. The acid was much higher than last season.

The Buffeljagsrivier fruit had good eating quality with very high sugars and sufficient acid. Maturity was from late May although the colour was still poor. The tests in May and 23 June were very good, still dark green stylar ends in June. Clemlate on these dates had much lower acid, better colour in May but similar in June and less green stylar ends.

The Clementarde are round with a slight nipple, smooth to pebbly rind, fairly easily peeled and oily, odd to some seed in mixed blocks. The trees are fairly flat and dense with a black stem. Estimated maturity varied widely between sites from late May at Buffeljagsrivier to early July at Uitenhage.

Hernandina

Trees were only evaluated at Buffeljagsrivier. The fruit was not mature on 20 May, although a good test. Eating quality was good on 23 June although low acid, at or past peak maturity. The test was good but acid fairly low, lower than Nules. Clemlate colour was slightly ahead, acid levels virtually the same, peak maturity early June.

Fruit shape is fairly round with a finely pebbled rind, fairly easily peeled to brittle and very oily. Some fruit had a ring on the stylar end with some green stylar ends. Stems are dark and some seed (mixed block).

Tardivo

At Buffeljagsrivier the fruit were not yet mature on 20 May, although acceptable quality. They were past peak by 1-2 weeks on 23 June with good quality but lowish acid. The tests were good, acid lower than Nules in June. Fruit colour was similar to Clemlate, developing a good orange red colour with some green stylar ends. There was some seed (mixed block).

On 17 June the fruit at Citrusdal was sweeter than CELL and Nour with slightly low acid and about at peak maturity internally, but overmature externally. No yield, fruit size or tests were recorded or done.

Fruit shape is round with finely pebbled rind, fairly easily peeled and oily. The tree has a dark stem.

Nour

The trees at the CFB had good eating quality on 1 June and mature in July. The tests were good in June and 10 July, overmature and with some green stylar ends on 28 July.

The young, semi commercial trees at Addo have been topworked except for three trees which bore no fruit.

At Buffeljagsrivier the fruit had almost insipid eating quality on 20 May, improving to good – very sweet with lowish acid. Peak maturity was about mid June, with some green stylar ends, although general rind colour better than Clemlate. The tests were good, acid higher than Clemlate.

The fruit at Citrusdal had good eating quality on 19 May, but only fair on 1 June. The acid was low on 17 June, the fruit dry and overmature. Maturity around end May/early June, but colour still poor, the majority with green stylar ends. The tests were good, acid higher than Clemlate. Compared to the other selections at the same site on 1 June, the fruit had less intense rind colour and slight lighter weight (but quite acceptable juice percentage). There was minor oleo in early and mid June.

The trees at Stellenbosch had a poor crop with medium fruit size, colour T8 and T4-6 with green stylar ends on 5 and 22 May respectively. The fruit was not quite mature in late May, a week or two short of peak maturity. Eating quality was fairly good on 22 May, slightly raggy and firm to slightly puffy. The tests were good with relatively high acid levels, as well as the Nules. Nules had a fair to good crop of smaller fruit size, T5-6 and T1 on 5 and 22 May respectively, peak maturity early to mid May. Both selections had the occasional seed.

The fruit is generally round with a nipple, ring at the stylar end, smooth to pebbly rind, orange flesh, basically seedless when not pollinated, fairly easily peeled and oily. The stylar ends tended to be green. The trees have a dark stem, are vigorous and dense and the fruit borne mainly on the inside of the tree.

Conclusion

Early Marisol

The trees bore an excellent crop of good fruit size. The quality was not as good as Marisol with much lower acid and similar colour. Maturity around mid-late April, Marisol similar to slightly later except for higher acid. This selection has improved a lot since last season and another evaluation is recommended.

Eskal

Production varied between sites with smaller fruit size than Nules. The smaller fruit size is of concern and will have to be manipulated, especially in the cooler Cape production areas. Fruit colour is ahead of Nules, maturity around the end of April to 1st week of May at Clanwilliam. Internal quality is good with slightly higher acid than Nules. Evaluations to continue.

Cadoux

Although the fruit has excellent quality, the fruit size tends to be too small. Not recommended due to the small fruit size and evaluations to be discontinued.

Tardif de Janvier I and II

Maturity of the T de Jan I appears to be early to late June, yield and fruit size not encouraging. Internal quality was good, although different acid levels between sites. The T de Jan II matured early June, with borderline acid. Due to the limited information available on both the selections, no recommendations can be made. Evaluations to continue.

Sidi Aissa and Ain Toujdate

Sidi Aissa had slightly better production, both similar smallish fruit size. Maturity varied from May to June but could be picked in May. Good quality. Evaluations to continue.

CELL

Production was generally poor, variable fruit size between sites, although not large. Eating quality was generally good but raggy with good tests. Colour tends to be retarded with some green styler ends. Maturity around the end of May. The CELL appears similar to the Clemlate in many respects. Evaluations to continue.

Clementarde

Production varied from poor to excellent, fruit size tending to be on the smaller side. Maturity was late but variable, the fruit having green styler ends. Internal quality was very good. Due to the variability of the results no conclusions can be drawn and further evaluations are necessary.

Hernandina

The yield was acceptable but fruit size on the small side. Other characteristics like low acid were similar to Clemlate. Evaluations to continue.

Tardivo

Production was very good with relatively small fruit size. The fruit had good quality but lowish acid, maturity around early June. Colour was similar to Clemlate. Further evaluations are necessary.

Nour

Production was generally poor, fruit size variable between sites but generally on the small side. The fruit quality was generally good, sometimes lowish acid and good tests. Maturity varied between sites from early to mid June, to July, although the colour may be retarded. There were a lot of green styler ends. The trees are vigorous and dense. Evaluations to continue.

Table 6.2.2.3.3. Internal fruit quality data of the various Clementine selections for the Eastern and Western Cape during the 2004 season.

Selection	Root-stock	Site	Harvest date	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Marisol Early	CC	L'Avenir	16/04	5-7	2	60.4	9.5	1.18	8.1	0
Esbal	CC	CFB	15/05	4	3	61.3	11.6	0.86	13.5	1.5
Esbal	CC	Twaktuin	19/04	6	4-5	61.9	11.3	1.30	8.7	0
Esbal	CC	Twaktuin	19/04	6-7	2-3	60.0	11.3	1.25	9.0	0
Cadoux	SC	ITSC Addo	14/05	1	3	59.7	13.0	0.95	13.7	1.7
Tardif de Jan I	TC	CFB	10/07	1	1-1X	49.8	14.2	0.78	18.2	0.1
Tardif de Jan I	SC	Sovereign	20/05	5	4	56.4	15.0	1.43	10.5	1.9
Tardif de Jan I	SC	Sovereign	23/06	1-3	4	56.8	16.0	1.22	13.1	0.6
Tardif de Jan II	CC	CFB	01/06	3-5	1X	56.3	12.3	0.73	16.8	6.9
Sidi Aissa	TC	CFB	15/05	4-5	3	54.4	12.6	1.04	12.1	2.6
Sidi Aissa	TC	CFB	01/06	3	3	59.5	12.5	0.98	12.8	4.2
Sidi Aissa	TC	CFB	10/07	1	3	57.7	13.8	1.01	13.7	3.6
Sidi Aissa	SC	Sovereign	20/05	4	4	59.3	11.6	0.91	12.7	0.7
Sidi Aissa	SC	Sovereign	23/06	1	3	55.2	13.4	0.97	13.8	0.5
Ain Toujdate	TC	CFB	15/05	5	2	58.6	13.0	1.08	12.0	5.3
Ain Toujdate	CC	CFB	01/06	2-3	3	56.5	13.6	1.08	12.6	3.9

Selection	Root-stock	Site	Harvest date	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Ain Toujdate	CC	CFB	10/07	1	3-4	60.3	14.8	1.09	13.6	2.2
Ain Toujdate	SC	Sovereign	20/05	2-3	2	55.1	12.0	0.95	12.6	2.5
Ain Toujdate	SC	Sovereign	23/06	1	2	49.3	13.4	1.01	13.3	2.3
CELL	TC	CFB	01/06	5-6	2	54.2	13.1	0.99	13.2	6.3
CELL	TC	CFB	10/07	2	1	57.9	13.6	0.81	16.8	7.7
CELL	SC	Sovereign	20/05	5-6	3	54.8	12.9	1.08	11.9	8.7
CELL	SC	Sovereign	23/06	3-4	3	54.6	13.7	0.88	15.6	5.4
CELL	CC	Brakfontein	19/05	6-7	2	57.2	11.5	1.27	9.1	0
CELL	CC	Brakfontein	01/06	5-6	1	54.4	12.6	1.03	12.2	0.1
CELL	CC	Brakfontein	17/06	4-5	1X	51.2	12.4	0.93	13.3	0
CELL	SC	Brakfontein	19/05	6-7	1	59.0	11.7	1.17	10.0	0.6
CELL	SC	Brakfontein	01/06	5	1	60.8	12.4	0.99	12.5	0
CELL	SC	Brakfontein	17/06	4-5	1	59.8	12.2	0.94	13.0	0.3
Clementarde	CC	CFB	10/07	3-4	3-4	51.0	14.6	1.16	12.6	0.5
Clementarde	SC	Sovereign	20/05	6-7-7	4	59.0	14.4	1.29	11.2	0.3
Clementarde	SC	Sovereign	23/06	3-5	3-4	58.4	15.5	1.07	14.5	0.9
Hernandina	SC	Sovereign	20/05	6-7-7	3	56.6	12.6	0.99	12.7	2.1
Hernandina	SC	Sovereign	23/06	4	3	58.1	13.5	0.75	18.0	1.8
Tardivo	SC	Sovereign	20/05	7	4	60.4	12.6	1.12	11.3	2.3
Tardivo	SC	Sovereign	23/06	4	3	60.9	13.9	0.83	16.7	2.9
Nour	CC	CFB	01/06	6-7	3	56.0	14.3	1.08	13.2	2.1
Nour	CC	CFB	10/07	2-3	3	60.2	14.5	0.96	15.1	1.3
Nour	SC	Sovereign	20/05	4-5	3	58.1	14.0	1.04	13.5	2.6
Nour	SC	Sovereign	23/06	1-2	3	58.5	15.0	1.02	14.7	0.5
Nour	CC	L'Avenir	05/05	7-8	3	51.9	11.3	1.26	9.0	0
Nour	CC	L'Avenir	22/05	4-5	3	59.5	12.7	1.20	10.6	0.1
Nour	CC	Brakfontein	19/05	6	3	54.5	11.5	1.35	8.5	0.2
Nour	CC	Brakfontein	01/06	5	2	56.0	12.0	1.06	11.3	0.2
Nour	CC	Brakfontein	17/06	4-5	2	52.9	11.4	1.01	11.3	0
Marisol	CC	L'Avenir	16/04	5-6	3	58.4	11.2	1.47	7.6	0
Nules	SC	CFB	15/05	3-5	1XX	47.5	12.0	0.79	15.2	4.6
Nules	SC	Sovereign	20/05	2-3	3	58.0	13.8	0.92	15.0	2.0
Nules	SC	Sovereign	23/06	1	2	57.0	15.1	0.88	17.2	1.9
Nules	SC	L'Avenir	05/05	5-6	3	58.7	12.5	1.16	10.8	0
Nules	SC	L'Avenir	22/05	1-3	3	53.6	14.5	1.22	11.9	0.3
Nules	TC	Twaktuin	19/04	6	3-4	59.8	11.3	1.23	9.2	0
Nules	TC	Twaktuin	19/04	6	1-2	56.8	12.0	1.20	10.0	0
Nules	CC	Brakfontein	19/05	4-5-5	1	57.3	10.0	0.9	11.1	0
Nules	SC	Brakfontein	19/05	4-5	2	55.0	12.5	1.09	11.5	0.2
Clemlate	SC	Sovereign	20/05	5-6	4	59.3	12.7	0.93	13.7	2.3
Clemlate	SC	Sovereign	23/06	3	3-4	60.5	14.5	0.77	18.8	1.8
Clemlate	SC	Brakfontein	19/05	6-7	1	55.5	11.6	1.05	11.0	3.1
Clemlate	SC	Brakfontein	01/06	6	1	58.3	11.8	1.02	11.6	3.0
Clemlate	SC	Brakfontein	17/06	4-5	1	60.0	11.5	0.83	13.9	0.3

Future research

Continue evaluations on mainly the later maturing selections.

6.2.2.4 Evaluation of mandarin hybrids in the Cape areas

Experiment 73 by C J Alexander (Private)

Opsomming

Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vruggrootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. Nova SL is soortgelyk aan Nova, maar nie heeltemal saadloos in gemengde blokke nie; B17 lyk belowend maar het hoër suur en effense vrugsplit; B24 is saadloos, met minder suur as B17, maar vrugte kan te groot wees met 'n plat vrugvorm; M37 het 'n lemoen tipe geur en sagte vesel. H25 het min, aantreklike vrugte gedra, maar die geur is swak en lyk nie belowend nie; H36 smaak soos 'n Nouvelle met goeie gehalte alhoewel die sap persentasie laag was en skilkleur vertraag; K33 is feitlik saadloos, maar lyk nie belowend nie weens sy swak voorkoms; Roma het goeie gehalte met 'n laer sap persentasie gehad met 'n naartjie tipe voorkoms; Ora het wisselende produksie en kleur gehad, goeie vruggrootte, maar vol saad. Or 2 het goeie produksie en aanvaarbare vruggrootte gehad, goeie gehalte, maar 'n grenslyn sap persentasie en pitte terwyl die Mor 22 aanvaarbare produksie en goeie vruggrootte gehad het, maar 'n swak toets. Afourer (B64) het swak produksie met medium vruggrootte gehad en goeie gehalte. Murcott x Clem het goeie produksie en aanvaarbare gehalte (goeie toets) gehad met goeie skilkleur en lyk belowend behalwe vir groterige vruggrootte en pitte. Bay Gold het swak produksie met goeie vruggrootte gehad, maar lyk nie belowend in die koeler gebiede nie weens hoër suur; Hadas het goeie produksie en gehalte, maar vrugte aan die klein kant. Winola het swak produksie met medium vruggrootte en goeie vrugkleur gehad, maar die suur was te hoog in Augustus. Die meeste van die seleksies is nog jonk en moet verder evalueer word.

Introduction

The objective of the mandarin hybrid project is to find high quality, well coloured, seedless selections with good production and fruit size, with special emphasis on extending the soft citrus season earlier and particularly later.

Materials and methods

The trees were either planted or topworked within commercial orchards where possible (to prevent cross pollination), or established on a semi commercial scale. Comparisons were made with a range of existing commercial selections or Clementines where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following standards for Clementines and Novas previously considered most acceptable by the market place: 48% juice; 9.5% TSS; 0.7 – 1.5% acid; 8.0:1 ratio; colour T3 of set 36. Seed maximum average 3.0 seeds per fruit. A list of selections and sites evaluated is given in Table 6.2.2.4.1.

Table 6.2.2.4.1. Mandarin hybrid trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Nova Seedless	Uitenhage	CFB Block	1999	CC	2
Nova Seedless	Patensie	Paksaam	1999	SC	semi com
Nova Seedless	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
Valley Gold (B17)	Addo	Dunbrody	1998	CC	semi com t/w
Valley Gold (B17)	Fort Beaufort	Baddaford	1998	TC	3
Valley Gold (B17)	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
Valley Gold (B17)	Citrusdal	ALG	1999	BN/RL	5
African Sunset (B24)	Addo	Dunbrody	1998	CC	semi com t/w
African Sunset (B24)	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
African Sunset (B24)	Citrusdal	ALG	1999	BN/RL	5
ITSC M37	Fort Beaufort	Baddaford	1998	TC	4
ITSC M37	Citrusdal	ALG	1999	BN/RL	5
ITSC H25	Citrusdal	ALG	1999	BN/RL	5
ITSC H36	Citrusdal	ALG	1999	BN/RL	5
ITSC K33	Citrusdal	ALG	1999	BN/RL	5
ITSC K33	Citrusdal	Hexrivier	1997	RPL	1
Roma	Citrusdal	ALG	1998	BN/RL	5

Selection	Area	Site	Plant Date	Rootstock	No of trees
Ora	Citrusdal	ALG	1998	BN/RL	5
Or 2	Citrusdal	ALG	1998	BN/RL	5
Mor 22	Citrusdal	ALG	1998	BN/RL	5
Afourer (B64)	Citrusdal	ALG	1998	BN/RL	5
Murcott x Clem	Uitenhage	CFB	1999	TC	2
Murcott x Clem	Citrusdal	Brakfontein	2000	RL	3 topwork
Bay Gold	Grabouw	Whitehall	1998	SC	5
Bay Gold	Clanwilliam	Jansekraal	2001	CC	48 semi com
Hadas	Uitenhage	CFB Block 8	1999	TC	2
Winola	Uitenhage	CFB Block 8	1997	TC	2
Nova	Uitenhage	CFB	1991	SC	
Nova	Patensie	Paksaam	1999	SC	commercial
Nova	Citrusdal	Ebenhaeser		RL	commercial

Results and discussion

A discussion of each selection follows. Various tables also need to be referred to when reading the text. Table 6.2.2.4.3. is a comparison of the various selections at ALG, Citrusdal and Table 6.2.2.4.4. internal quality data.

Nova Seedless. The CFB trees carried an excellent crop of medium fruit size, colour T2-4 on 15 May and T1 on 1 June. Eating quality was good, better than Nova. The tests were good, slightly higher acid than Nova. Estimated maturity is early June. There were 2,7 seeds/fruit (mixed block) and Nova 3,5 seeds/fruit.

At Patensie both the Nova Seedless and Nova had a fair to good crop with good, medium-large fruit size. Visually there was no difference in fruit size, but measurements indicated Nova to be slightly larger. Refer table 6.2.2.4.2. Colour of both was T6 on 27 April. Both had good quality, ready to be picked around 10 May. Both tests were similarly good, Nova SL with slightly higher juice and lower acid. Nova had few, but more slightly ricey fruit. There was no fruit split but signs of *Alternaria* on both selections. The fruit had far more seed than last season: Nova SL, average 0,3 seeds/fruit, 89% seedless, 2% exceeding 3 seeds/fruit; Nova average 0.8 seeds/fruit, 69% seedless, 7% exceeding 3 seeds/fruit.

Table 6.2.2.4.2. Average fruit size and count distribution (100 fruit) of Nova Seedless and Nova measured at Paksaam, Patensie on 27 April and Ebenhaeser, Citrusdal on 19 April, 2004.

Selection	Fruit size (mm)	Percentage fruit per count						
		1XX	1X	1	2	3	4	5
Patensie								
Nova SL	62.9	5	10	22	45	17	1	
Nova	65.4	8	17	47	23	3	1	1
Citrusdal								
Nova SL *	65.5	7	23	40	26	4		
Nova	62.4	2	11	28	40	13	6	

* younger trees than Nova

The Nova SL and Nova at Citrusdal are in separate blocks, the Nova older and subjected to some cross pollination. The Nova SL had a good to excellent crop of medium to medium-large fruit size, colour T4-6 on 19 April. Eating quality was good to slightly high acid, raggy and some slightly ricey. Novas had a similar crop with variable fruit size, T5-6, good quality but acid slightly lower. Nova SL tests had higher sugars and acid, the acid too high (this could be stress related). There were five seeds (one seed/fruit) found in 100 fruit. Nova tests were good. Oleo in both selections was minimal. Both selections about one week short of full maturity.

Fruit shape was flattish, a smooth rind but slightly coarser at Citrusdal, orange internal colour, peelable to difficult and oily and thin rinds. There was some riceyness in both selections at Patensie but just in the Nova SL at Citrusdal, although the juice was not so free. Although not totally seedless, the Nova SL had less seed than the Nova (mixed blocks). There was the occasional split fruit in Citrusdal and some creasing at the CFB.

Valley Gold (ITSC B17)

The topworked semi commercial block at Addo had a fair yield of about 44t/ha with good fruit size, mainly count counts 1,2 and 3. The fruit was attractive and clean with an 84% packout (apparently less susceptible to thrips) with good colour, a good red colour on the east and paler on the western side. The quality was good but tart, TSS between 11-13% and acid 1.2-1.4%. The fruit was picked at the end of June, early July, a week too late. It was firmer and more raggy than a Clementine, juicy and easily peeled. There was little fruit split (also ITSC B24 and Nova).

Trees at Fort Beaufort bore a poor crop of medium small fruit size, colour T1 on 17 July. Quality was good, acid slightly high. The test on 5 July was good with good eating quality, maturity around mid July. There were about 2 seeds/fruit (mixed block).

The semi commercial topworked trees at Citrusdal were pale with some leaf drop which could be stress related. There were only odd fruit of medium-large fruit size, picked by 30 June. Fruit colour on 17 June was very good, T1-3. Sugars were high and acid excessive and test unacceptable due to high acid and low ratio. If the acid was lower it could have been picked early June. The fruit looked watery with wide open cores, albedo starting to pull away from segments, large juice vesicles. There was no oleo in mid June.

ALG trees fruit were probably at peak maturity possibly in late June but the acid was still high, but externally overmature on 1 July. The fruit was tart on 17 June and July with a good flavour and high sugars but an aftertaste in July. The test was unacceptable in June (high acid and low ratio), quite acceptable in July. There was minor oleo.

Fruit shape varied from round to flat with occasional slight neck in Citrusdal, smooth and mainly smooth to slightly pebbly in Citrusdal, easily peeled and slightly oily with a thin rind, deep orange internal colour and open cores, mainly seedless to odd seed. There was odd fruit split.

African Sunset (ITSC B24)

The topworked semi commercial block at Addo had a slightly poorer crop than B17 at 40 tons/ha, but fruit size too large, about 90% counts 1X – 1XXX, picked at the end of June/early July. The fruit had a 72% packout (also less susceptible to thrips). The colour and quality was good having a different flavour and lower acid than B17 and soft rag. There was minor fruit split. Due to the flat nature of the fruit it tended to be underweight and difficult to pack.

The semi commercial topworked trees at Citrusdal had a variable fair to good crop of large to extra large fruit size (count 1XXX and larger), colour T2-4 on 17 June, picked by 30 June. The taste was better than adjacent B17 but also had high, but lower acid. The test was good, maturity probably end June/early July. There was odd sunburn and no oleo in mid June.

Fruit at ALG had good quality on 17 June, losing some of its flavour by 1 July and slightly dry. The tests were good but juice becoming borderline (oversize fruit), 1.0% lower TSS and 0.51% lower acid than B17 but a higher ratio. The fruit was raggy, one seed found and no oleo. Maturity mid to late June.

Fruit shape was flat sometimes fluting on the stem end. The rind was smooth to slightly pebbly at Citrusdal with occasional closed protruding navel ends. Internal colour was good with slightly open to open cores, thinnish rinds, fairly easily peeled and oily. The fruit is seedless (except for one seed at Citrusdal) and not as tart as B17.

ITSC M37

Fort Beaufort trees had a good crop of medium fruit size, colour T1-3 on 17 July. Eating quality was good and fruit mature with good tests but seedy (mixed block) and some split fruit. Estimated maturity end June/early July.

Fruit at Citrusdal had a good almost orange like flavour on 17 June, still good on 1 July with lower acid, peak maturity about 3rd week of June but colour only acceptable in July. The test in June was very good but the sugars had dropped considerably and the acid slightly (both acceptable) in July. There was slight creasing and minor oleo.

Fruit shape was round with a smooth rind, slightly coarse at Citrusdal. Internal colour was deep orange with closed to open cores, a soft rag and juicy, fairly easily to easily peeled and oily. There was some splitting at Fort Beaufort and some creasing and odd furrowing of the stem end at Citrusdal. Seediness varied from odd to seedy.

ITSC H25

There were only a few fruit to evaluate. The fruit was not quite mature on 4 May, around peak on 19 May but lacking flavour, soft, watery and lacking flavour but not insipid and poor on 17 June. The colour was acceptable late May. The tests were mediocre, acid too low by mid June. There was minor oleo.

Fruit shape is round with a slight nipple, fairly smooth rind and attractive although there can be slightly green styler ends. Internal colour is orange, soft, closed to slightly open cores, fairly easy to difficult to peel with some albedo adhering to the segments and oily. The tree is dense with an atypical mandarin leaf shape and some leaf drop in mid June.

ITSC H36

The quality was good on 17 June (although could be slightly richer) and 1 July with a slight Nouvelle-like taste, slightly soft, peak maturity around mid June although rind colour delayed. The tests were good although the juice percentage too low (large fruit).

The fruit is not attractive (too green) with a round fruit shape, pebbly rind and a ring on the styler end. Fairly easily peeled but very oily with a soft flesh, a good orange colour, slightly open cores and seedy. There was odd out of season fruit, some creasing and minor oleo.

ITSC K33

The Hexrivier tree had a poor crop of medium fruit size, colour T4-5 with green stem ends on 17 June, T3-4 with some green nipples on 30 June, fully orange coloured, T1-2 on 20 July. Quality was good, but acid fairly high on 30 June, more or less at peak maturity, lacking flavour in July and past peak maturity. All three tests had unacceptably high acid levels. The fruit was firm with a fine texture, slightly soft in July. There were odd seed, counts varying from zero (66%) to four seeds/fruit (3%). There was bark scaling above the bud union (also seen on adjacent navels on Rangpur). There was no oleo in mid June or July.

The fruit at ALG had good quality but quite high acid on 17 June, still high acid and raggy on 1 July. Peak maturity probably mid July. The June test had unacceptably high acid, a good test in July, and the acid dropping by 0,53% over the two weeks. There was slight creasing and no oleo.

Fruit shape is round with a green nipple, pebbly rind, and ring on the styler end and not very attractive. Peelability is fairly easy but oily. Flesh colour is a good orange with some soft flesh but overall raggy, closed to slightly open cores and seedless to odd seed in a mixed block. The trees are possibly cold sensitive.

Roma

The fruit had good quality on 17 June, at or close to peak maturity, although the fruit looked overmature like a naartje and slightly soft. The test was good, except the juice percentage was too low. There was no oleo.

The fruit is not very attractive with some green styler ends and fruit shape is roundish with a small nipple, a fairly smooth rind and occasional navel. Peelability was fairly easy to difficult with a thin rind and oily, the fruit slightly raggy and seedy. The trees are fairly vigorous and dense with small thorns and leaves.

Ora

The quality was not so good on 17 June, not quite mature, acceptable but watery on 1 July, about at peak maturity, slightly soft. The tests were good. The fruit colour was paler in June with quite a wide colour range on both dates. There was no oleo.

Fruit shape is flattish with a fairly smooth rind, star like navel end and slightly furrowed stem end, fairly easily peeled and oily. Flesh colour is orange with closed cores and seedy. Trees are very vigorous.

Or 2

The fruit had high sugars and fairly high acid on 1 July with a good test but borderline juice, lower tests than Ora in July. Maturity estimated around mid July. Fruit shape is slightly flat with a slightly coarse rind, easily peeled and oily. Flesh colour is orange with signs of riceyness and a closed core. The average seed count was 2,4 seeds/fruit in a mixed block. There was no oleo.

Mor 22

The fruit had high sugars and fairly acid with a strange aftertaste on 1 July. The test was not acceptable with unacceptably low juice and borderline (low) acid, maturity around mid July. Fruit shape is flat with a smooth to slightly pebbly rind and ribbing, fairly easily peeled, thin rind and slightly oily. Flesh colour is deep orange with a fine texture, closed to slightly open cores and some seed. The fruit is firm with no oleo.

Afourer (B64)

The fruit had good high sugars with high acid and a good test on 1 July, maturity estimated mid to late July. Fruit shape is flat with a smooth, fairly thin rind, easily peeled and slightly oily. Flesh colour is deep orange with an open core and virtually seedless. There was some out of season fruit and no oleo.

Murcott x Clementine

The semi commercial block at Kirkwood (700 trees planted 2003 on Carrizo) is not yet in production. The CFB trees had a good crop of good looking, medium-large fruit size, colour T1 on 10 July. Eating quality was acceptable with a good test and mature on 10 July. Eight seeds/fruit.

The Citrusdal trees had a good crop of medium-large to large fruit size, colour T5-6 on 19 May, T3-4 on 1 June, T1-3 on 17 June and all T1 on 30 June. There were initially some green styler ends in early June whereafter the fruit developed a deep orange red colour with occasional tinges of green. The fruit was immature on 4 May, good eating quality on 19 May, losing a bit of flavour from early June, then seemingly improving at the end of June, the acid always evident. Peak maturity around mid June, slightly puffy at the end of June. The tests were good, acid remaining steady, and the fruit seedy. There was odd creasing and no oleo recorded in mid or late June.

Fruit shape is round to slightly flat, rounder and smoother at the CFB, with a smooth to slightly pebbly rind, although the styler ends can be coarser with a slight star and some furrowing at the stem end. The fruit is fairly easily peeled and oily. The flesh has a deep orange colour, open cores and seedy (mixed blocks). The trees are vigorous.

Bay Gold

The Grabouw trees had been picked by 5 May and the few fruit left were T6 and better coloured. The fruit had high acid.

The Clanwilliam trees bore a poor to fair crop of medium to medium-large fruit size. The average fruit size on 1 June was 69.7mm, 92% counts 1XX, 1X and 1. The colour on 4 May was T6-7 but juice not yet free. On 1 June the colour was T5-7 (adjacent Nules just finished harvesting), fruit slightly dry, acid still very high and fruit slightly soft. By 30 June the colour was good, T1-3, acid still fairly high and sugars probably good although masked by the acid, overmature and pick easily. All the tests had excessive acid levels and low juice in the first and last test. Maturity estimated around mid June.

The fruit has an obovoid shape (almost Minneola-like), some lopsided, with a smooth rind, slightly pebbly in Clanwilliam. The fruit is fairly easily peeled and slightly oily, pale orange flesh with a pale juice colour and slightly open to open cores. Seed counts vary from zero to 22, averaging 4.7 seeds/fruit. There are up to 18% internal navels. There were odd split fruit and 28% and 10% minor oleo in May and early June respectively. Some of the fruit developed brown spots on the sunny side, which could be sunburn. Tree colour is slightly pale.

Hadas

Trees at the CFB had a good crop of medium fruit size, colour T1 on 30 August. The fruit borne more on the terminals of branches. Quality was good and peak maturity around early - mid August. The test on large fruit was good, easily meeting Ellendale standards, except for the high seed count (mixed block). The fruit shape is flattish with a very smooth thin rind, peelable, oily and open cores. There was odd split seen on trees on Swingle at the end of April.

Winola

Trees at the CFB had a poor crop of medium fruit size, colour T1 on 30 August. The taste was fair with high acid and overmature in August. The test had very high sugars and excessive acid. The fruit shape is round with a smooth rind, peelable, oily and slightly open cores, bright orange flesh colour and seedless.

Table 6.2.2.4.3. Comparison of production, fruit size and colour of various Mandarin hybrids topworked on Bahianinha/Rough lemon at ALG, Citrusdal and evaluated during 2004.

Selection	Production	Fruit Size	Colour transparency			Maturity	Comment	Ave Seed
			19 May	17 June	1 July			
Valley Gold ITSC B17	Good	Medium, uniform		3-5, some green blotches	2-4, good, red colour	Late June?	Good flavour but tart, acid can be excessive	Odd, mainly 0
African Sunset ITSC B24	Fair-good	Large		2-3	1	Mid-late June	Good quality and tests, very large fruit	1 seed found
ITSC M37	Poor	Medium-medium small		4-5	2-5	3 rd week of June	Good, orange like flavour, soft rag.	Odd-seedy 8.7
ITSC H25	Odd fruit, overgrown by navels	Medium-large	5	1 June 1,4 17 June 1-3		3 rd week of May	Soft, low quality fruit, attractive	Mainly 0 to seedy 3.3
ITSC H36	Poor – good, variable	Medium – large		5-7	5-6	Mid June	Good, slight Nouvelle like flavour, good tests although low juice	Seedy 7.0
ITSC K33	Fair	Medium, some medium-large		5-6 green nipples	4-5	Mid July	Initially very high acid high, green stem ends	0
Roma	Poor-fair	Medium		4 variable, some green blotches		Mid-late June	Good quality but low juice, naartje like appearance	Seedy 9.8
Ora	Variable, poor-excellent	Medium – medium-large		3-6 fairly pale yellow	2-5	End June /early July	Good quality	Seedy 12.1
Or 2	Fair-good	Medium			3-5	Mid July	Good quality, borderline juice	2.4
Mor 22	Fair-good	Medium-medium-large			2-5	Mid July	Fruit looks good, low test and strange aftertaste.	Some seed 1.5
Afourer (B64)	Poor-fair mainly on top	Medium, occasionally large			2-5	Mid – late July	Good looking fruit	Almost no - 0 seed

The trees are topworked next to each other in a navel block.

Conclusion

Nova Seedless

Production was good with generally good fruit size. Both had good quality and tests, acid too high in Citrusdal (possibly stress related). There was no consistency regarding differences in tests between the selections. There was some riceyness. Maturity varies per area from about late April to early June. The Nova SL is not totally seedless in mixed blocks but has less seed than Nova. There was the occasional split fruit in Citrusdal and some creasing at the CFB. The two selections appear suitable for planting commercially, the Nova SL having less seed. Evaluations to be finalised next season.

Valley Gold (ITSC B17)

Production and fruit size varied between sites and generally good fruit colour. Fruit sugars are high while the acid also tends to be high to excessive. Maturity around to 3rd week of June to mid July but the acid can still be high. The selection looks promising, is virtually seedless but has the drawback of slight fruit split and particularly high acid. Further evaluations are necessary, especially on the semi commercial blocks. The selection should also be evaluated in hotter areas with lower acid levels.

African Sunset (ITSC B24)

Production at the various sites was fair to good, the fruit size tending to be very large and flat shape. Fruit quality was good with good tests (borderline juice at Citrusdal), good rind and flesh colour and seedless. Maturity varied from mid to later June with lower acid and sugars but higher ratio than B17. The selection looks promising as a later maturing mandarin but has the drawback of large fruit size. Further evaluations are necessary, especially on the semi commercial blocks.

ITSC M37

Production varied from poor to good, fruit size medium to smaller. Fruit quality was good with an orange like flavour and soft rag, although sugars dropping at Citrusdal. Maturity around 3rd week to end of June, seedy in mixed blocks. Further evaluations are necessary.

ITSC H25

Evaluations were limited as there were only a few fruit of medium-large fruit size, attractive but lacked flavour. The selection does not look promising and one further evaluation is necessary.

ITSC H36

Production was variable, poor to good with medium-large fruit size and seedy. Maturity is around mid June although retarded rind colour and good quality with Nouvelle like flavour, but low juice. Further evaluations are necessary and it needs to be established whether the fruit is seedy in the absence of cross pollination.

ITSC K33

Production was poor to fair with generally medium fruit size. Fruit quality was good but high to unacceptably high acid, peaking around end June/mid July. The fruit are not attractive and have green stem ends. The selection does not look promising except for its virtual seedlessness but further evaluations are necessary as the trees are still young.

Roma

Production was poor to fair with medium fruit size. Quality was good, but very low juice and seedy, maturing mid to late June, variable colour and some green blotches with a naartje like appearance. The growth habit is dense. Further evaluations are necessary.

Ora

Production was variable with good fruit size. Maturity end June/early July and initially good flavour. Rind colour is variable and fruit seedy. Further evaluations are necessary.

Or 2

The production was acceptable with good fruit size, good quality but borderline juice. Maturity around mid July, later than Ora. Some seed in a mixed block. Further evaluations necessary.

Mor 22

Production was acceptable with good fruit size but unacceptable test, maturity around mid July. Some seed in a mixed block. Further evaluations necessary.

Afourer (B64)

Production was poor with fruit borne towards the top of the trees and fruit size medium. Quality was good but not outstanding. Maturity around mid to late July. Further evaluations necessary.

Murcott x Clementine

Production was good with good to large fruit size. Eating quality was acceptable to good with good tests, maturity late June/early July for the CFB and mid June for Citrusdal. The fruit develops a good rind colour. The fruit is seedy in a mixed block and further evaluations are necessary to determine the seed status in the absence of cross pollination. The fruit can tend to be a bit on the large side. The selection looks promising.

Bay Gold

Production was poor to fair with good fruit size. Quality was poor due to high acid levels and seed counts varied from seedless to seedy. There was odd fruit split and possible sunburn. The selection does not look promising and further evaluations are necessary but it should be tried out in hotter areas in an attempt to reduce acid levels.

Hadas

Production was good but fruit size a little on the small side. The quality and test was good but fruit seedy in a mixed block. Maturity around early to mid August. Evaluations to continue.

Winola

Production was poor with medium fruit size and good colour. The fruit was overmature at the end of August, but the acid level still excessive. Further evaluations are necessary.

Table 6.2.2.4.4. Internal fruit quality data of mandarin hybrid selections for the Eastern and Western Cape during the 2004 season.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Nova Seedless	CC	CFB	15/05	2-3	1	60.7	12.6	0.84	15.0	2.6
Nova Seedless	CC	CFB	01/06	1	2	60.1	13.2	0.82	16.1	2.8
Nova Seedless	SC	Paksaam	27/04		2	62.4	11.0	0.95	11.6	0
Nova Seedless	SC	Paksaam	27/04	6-7	1	58.9	10.9	0.91	12.0	0.3
Nova Seedless	RPL	Ebenhaeser	19/04	5	2	55.4	13.7	1.66	8.3	0
Nova Seedless	RPL	Ebenhaeser	19/04	5	1	53.5	13.8	1.59	8.7	0.1
Valley Gold	TC	Baddaford	05/07				10.1	1.10	9.7	
Valley Gold	RPL	Ebenhaeser	17/06	1-2	1X	56.2	12.8	1.66	7.7	1.1
Valley Gold	BN/RL	ALG	17/06	3-5	2	62.9	12.5	1.65	7.6	
Valley Gold	BN/RL	ALG	01/07	2-4	1	52.5	11.2	1.20	9.3	
African Sunset	RPL	Ebenhaeser	17/06	2	1XXX	57.8	12.5	1.04	12.0	0
African Sunset	BN/RL	ALG	17/06	2-3	1XXX	49.1	11.8	0.98	12.0	
African Sunset	BN/RL	ALG	01/07	1	1XXX	50.0	10.0	0.85	11.8	
ITSC M37	TC	Baddaford	17/07	1	1-2	61.9	13.3	0.82	16.2	9.1
ITSC M37	BN/RL	ALG	17/06	4-5	1-2	52.0	13.2	1.14	11.6	
ITSC M37	BN/RL	ALG	01/07	2-5	1	51.7	9.7	0.94	10.3	
ITSC H25	BN/RL	ALG	19/05	5		50.0	9.8	0.80	12.3	0.7
ITSC H25	BN/RL	ALG	01/06	1,4	1-1X	48.3	10.0	0.75	13.3	0.9
ITSC H25	BN/RL	ALG	01/07	1-3	1X	50.0	10.0	0.63	15.9	
ITSC H36	BN/RL	ALG	17/06	5-7	1XX	47.8	12.2	0.94	13.0	
ITSC H36	BN/RL	ALG	01/07	5-6	1XX	45.5	10.8	1.03	10.5	
ITSC K33	RPL	Hexrivier	17/06	5	1	61.7	11.7	1.64	7.1	0.7
ITSC K33	RPL	Hexrivier	30/06	3-4	1X	61.5	12.1	1.62	7.5	0.6
ITSC K33	RPL	Hexrivier	20/07	1-2	1	60.2	12.1	1.60	7.6	0.7
ITSC K33	BN/RL	ALG	17/06	5-6	1X-1XX	55.3	13.7	1.53	9.0	
ITSC K33	BN/RL	ALG	01/07	4-5	1XX	50.0	11.0	1.00	11.0	
Roma	BN/RL	ALG	17/06	4	1	41.4	12.3	1.04	11.8	
Ora	BN/RL	ALG	17/06	3-6	1X-1XX	54.3	12.0	1.00	12.0	
Ora	BN/RL	ALG	01/07	2-5	1XX	50.0	12.1	0.87	13.9	
Or 2	BN/RL	ALG	01/07	3-5	1X	48.5	10.3	1.06	9.7	
Mor 22	BN/RL	ALG	30/06	2-5	1X	44.1	10.9	0.75	14.5	
Afourer	BN/RL	ALG	30/06	2-5	1X	51.4	10.6	1.15	9.2	
Murcott x Clem	TC	CFB	10/07	1	1XX	59.4	12.4	0.99	12.5	8.0
Murcott X Clem	RL	Brakfontein	19/05	6-7	1XX	55.1	10.8	1.31	8.2	13.7
Murcott X Clem	RL	Brakfontein	01/06	4-5	1XX	54.5	12.7	1.32	9.6	14.9
Murcott X Clem	RL	Brakfontein	17/06	1-2	1XXX	54.8	13.3	1.20	11.1	11.8
Murcott X Clem	RL	Brakfontein	30/06	1-2	1XXX	53.1	13.1	1.20	10.9	11.7
Bay Gold	CC	Jansekraal	04/05	7	2	42.2	11.4	2.90	3.9	3.3
Bay Gold	CC	Jansekraal	01/06	6-7	1X	52.5	12.1	2.12	5.7	4.9
Bay Gold	CC	Jansekraal	30/06	1-3	1XX	47.6	12.6	2.01	6.3	4.2
Hadas	TC	CFB	30/08	1	1XX	57.3	12.9	1.34	9.6	10.6
Winola	TC	CFB	30/08	1	1	57.6	15.4	1.68	9.2	0
Nova	SC	CFB	01/06	3	1X	61.3	11.9	0.78	15.3	3.5

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Nova	SC	Paksaam	27/04		2	58.4	11.4	1.02	11.2	0.3
Nova	SC	Paksaam	27/04	6-7	1X	56.3	11.2	0.99	11.3	0.8
Nova	RL	Ebenhaeser	19/04	5-6	2	55.7	12.0	1.38	8.7	6.5
Nova	RL	Ebenhaeser	19/04	6	1	54.4	11.8	1.27	9.3	10.9

Future research

Continue evaluating all the above selections. Gather as much semi commercial data of Nova Seedless, ITSC B17 and B24.

6.2.2.5 Evaluation of navel oranges in the Cape areas

Experiment 74 by C J Alexander (Private)

Opsomming

Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder, asook om seleksies te vind met meer gevorderde vrugkleur, veral aan die begin van die seisoen en vrugte te kry met verbeterde vrugset wat binne die gesogte vruggrootte reeks val. Die Atwood en Dream het min of meer saam met die Lina rypgeword, die Dream het enkele pitte ingehad. Die Fukumoto het saam tot effens vroeër as die Lina rypgeword. Daar is geen duidelikheid oor die onverenigbaarheid van die Fukumoto op trifoliaat of sy baster onderstamme nie en aanplantings op sulke onderstamme is nie aanbeveel nie. Daar was te min Letaba Early vrugte beskikbaar om ordentlik te evalueer. Die Cliff Early lyk vroeg en proewe moet uitgebrei word. Die Washington (SGB materiaal) het goed gevaar, maar het groot vrugte en kan swak gehalte op jong bome gee. Cambria (SGB materiaal) het beter gehalte as verlede seisoen gehad. Die Coetzee Late, Renken Late, Mouton Late 1 en 2 het min gedra en kon nie volledig gevalueer word nie. Glenora Late en Witkrans is nog nie in produksie nie. Verdere evaluasies van al die seleksies is nodig.

Introduction

The aim is to find cultivars to spread the navel season both earlier and later and to minimise production peaks, also with more advanced rind colour, particularly at the commencement of the season and with improved fruit set potential in the desired fruit size range.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale. Palmer, Tuligold, Lina, Newhall and Lane Late navels were used as controls. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is based on subjective tasting. Fruit quality was compared with the following standards previously considered most acceptable by the market place (higher standard for Navelates in brackets): 48% juice; 9.0% (10.0%) TSS; 0.6 – 1.8% (0.85 – 1.50%) acid; 7.5:1 (8.0:1) ratio; colour T3 + 20% T4 of set 34 (T3). Zero seeds/fruit.

A list of selections and sites evaluated during 2004 is given in Table 6.2.2.5.1.

Table 6.2.2.5.1. Navel trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Atwood	Uitenhage	CFB Block 7	1999	RL	2
Atwood	Sunland	Paterson	2000	CC	5 topwork
Atwood	Heidelberg	Kruisrivier	2001	C35	5
Dream	Uitenhage	CFB Block 7	1999	CC	2
Dream	Sunland	Paterson	2000	CC	5 topwork
Dream	Heidelberg	Kruisrivier	2001	C35	5
Fukumoto	Uitenhage	CFB Block 4	2001	CC	2
Fukumoto	Sunland	Paterson	2000	CC	5 topwork
Fukumoto	Heidelberg	Kruisrivier	2001	C35	4

Fukumoto	Citrusdal	ALG	2002	RL	20 topwork
Letaba Early	Sunland	Paterson	2000	CC	5 topwork
Letaba Early	Heidelberg	Kruisrivier	2001	C35	7
Cliff Early	Fort Beaufort	Riverside	1996	TC	4
Fenix Early	Addo	ITSC	1999	SC	2 topwork
Krajewski Early	Addo	ITSC	1999	CM	2 topwork
Mistkraal Early	Addo	ITSC	2000	MxT	2 topwork
Sundays River Early	Addo	ITSC	1999	VA	2 topwork
Round Lina	Patensie	Paksaam			1
Washington	Addo	Willowtree	1998	CC	commercial
Washington	Patensie	Melverne	1996	RL	commercial
Washington	Patensie	Ripplehill	1999	RL	commercial
Cambria	Uitenhage	CFB Block 7	1999	CC	2
Cambria	Patensie	Patensie Acht	2000		commercial
Coetzee Late Navel	Citrusdal	Hexrivier	1997	RPL	9 topwork
Renken Late Navel	Citrusdal	Hexrivier	1997	RPL	8 topwork
Mouton Late Navel 1	Citrusdal	Hexrivier	1997	RPL	4 topwork
Mouton Late Navel 2	Citrusdal	Hexrivier	1997	RPL	2 topwork
Tuligold	Fort Beaufort	Riverside	1995	TC	5
Tuligold	Citrusdal	ALG	1998	RL	17
Lina (control)	Uitenhage	CFB Block 4	2001	SC	2
Lina (control)	Fort Beaufort	Riverside			
Lina (control)	Citrusdal	ALG	1997	RL	commercial
Newhall (control)	Sunland	Paterson	1999	CC	commercial
Newhall (control)	Citrusdal	ALG	1997	RL	commercial
Palmer (control)	Patensie	Melverne	1996	RL	commercial
Californian Lane Late (control)	Uitenhage	CFB Block 4	2001	SC	6
Californian Lane Late (control)	Citrusdal	Hexrivier	1997	RPL	semi comm
Royal Late	Uitenhage	CFB Block 4	2001	RL	

Results and discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.5.5 which needs to be referred to when reading the text.

Table 6.2.2.5.2. Comparison of production, fruit size and colour of various navel selections evaluated at the CFB during 2004.

Selection	Yield	Fruit Size	Colour transparency				Maturity	Comment
			19 April	4 May	15/19 May	1 June		
CFB								
Atwood	Good	Medium-large			5	1-2	Early June	
Dream	Good	Medium-large			4	2	Early June	
Fukumoto	Poor	Medium			2-3		Late May	
Lina / SC	Fair	Medium-large			5		Early-later June	
Palmer	Fair	Large			7		Mid-later June	
Citrusdal								
Fukumoto	poor	Medium-large – extra large	6	5-6	3-4 odd 6, deep orange	1-3 odd 5, later set?	1 st week May	All similar, mediocre taste on 5 May, Tuligold later
Tuligold	Good	Medium - medium-large	6-7	6-7	5-6 green heads	5 green heads	1 st week May	

Selection	Yield	Fruit Size	Colour transparency				Maturity	Comment
			19 April	4 May	15/19 May	1 June		
Lina	Variable, fair	Medium-large – large	7	6	4-5, 5-6 green heads	4-5 green heads	1 st -2 nd week May	
Newhall	Variable, poor	Medium – extra large	6	5-6	4		1 st -2 nd week May	

Atwood. The quality at the CFB on 15 May was fair, ready for picking on 1 June. The test was just acceptable (except colour) in May, Navelate quality in June.

The trees at Addo had a poor crop (partly picked) of large fruit size, T6 on 14 May. The quality was poor with low sugars and acid and about 3 weeks short of maturity. The test was acceptable but colour poor.

The young trees at Heidelberg only had odd fruit of medium and slightly larger fruit size, colour T7-8 on 26 April. There were too few fruit to evaluate properly.

Fruit shape is round, with a smooth rind, variable navel ends, peelable but oily, orange flesh and slightly open to open cores. The trees had some thorns at Heidelberg.

Dream. Quality at the CFB was fair, similar maturity to Atwood. Both tests met Navelate standards (except colour initially and some seed), better than Atwood but on a better rootstock. The acid maintained the same level in May and June.

The trees at Addo had a poor crop of large fruit size, mainly T5-6 on 14 May. The quality was poor with low sugars and acid and about 3 weeks short of maturity. The test was acceptable, similar to Atwood but colour poor.

The young trees at Heidelberg only had odd fruit of medium fruit size, colour T7-8 on 26 April and raggier than the other selections. There were too few fruit to evaluate fully.

The fruit shape is round with a smooth rind, peelable to difficult to peel and oily, orange flesh and slightly open to open core.

Fukumoto. Young trees at the CFB had fair quality with slightly high acid on 15 May, still about two weeks short of full maturity. The test was good, although acid not high, easily meeting Navelate standards. Similar aged Linas on Swingle had fair quality in May, slightly later maturing and a slightly poorer test.

There was no fruit at Addo. The young trees at Heidelberg only had odd fruit of medium to medium-large fruit size, colour T7 on 26 April, slightly ahead of Atwood and Dream. There were too few fruit to evaluate fully.

At Citrusdal a comparison was done with young topworked Fukumotos versus older Tuligold, Lina and Newhall, all on Rough lemon rootstock.

There was little difference in taste between the selections on 4 May, acceptable with sufficient sugars and acid, Tuligold later. The tests were unacceptable due to low juice (except Tuligold) and poor colour although the TSS and acid were good. An evaluation of samples a few days later had the following results (best to worst):

Rind colour: Newhall, Fukumoto, Tuligold, Lina
Fruit shape: Tuligold, Fukumoto, Newhall, Lina (most elongated)
Protruding navels: Fukumoto, Tuligold, Newhall, Lina
Malformed navels: Lina, Fukumoto, Tuligold

On 19 May the quality of all was only fair, sweetish with little acid, past peak maturity by 2 weeks. Tuligold and Lina slightly better taste, Newhall the best. Only Newhall met navel standards, the others having too low juice.

On 1 June all were overmature and poor quality, both Tuligold and Lina meeting Navelate standards, except for colour. Fukumoto had low juice (large fruit) and Newhall not tested. There was no oleo seen on Fukumoto or Lina, but some on Tuligold although exportable.

Fruit shape was mainly round, some elongated and some with a flat navel end, reasonably smooth to a slightly pebbly rind and variable navel ends. The flesh colour is orange with slightly open to closed cores sometimes a large internal navel. At Citrusdal the trees are vigorous with some chimeras and thicker rinds than Tuligold.

A survey of rootstock compatibility was done on two year old trees topworked to Fukumoto navel at ALG, Citrusdal. The scion had been budded onto rootstock shoots. There was no conclusive evidence to suggest that there is incompatibility with rootstocks other than Rough lemon, however the observations made were not consistent with observations made in California. For more detail refer to the following article: du Toit, Thys. 2004. Fukumoto Navel. SA Fruit Journal, vol 3(5):67. Oct/Nov.

Letaba Early

There was a poor crop at Addo with medium-large fruit size, colour T5-6 on 14 May similar to Atwood and Dream. The quality was poor with low sugars and acid about 2 weeks short of maturity. There were insufficient fruit for a test. Fruit shape was round with a smooth rind and variable navel ends. The young trees at Heidelberg were not yet in production.

Cliff Early (Painter Early II)

The young trees were compared with slightly older Tuligold and Lina navels. The fruit colour was T4-6, similar to Tuligold on 6 - 8 April, slightly ahead of Lina. Tuligold and Lina had a slightly better test and slightly higher acid. All had a very low juice percentage. On 5 May, both Cliff Early and Tuligold had some creasing. Estimated maturity around mid to end April and the fruit is reported to degreen well.

Fenix Early

This budsport was selected in the Addo area for its early maturity. The topwork trees had their first fruit, a poor crop with medium small fruit size and poor quality, colour T6 on 14 May. The test was good. The control Lina/Newhall had already been harvested. The fruit is round with a smooth, thin rind, almost closed navel end and closed core. The flesh was pale and coarse on 28 April.

Krajewski Early

This budsport was selected in the Kirkwood area for its early maturity. The topwork trees had their first fruit, a fair crop with medium-large fruit size and fair quality but acid slightly high. The colour was T6-7 on 14 May. The test was acceptable. Externally maturity appears to be much later, around mid June although the acid level is not high. The fruit is round with a fairly smooth rind, average navel ends, difficult to peel and oily. There is budwood available at the CFB.

Mistkraal Early

This budsport was selected in the Kirkwood area for its early maturity. The topwork trees had their first fruit, a poor crop with medium fruit size and fair quality, colour T5 on 14 May. No test was done. The fruit is round with a smooth rind and some splitting, peelable and oily with orange flesh, raggy and a closed core.

Sundays River Early

This budsport was selected in the Kirkwood area for its early maturity. The topwork trees had their first few fruit in 2003 on Carrizo, with no fruit last season. This season there was a fair crop on Volckameriana with medium-large fruit size and poor quality (low sugars and acid), colour T5-6 on 14 May. The test had unacceptably low TSS. Maturity estimated towards the end of May. The fruit is round with a smooth rind, difficult to peel and oily with orange flesh and a closed core.

Round Lina

This tree discovered in a Satsuma block and has the same characteristics as a Lina except that a lot of the fruit is round. The crop was good. Buds were marked for daughter trees to be made.

Washington

Commercial trees at Addo had a good crop of medium-large fruit size, colour mainly T3-5, some T1-2 on 1 June. The quality was fair with slightly high acid. The test was very good, acid level 1.21% and colour not quite good enough. Peak maturity around 3rd week of June. There were 3.0% fruit with protruding navels and 26% malformed navels (both class 1) and some oleo in the subsample. Harvesting of the orchard commenced on 27 May and finished 28 June which was a bit late resulting in some overmaturity.

Table 6.2.2.5.3. Packhouse data for yield, percentage per class and count distribution of class 1 fruit for Washington navels on Carrizo citrange planted in 1998 (5.5 x 2.5m) at Willowtree Farm, Addo.

Year - Age	Tons /ha	% export grd 1&2	% grade 1	Over size	Percentage fruit per count in grade 1							Local fruit	Factory fruit
					36	40	48	56	64	72	88		
2002 -4	10.5	66	44	0	5	16	25	25	14	12	3	29	4
2003 -5	24.9	42	26	1	12	24	24	20	12	8		42	15
2004 -6	27.0	57	38	1	11	13	24	18	18	13	3	29	13

2004 cull factors due mainly to wind blemish (lack of good winbreaks), some pests and overmaturity. With additional irrigation lines the production could have been increased.

Production at Malverne of both Washington and Palmer were good with medium-large fruit size, 86% of the colour T2-5 on 31 May. The average fruit size of a subsample was 81.6mm, 80% falling into counts 56-72, 17.5% count 48 and larger. The quality of both were poor with low sugars and acid, the tests acceptable, Washington initially better, Palmer far better in July when most of the fruit had already been harvested. Maturity probably late June. There were fruit with malformed navel ends, all within class 1, 67% rated zero.

The trees at Ripplehill bore a good crop of medium-large fruit size, colour 87% T4-6 and 12% T2-3 on 31 May, the odd fruit left on 7 July T1. Quality was poor (young trees) and the May test just acceptable (borderline TSS), except colour and July test just failing on TSS. Maturity probably late June. Two hundred fruit were evaluated in May. 64.5% fell between counts 56-72 and 35.5% counts 36-48, average 84.5mm. Three percent of the fruit had protruding navel ends and 37% class 1 malformed navels.

The Washington fruit are round with a smooth rind, peelable to difficult and oily, orange flesh with a closed to open core.

Cambria

The trees at the CFB had a poor crop of medium fruit size, colour T1 on 30 August. Slightly younger Royal Late navels on Rough lemon had a similar crop and colour with slightly larger fruit size. Quality was poor with low sugars and acid, Royal Late fair, both mature. The tests however were good, similar to Royal Late. The Cambria had some seed which is unexplained, however, seed has been seen in the Royal Late previously at the CFB.

The young trees at Patensie had a good crop of medium-large fruit size, T2-4 on 7 July. The quality was good. The colour was T1-3, odd T4 on 27 July, developing a good orange colour. The quality was still good, around peak maturity. The tests were good on both dates, Navelate quality in early July, acid just a little too low late July.

The count distribution of 200 fruit measured was 24.5% count 48 and larger, 40.5% 56, 20% 64, 12% 72 and 3% 88 average 82.0 mm. Some fruit had protruding navel ends, odd malformed navels and very little oleo seen.

The Cambria generally has an elongated fruit shape with some round fruit. Some trees or branches tend to have more elongated shape than others. The rinds are smooth occasionally slightly pebbled. Peelability is acceptable to difficult and oily, the fruit still firm in late July. The flesh has a fine texture, orange with closed to slightly open cores.

Evaluation of late maturing Coetzee Late, Renken Late, Mouton Late 1 and Mouton Late 2 navels at Hexrivier, Citrusdal

These trees suffered severe frost damage the previous season and have not performed well. The results must be treated with caution due to the small sample size. Only the Mouton Late 1 trees were evaluated on 17 June, while all the trees were picked on 30 June prior to fruit evaluations. Adjacent Californian Lane Late navels were used for comparative purposes. Comments on the evaluations are presented in Table 6.2.2.5.4.

Table 6.2.2.5.4. Evaluation of tree size, yield, fruit size, colour and fruit characteristics of various late maturing navel selections on Rangpur lime rootstock at Hexrivier, Citrusdal on 17 June, 2004.

Selection	Tree size	Yield	Fruit size	Colour	Fruit characteristics
Coetzee Late	Small – medium-large	Poor	Medium-large	4-5	Round – slightly elongated
Renken Late	Small tree, thorns	0–odd	Medium-large	3-5	
Mouton Late 1	Medium-large upright	Poor – fair	Medium-large. Ct 56/48	3-4	Unattractive, slightly elongated, coarse, high shoulders, slightly soft. Some protruding and malformed navels, ribbing
Mouton Late 2	Medium–medium small	Poor – fair	Medium	5	Round
Californian Lane Late	Medium-large - large	Fair	Medium-large. Ct 56/48	4-5	Fairly round to slightly elongated, slightly pebbly. Some protruding and malformed navels

Mouton Late 1

The few fruit evaluated were poor quality, lacking flavour on 17 June, soft yellow orange flesh, closed cores and easily peeled but very oily. Except for low juice (thick rinds), the test was acceptable. There was virtually no oleo, more for the Californian Lane Late.

Californian Lane Late (control)

The quality on 17 June was better than Mouton Late 1, slightly raggy, yellow orange flesh, not as easily peeled, oily and about two weeks short of maturity. The rinds were thinner, a fairly similar test having slightly higher acid but also not acceptable due to low juice.

Conclusion

Atwood

The trees had a poor to fair crop between sites with good to large fruit size. Fruit quality was poor to fair with similar maturity to Lina, later meeting Navelate standards. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Dream

Production, fruit size and quality varied between sites. The tests varied between acceptable to good although there was some seed. Maturity appears similar to Lina. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Fukumoto

Production was poor (young trees) and fruit size varied from medium to extra large. Fruit had good orange rind colour, slightly ahead of Lina and Newhall. Maturity was early May in Citrusdal, similar to slightly ahead of Lina and Newhall. Quality was fair, similar to the other early navels with acceptable to good tests but all failing on juice (large fruit). It is not recommended to plant Fukumoto on trifoliate or its hybrid rootstock at this stage until there is more clarity on the compatibility issue. As the trees are still young and vigorous further evaluations are necessary before any recommendations can be made.

Letaba Early

Production was poor, good fruit size and poor quality. Based on the limited data no recommendations can be made and further evaluations are necessary.

Cliff Early (Painter Early II)

The selection is early maturing, slightly ahead of Lina, peaking around mid to end April. Further trial plantings need to be established and further evaluations needed.

Fenix Early

There is too little data available to draw any conclusions. Further evaluations are necessary.

Krajewski Early

There is too little data available to draw any conclusions. Further evaluations are necessary.

Mistkraal Early

There is too little data available to draw any conclusions. Further evaluations are necessary.

Sundays River Early

There is too little data available to draw any conclusions. Further evaluations are necessary.

Round Lina

Buds were marked to be used later for making daughter trees.

Washington

Production was generally good, with medium-large to large fruit size. Colour was acceptable at maturity which is around 3rd week June. Eating quality varied between sites from poor to fair, tests acceptable to good. Although the trees are still young, the Washington can be considered for commercial planting although the fruit tends to be on the large side and quality not always good on young trees. More commercial production data needed.

Cambria

Production in the commercial block was good with medium-large fruit size, good quality and tests. Fruit at the CFB was smaller with poorer quality. The fruit is firm with a generally elongated fruit shape, maturing around late July. Further evaluations of trees from Foundation Block material are necessary before recommendations can be made.

Coetzee Late Navel

Production was poor with medium-large fruit size. Further evaluations are necessary.

Renken Late Navel

Production was very poor with medium-large fruit size. Further evaluations are necessary.

Mouton Late Navel 1

Production was poor to fair with medium-large fruit size and poor quality. The fruit was unattractive. Further evaluations are necessary.

Mouton Late Navel 2

Production was poor to fair with medium fruit size. Further evaluations are necessary.

Glenora Late, Witkrans, Royal Late and Californian Lane Late

These topworked trees in the Clanwilliam area are not yet in production and should bear their first few fruit next season. All are growing vigorously, the Glenora Late with long thorns, especially on the most vigorous branches (also at the CFB).

Table 6.2.2.5.5. Internal fruit quality data for navel orange selections for the Eastern and Western Cape areas during the 2004 season.

Selection	Root-Stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Atwood	RL	CFB	15/05	5	56	54.3	9.5	1.18	8.1	0
Atwood	RL	CFB	01/06	1-2	56	54.1	10.6	1.04	10.2	0
Attwood	CC	Paterson	14/05	6	40	50.4	9.6	0.97	9.9	0
Dream	CC	CFB	15/05	4	56	54.0	11.0	1.13	9.7	0
Dream	CC	CFB	01/06	2	64	54.2	11.6	1.13	10.3	0.3
Dream	CC	Paterson	14/05	5-6	56	50.6	9.7	0.97	10.0	0
Fukumoto	CC	CFB	15/05	2-3	64	51.6	11.6	1.00	11.6	0
Fukumoto	RL	ALG	19/04	6	48	37.5	10.2	1.08	9.4	
Fukumoto	RL	ALG	04/05	5-6		47.4	10.8	0.94	11.5	
Fukumoto	RL	ALG	19/05	3-4		47.2	10.2	0.75	13.6	
Fukumoto	RL	ALG	01/06	1-3	40	46.6	9.7	0.70	13.9	
Cliff Early	TC	Riverside	06/04	4-5-6		40.4	10.9	1.16	9.4	
Cliff Early	TC	Riverside	08/04	4-5		43.2	10.8	1.07	10.1	

Selection	Root-Stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Fenix Early	SC	ITSC Addo	14/05	6	64	52.0	10.8	1.04	10.4	0
SundaysREarly	Va	ITSC Addo	14/05	5-6	56	50.2	8.9	0.84	10.6	0
Krajewski Early	CM	ITSC Addo	14/05	6-7	56	53.7	9.7	0.96	10.1	0
Washington	CC	Willowtree	01/06	4-5	56	51.3	12.8	1.21	10.6	0
Washington	RL	Melverne	31/05			51.1	9.8	0.81	12.1	0
Washington	RL	Melverne	07/07	1	56	53.2	9.3	0.64	14.5	0
Washington	RL	Ripplehill	31/05	5-6	56	49.2	9.0	0.82	11.0	0
Washington	RL	Ripplehill	07/07	2-3	56	49.8	8.9	0.65	13.7	0
Cambria	CC	CFB	30/08	1	72	56.2	10.7	0.73	14.7	1.8
Cambria		PatensieAcht	07/07	3-4	56	52.1	11.5	0.95	12.1	0
Cambria		PatensieAcht	27/07	2-3	56	54.5	11.4	0.83	13.7	0
Mouton Late 1	RPL	Hexrivier	17/06	3-4	56/48	43.8	9.7	0.90	10.8	
Tuligold	TC	Riverside	08/04	4-5		46.6	11.4	1.19	9.6	
Tuligold	RL	ALG	19/04	6-7	64	41.8	10.2	1.20	8.5	
Tuligold	RL	ALG	04/05	6-7		48.1	11.0	1.07	10.3	
Tuligold	RL	ALG	19/05	5-6		48.3	9.9	1.03	9.6	
Tuligold	RL	ALG	01/06	5	64	50.0	10.3	0.97	10.6	
Navelina	SC	CFB	15/05	5	56	55.2	10.2	0.96	10.6	0
Lina	TC	Riverside	07/04	5-6		40.7	11.0	1.26	8.7	
Lina	RL	ALG	19/04	7	64	40.9	10.3	1.13	9.1	
Lina	RL	ALG	04/05	6		47.0	11.6	1.06	10.9	
Lina	RL	ALG	19/05			48.5	9.9	0.95	10.4	
Lina	RL	ALG	01/06	4-5	56	50.7	10.0	0.90	11.1	
Newhall	RL	ALG	19/04	6	48	37.7	10.7	1.03	10.4	
Newhall	RL	ALG	04/05	5-6		43.0	10.5	0.98	10.7	
Newhall	RL	ALG	19/05			47.8	10.1	0.86	11.7	
Palmer	RL	Melverne	31/05		56	48.7	9.6	0.69	13.9	0
Palmer	RL	Melverne	07/07	1	56	49.0	11.2	0.72	15.6	0
Lane Late	SC	CFB Block 4	30/08	1	56	53.5	10.8	0.77	14.0	0.9
Californ L Late	RPL	Hexrivier	17/06	4-5	56/48	44.4	9.4	1.02	9.2	0
Royal Late	RL	CFB	30/08	1	56	53.6	10.7	0.64	16.7	0

Future research

Evaluate all sites and selections and accumulate data from commercial Washington and Cambria navels (CFB material).

6.2.2.6 Evaluation Midseason oranges in the Cape areas

Experiment 77 by C J Alexander (Private)

Opsomming

Die doel van die proef is om midseisoen seleksies wat beter in die koeler streke sal aard in terme van vrug grootte, gepigmenteerde vleis en saadloosheid, te vind. Die aanvaarbaarheid van die Salustiana se vrug grootte onder kommersiële toestande word ook bevestig. Kommersiële Salustianas in die Sondagsriviervallei het 'n goeie oes van goeie vrug grootte, aanvaarbare tot goeie gehalte gehad en word vanaf die derde week in Junie ryp. Die Sanguinello op Fort Beaufort het goeie produksie met klein vrug grootte gehad, redelike gehalte en sommige vrugte met gepigmenteerde vleiskleur. Tarocco produksie was redelik goed en vrug grootte goed tot kleinerig, eetgehalte redelik met goeie toetse. Die Tarocco Gallo en 57/1E/1 het soortgelyke resultate gehad, 57/1E/1 beter eetgehalte. 'n Vergelyking van huidige en vorige data tussen die Maltaise Half, Maltaise Half II en Maltaise Line G het enkele verskille tussen die seleksies uitgewys, maar die vrug grootte van al drie was nie na wense nie en dus word hulle nie aanbeveel nie. Bokhobza het beter produksie as Barlerin gehad, beide medium vrug grootte en Bokhobza weereens met rooi vleiskleur. Verdere evaluasies is nodig.

Introduction

The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless. Confirm fruit size acceptably of the non-pigmented Salustiana in commercial orchards that are in production.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following Tomango standards previously considered most acceptable by the market: 52% juice; 9.0% TSS; 0.7 – 1.8% acid; 7.0:1 ratio; colour T3 + 20% T4 of set 34. Seed maximum average 6.0 seeds per fruit.

A list of selections and sites evaluated is given in Table 6.2.2.6.1.

Table 6.2.2.6.1. Midseason orange trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Salustiana	Uitenhage	CFB	2001	TC	4
Salustiana	Addo	Dunbrody	1997	CC	commercial
Salustiana	Fort Beaufort	Baddaford	1996	TC	10
Sanguinello	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Uitenhage	CFB	1994	TC	2
Tarocco	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Adelaide	Saxfold Park	1996	TC	Commercial
Tarocco	Ashton	Excelsior	2002	TC	com topw
Tarocco Gallo	Uitenhage	CFB	1999	CC	2
Tarocco 57/1E/1	Uitenhage	CFB	1999	CC/SC	4
Maltaise Half	Uitenhage	CFB	1997	TC	2
Maltaise Half II	Uitenhage	CFB	1997	TC	2
Maltaise Line G	Uitenhage	CFB	1997	TC	3
Maltaise Line G	Fort Beaufort	Baddaford	1998	TC	5
Maltiase Barlerin	Uitenhage	CFB	1997	TC	2
Malktaise Bokhobza	Uitenhage	CFB	1997	TC	2

Results and Discussion

A discussion of each selection follows with yield, fruit size, colour and estimated maturity and internal quality results for the various selections presented in Tables 6.2.2.6.3–4 that need to be referred to when reading the text.

Salustiana

Quality at the CFB was fair to good and mature on 10 July, peak around mid July, over-mature on 30 August. The test was unacceptable due to low juice (large fruit), otherwise good.

The commercial trees at Addo had a good crop of medium-large fruit size, harvested from mid-June. There was alternate bearing among the trees resulting in a good fruit size spread. The majority of the fruit were T2-4 on 1 June, the eating quality good, poorer than last season with slightly high acid. The test was acceptable and the fruit seedless. There was some oleo recorded in a small subsample, 80% class 1 and 20% class 2, but none commercially and no creasing. The average fruit size and count distribution results of fruit measured are given below.

Table 6.2.2.6.2. Average fruit size and count distribution for Salustianas measured at Dunbrody Estates, Addo on 22 May 2002, 30 May 2003 and 1 June 2004.

Year	Production tons/ha (6 x 3m)	Export % Class 1 & 2	Ave fruit size (mm)	Percentage fruit per count distribution						
				≥40	48	56	64	72	88	105
2001	51	78								
2002	82	82	79.1	2	9	23	32	28	6	
2003	Estim 55-60		80.8	8.5	11.5	25	32	19	4	
2004	75	81	77.6	2	7	17	26	33	13.5	1.5

Sample size 100 in 2002; 200 in 2003/4.

The actual orchard count distribution was mainly count 64 – 88 odd 105 and very few 40/48 tests varying between 9.0 – 9.5% TSS and 0.8 – 1.1% acid. The fruit was very juicy with a soft rag, soft fruit and rind colour pale orange.

The fruit quality at Fort Beaufort was fair on 17 July with a good test and mature.

The fruit shape is round, sometimes a flat stylar end, a smooth rind although the larger fruit at Addo had some sheep nose on the larger fruit and coarser. The skin tended to be thick at Addo. The fruit was peelable but oily, orange flesh, mainly open cores and seedless in a solid block.

Sanguinello

Fruit quality at Fort Beaufort was fair with highish acid on 17 July and mature. The test was good, far better than Salustiana with higher acid and red juice. There were red flecks in the flesh which improved to T5 of Star Ruby set 47 after 5 days.

Fruit shape is round with a smooth rind and peelable but oily. The cores were open and only odd seed in a mixed block.

Tarocco

The CFB quality was poor to fair with high acid on 1 June, overmature on 10 July with variable red flecks in the flesh, better than 57/1E/1. The test in June was unacceptable due to high acid, good in July. Gallo and 57/1E/1 both had better quality than Tarocco on 28 July. Zero to odd seed.

Fruit quality at Fort Beaufort was fair, and mature by 17 July, the test very good. There were some red flecks in the flesh and the juice red.

Commercial Taroccos at Adelaide (not evaluated) were ready for harvesting end June/early July. Except for two instances where the juice level was too low, the tests were very good from May through to July, pigmentation starting to show up in early July. The commercial block in the Western Cape bore only odd fruit, good quality and colour T1-2 on 23 June with no pigmentation. The trees should bear their first small crop next season.

Fruit shape is round to necked with a smooth rind. The fruit is peelable but oily, open cores and few seeds. There was some creasing at the CFB and Fort Beaufort. Trees are large and vigorous and appear to outgrow the large thorns over time, also Tarocco Gallo and 57/1E/1.

Tarocco Gallo

The CFB quality was poor to fair with slightly high acid on 1 June, overmature on 10 July with more surface blush and firmer than 57/1E/1. Both tests were good except colour not good enough in June. Trees on Swingle rootstock had smaller tree size. Thorns varied from large to none. No pigmentation seen.

The fruit shape is round with a smooth rind, peelable and oily, orange flesh and open cores and odd seed. There was some creasing.

Tarocco 57/1E/1

Fruit quality at the CFB was fair with high acid on 1 June not yet mature, good quality but overmature (more so than Gallo) on 10 July, acid still high and fruit soft. The tests were good, acid still fairly high in July. The rind colour was deep red in July, some fruit had no pigmentation, others speckled in late July. There was less pigmentation on Swingle trees in late July.

The fruit shape is round with a smooth rind, peelable and oily, orange flesh and open cores. There was some creasing and odd seed. Trees are vigorous with variable thorniness from zero to large.

Maltaise Half

Quality at the CFB was fair and mature on 1 June, better taste than Half II, overmature at the end of August. The tests were good but acid level still 1.31% on 30 August. The fruit shape is round with a smooth rind, peelable and oily, open cores, orange flesh and odd seed. Refer comparison of Maltaise Half, Half II and Line G.

Maltaise Half II

Quality at the CFB was fair and mature on 10 July, overmature at the end of August. The tests were good except slightly low juice and acid still 1.19% at the end of August. The fruit shape is round with a smooth rind, peelable and oily, open cores and orange flesh. There were odd seed. Refer comparison of Maltaise Half, Half II and Line G.

Maltaise Line G

Fruit quality at the CFB was fair with fairly high acid and not quite mature on 1 June. On 10 July the fruit was overmature and acid low. The tests were generally good, some variation in the acid but juice too low in July and August. The fruit at Fort Beaufort had fair quality and mature on 17 July, no test done. The fruit shape is fairly round and smooth, peelable but oily, orange flesh and closed to open cores and odd seed.

Comparison of the Maltaise Half, Maltaise Half II and Maltaise Line G

Results of these three selections were compared for the past three seasons and revealed the following: all had similarly good crops, but all had a size problem around medium with Half II the smallest fruit - medium small. Both Half and Half II had some creasing. Rind colour was good with Line G fractionally behind the other two by half a transparency. Eating quality was fair, although this is not reflected in the tests. There were some differences between the internal quality tests (all acceptable to good), Line G the lowest juice (borderline), Half II 1.0% higher TSS, Half the highest acid and Line G the lowest acid resulting in the best ratio. The Line G at Baddaford had similar juice but lower TSS and acid than those at the CFB.

Maltaise Barlerin

The quality was fair but sugars and acid on the low side on 30 August and overmature. However, the test was very good. Fruit shape is round with a smooth rind, peelable but oily, open cores, orange flesh and seedless.

Maltaise Bokhobza

Quality was fair and overmature on 30 August, the test very good and juice red. The flesh was pigmented at the end of August.

Fruit shape is round with a smooth rind, peelable but oily and open cores. There was a lot of creasing and odd seed. The leaves have a wavy shape.

Table 6.2.2.6.3. Yield, fruit size, fruit colour, estimated maturity, average seed and comments of various midseason oranges evaluated during 2004.

Selection (year planted)	Yield	Fruit size	Colour transparency				Estimated maturity	Ave. seed/ Comment
			1 June	10 July	17 July	30 Aug		
CFB								
Salustiana ⁰¹	Fair-good	Medium-large		1		1	Mid July	0.3
Tarocco ⁹⁴	Fair	Medium	1	1		1	Mature by 1 June, overmature by 10 July	0 – 0.5 Red flecks
Tarocco Gallo ⁹⁹	Fair - good	Medium-large	4-5	1		1	Mature 3 rd week June, overmature by 10 July	0.2 – 0.3 No pigment, good blush
Tarocco 57/1E/1 ⁹⁹	Fair	Medium-large	3	1		1	2 nd -3 rd week June, overmature by 10 July	0.6 – 0.9 Odd pigment

Maltaise Half 97	Fair	Medium		1		1	Mature by 10 July	0 – 2.1 Highest acid of all
Maltaise Half II 97	Fair	Medium		1		1	Mature by 10 July	0.3 – 1.2
Maltaise Line G 97	Fair	Medium- large	1-2		1	1	2 nd -3 rd week June, overmature by 10 July	0.3 – 0.8 Soft flesh
Maltaise Barlerin 97	Poor	Medium				1	Overmature by 30 August	Seedless
Maltaise Bokhobza 97	Good	Medium				1 Red	Overmature by 30 August	0.3 Pigmented like T5 of Star Ruby
Dunbrody								
Salustiana 97	Good	Medium- large	2-4				3 rd week June	0
Baddaford								
Salustiana 96	Good	Medium- large			1		Mature by 17 July	0.5
Sanguinello 96	Good	Small			1		Mature by 17 July	0.3 Red flecks and red juice
Tarocco 96	Good	Medium- large			1		Mature by 17 July	0.5 Red flecks, soft flesh
Maltaise Line G 98	Good	Medium small			1		Mature by 17 July	Odd. Size too small

The CFB and Baddaford trees are in a mixed block.

Conclusion

Salustiana

Production and fruit size was generally good, good colour, fair to good quality and maturity from mid June to mid July depending on the site. Based on these results the Salustiana can be planted in the Addo and Fort Beaufort areas, although midseasons do tend to develop smaller fruit size when trees get older. Evaluations to be discontinued.

Sanguinello

Production was good but fruit size small. Quality was fair with highish acid and a good test. There was some flesh pigmentation which improved since picking. Evaluate one more year.

Tarocco

Production was fair to good with smaller to good fruit size. Quality was fair and tests good, although high acid initially. The fruit developed some flesh pigmentation on the trees. Fruit from commercial orchards in the Eastern Cape were pigmented with very good tests except for odd low juice. Commercial orchards to be evaluated in the Eastern and Western Cape next season.

Tarocco Gallo

Production was fair and fruit size good but quality poor although the tests were good, maturing around 3rd week of June. Thorns on the trees were variable in presence and size. Further evaluations are necessary.

Tarocco 57/1E/1

Production was fair with medium-large fruit size, fair eating quality and good tests maturing around 2nd-3rd week of June. Some fruit were pigmented. Further evaluations are necessary.

Maltaise Half

Production was fair with medium fruit size, fair quality and a good test. It had the highest acid of all the selections. Evaluations to be discontinued.

Maltaise Half II

Production was fair with medium fruit size and quality was fair with good tests. Evaluations to be discontinued.

Maltaise Line G

Production was fair to good with variable fruit size between sites. Quality was fair, with good sugars. Maturity from mid to late June at the CFB. Evaluations to be discontinued.

Comparison of the Maltaise Half, Maltaise Half II and Maltaise Line G

Although there were slight differences between the selections, of concern is the relatively small fruit size off young trees and only average eating quality although subjective. As none of these selections are outstanding in any way as a midseason they are not recommended for commercial planting.

Maltaise Barlerin

Production was poor with medium fruit size. Quality was fair although the tests were good at the end of August. Further evaluations are necessary.

Maltaise Bokhobza

Production was good with medium fruit size. The quality was fair with a good test at the end of August and some flesh pigmentation. There was a lot of creasing. Further evaluations are necessary.

Table 6.2.2.6.4. Internal fruit quality data of the various midseason orange selections for the Eastern Cape during the 2004 season.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Salustiana	TC	CFB	10/07	1	48	51.3	11.0	0.84	13.1	0.3
Salustiana	CC	Dunbrody	01/06	3-4	56	56.9	9.6	0.98	9.8	0
Salustiana	TC	Baddaford	17/07	1	56	53.3	10.6	0.81	13.1	0.5
Sanguinello	TC	Baddaford	17/07	1	72	58.4	12.9	1.24	10.4	0.3
Tarocco	TC	CFB	01/06	1		58.4	12.9	1.71	7.5	0.5
Tarocco	TC	CFB	10/07	1	64	56.3	11.8	1.21	9.8	0
Tarocco	TC	Baddaford	17/07	1	72	54.7	12.6	0.96	13.1	0.5
Tarocco	TC	Saxfold Pk	06/05			57.0	11.0	0.97	11.4	
Tarocco	TC	Saxfold Pk	06/05			59.0	11.6	1.04	11.2	
Tarocco	TC	Saxfold Pk	07/07			51.4	10.2	1.16	8.8	
Tarocco	TC	Saxfold Pk	08/07			56.1	11.4	1.17	9.7	
Tarocco	TC	Saxfold Pk	08/07			57.3	11.3	1.27	8.9	
Tarocco	TC	Saxfold Pk	09/07			49.3	11.5	1.12	10.2	
Tarocco Gallo	CC	CFB	01/06	4-5	56	56.3	10.7	1.28	8.4	0.3
Tarocco Gallo	CC	CFB	10/07	1	64	58.5	11.4	1.07	10.7	0.2
Tarocco 57/1E/1	SC	CFB	01/06	3	48	58.3	10.3	1.14	9.0	0.6
Tarocco 57/1E/1	CC	CFB	10/07	1	64	53.5	11.5	1.28	9.0	0.9
Maltaise Half	TC	CFB	10/07	1	105	58.6	12.4	1.49	8.3	2.1
Maltaise Half	TC	CFB	30/08	1	64	54.6	12.4	1.31	9.5	0
Maltaise Half II	TC	CFB	10/07	1	72	57.0	12.6	1.24	10.2	0.3
Maltaise Half II	TC	CFB	30/08	1	56	51.7	12.8	1.19	10.8	1.2
Maltaise Line G	TC	CFB	01/06	1-2	56	52.6	11.4	0.83	13.7	0.3
Maltaise Line G	TC	CFB	10/07	1	72	51.7	11.4	1.04	11.0	0.8
Maltaise Line G	TC	CFB	30/08	1	64	50.6	11.8	0.98	12.0	0.7
Barlerin	TC	CFB	30/08	1	56	55.7	12.5	1.08	11.6	0
Bokhobza	TC	CFB	30/08	1	72	55.7	12.4	0.94	13.2	0.3

Future research

Continue evaluations of all the above selections except Salustiana, Maltaise Half, Maltaise Half II and Maltaise Line G.

6.2.2.7 Evaluation of Valencia oranges in the Cape areas

Experiment 77 by C J Alexander (Private)

Opsomming

Die doel van die Valencia proef is om vroeër, mid en laat seleksies met groot vruggrootte, saadloos en verbeterde vrugset as alternatiewe vir die huidige seleksies te soek, en die verenigbaarheid van Turkey op Rangpur Lime onderstam te ondersoek. Die Mouton Early lyk belowend as 'n vroeër seleksie met goeie produksie, vruggrootte en gehalte en die skil is gladder as die vorige seisoen. Dit moet op onderstamme of grond gevestig word wat 'n gladder skil kan gee. Die Turkey kan as 'n vroeër seleksie geplant word, maar die interne gehalte is 'n kwessie. Die verenigbaarheid op Rangpur Lime is twyfelagtig en ander onderstamme moet oorweeg word. Verskeie mutasies van virusvrye materiaal was, van hulle vir die eerste keer, by die CFB geëvalueer. Die meeste het vir die eerste keer gedra, die meerderheid saadloos en moet verder geëvalueer word. Dit sluit die Rietspruit, Portsgate, Bend 8A, G5, McClean SL, Delicia en Kleinhans in. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate early, mid and late maturing Valencia selections in terms of their maturity, rootstock compatibility, colour, fruit size and seediness. A number of mutations that have undergone STG have borne their first fruit at the Foundation Block.

Materials and methods

The trees were either planted or topworked within commercial orchards, established on a semi commercial scale where possible or evaluated at the CFB. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following Valencia standards previously considered most acceptable by the market (Delta and Midnight in brackets): 48% (52%) juice; 9.0% (10.5%) TSS; 0.6 – 1.8% (0.85 – 1.5%) acid; 7.0:1 (7.5:1) ratio; colour T3 + 20% T4 of set 34. Seed maximum average 9.0 seeds per fruit (Delta 0, Midnight 1).

A list of selections and sites evaluated is given in Table 6.2.2.7.1.

Table 6.2.2.7.1. Valencia orange trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Root Stock	No of trees
Mouton Early upper orchard	Citrusdal	Sewe Oliene	1997	RL	5 topwork
Mouton Early lower orchard	Citrusdal	Sewe Oliene	1999	RL	commercial
Midnight upper (control)	Citrusdal	Sewe Oliene	1986	RL	1
Valencia Late upper (control)	Citrusdal	Sewe Oliene	1986	RL	commercial
Turkey lower (control)	Citrusdal	Sewe Oliene	1999	RL	semi com
Turkey	Citrusdal	Hexrivier	1995	RPL	commercial
Rietspruit	Uitenhage	CFB Block 4	2001	2	RL
Portsgate	Uitenhage	CFB Block 7	1999	2	TC
Bend 8A	Uitenhage	CFB Psylla house	1999	1	TC
G5	Uitenhage	CFB Psylla house	1999	3	TC
McClean Seedless SL	Uitenhage	CFB Block 7	1999	2	SC
Delicia	Uitenhage	CFB Block 7	1994	2	TC
Kleinhans	Uitenhage	CFB Block 7	1997	2	TC
Midnight (control)	Uitenhage	CFB Block 4	2001	2	TC
ValenciaLate (control)	Uitenhage	CFB Psylla house	1999	2	TC
ValenciaLate (control)	Uitenhage	CFB Block 7	2001	2	SC

Results and Discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.7.4. which need to be referred to when reading the text.

Mouton Early

Trees were evaluated in two orchards on the same farm – upper and lower orchards, with their own controls. Results are presented in Table 6.2.2.7.2 below.

Table 6.2.2.7.2. Yield, fruit size, colour transparency, taste, test comments and estimated maturity of three Valencia selections on Rough lemon rootstock at Sewe Oliene, Citrusdal, evaluated on 17 June and 20 July 2004.

Selection	Yield	Fruit size	Colour		Taste	Test (July)	Estimated maturity
			17/6	20/7			
Upper orchard							
Mouton Early 1997	Good	Medium-large, odd medium. 68% count 72, ave 74.0mm	T3	T1, paler on east side	June – still fairly acid. July - good quality, past peak by 1-2 weeks	June – good, easily meets Valencia standards 0.2 seeds	Early July
Midnight 1986	Poor	Medium-large - mainly large		T1-2 pale	July – good quality, slightly tart, raggy, not at peak, 1-2 weeks to go	Not meeting Valencia standards due to low ratio (high acid)	End July - early August
Valencia Late 1986	Fair-good	Medium	T6-7	T1-2 pale	June – high acid. July – variable, sweet to very high acid, far from peak, 3-4 weeks to go	Not meeting Valencia standards due to high acid, good TSS	Mid – late August.
Lower orchard							
Mouton Early 1999	Poor-fair	Medium - medium-large		1	July – fair to good, past peak by 1-2 weeks	Acceptable Valencia test 0.7 seeds	Early July
Turkey Valencia 1999	Poor-fair	Medium-large -large		1	July – acceptable overmature taste, slightly tart, 1-2 weeks to go?	Not meeting Valencia standards due to low ratio	End July?

The lower orchard suffered severe frost damage in 2003 and light frost in 2004 and hence not all trees are in production.

Fruit shape is round, some with slight shoulders, with a fairly smooth to slightly pebbly rind (better than in the past). The younger trees had coarser rinds. The Midnights and Valencias had a slightly smoother rind. Flesh colour was orange, with closed to slightly open cores (others closed) and not as oily as the others. The fruit was slightly soft and the calyx removed on picking. Mostly seedless. The Turkey had the palest juice colour followed by Mouton Early and Valencia Late the darkest.

Turkey

The Turkey was evaluated at one site to determine its suitability to the Citrusdal area and compatibility on Rangpur Lime rootstock. The orchard suffered severe frost last season. The crop was good to excellent with fairly even, medium-large fruit size. The colour was T1-2 (pale) on 30 June and T1 on 20 July developing an orange colour. The sugars were good in June but tart, still tart in July, losing flavour and raggy. Peak maturity around late July. The test in June was unacceptable (low juice and ratio) and still unacceptable in July due to a low ratio – the TSS dropped by 1.2%.

Fruit shape is round with a fairly smooth to slightly pebbly rind. The fruit was slightly soft with little blemish and no oleo in June. The fruit was seedy, averaging 5.3 seeds/fruit with pale orange flesh, closed to slightly open cores and fairly thick rinds.

Some trees had odd suckers growing out the rootstock and other trees nodules on the rootstocks. There were no signs of rootstock incompatibility evident under the bark. The bud unions look good.

A lot of new selections coming into production were evaluated at the CFB. The results are tabulated below in Table 6.2.2.7.3.

Table 6.2.2.7.3. Yield, fruit size, colour, quality, maturity and fruit characteristics and average seed of various Valencia selections evaluated at the CFB on 30 August 2004.

Selection/ Block/ R-stock,age	Yield	Fruit size	Fruit colour	Taste	Test	Estimated maturity	Fruit characteristics	Ave. Seed
<u>Bend 8A2</u> Psylla house TC 99	Fair	Large	2-3	Poor, low sugars, slightly high acid	Meets Valencia standards	Mature	Round, rough rind	0
<u>G5</u> Psylla TC house 99	Good	Medium	3-4	Good	Meets Valencia standards	Mature	Round, smooth rind	0
<u>Val Late</u> (control) Psylla house TC 99	Poor – fair	Medium	4-5	Good	Meets Midnight standards except colour	Mature	Round, smooth rind	0.8
<u>Rietspruit</u> Block 4 RL 01	Poor	Medium-large	1	Poor, low sugars, high acid	Borderline TSS, juice too low	Mature, not mature 28 July	Round, smooth and thick rind	0
<u>Midnight</u> (control) Block 4 TC 01	Fair	Medium	1	Fair	Meets Valencia standards	Mature, not quite mature 28 July	Round, smooth rind	0
<u>Val Late</u> (control) Block 4 SC 01	Poor	Medium-large	1	Poor, high acid	Meets Valencia standards	Mature, not mature 28 July	Round, smooth rind	0
<u>Portsgate</u> Block 7 TC 99	Good	Medium	1	Fair	Just meets Valencia standards	Mature	Round with occasional slight shoulders, smooth rind	0
<u>McClean SL</u> Block 7 SC 99	Good	Medium	1	Fair, slightly high acid	Meets Valencia standards	Mature	Round, smooth rind	0
<u>Delicia</u> Block 7 TC 94	Fair	Medium-large	1	Good	Meets Midnight standards	Mature	Round, smooth rind	0.3
<u>Kleinhans</u> Block 7 TC 97	Fair small	Small	1	Poor, high acid	Acid far too high, probably stressed	?	Elongated, smooth rind	1.8

Rietspruit

This selection was included in the programme as the best seedless, mid maturing Valencia selection, superior to the Delta Valencia in the Nelspruit area. The fruit had poor quality and low juice due to thick rinds, cores slightly open, the Valencia Late closed. Both Midnight and Valencia Late had better quality.

Portsgate

This selection was included in the programme as a promising mid maturing seedless Valencia selection from the Hoedspruit area. The fruit had fair quality just meeting Valencia standards, cores slightly open.

Bend 8A2

This selection was included in the programme as a large, seedless, late maturing Valencia selection. The fruit had acceptable but relatively low TSS with a good acid level and high juice. The fruit colour could be delayed as the trees are in the psylla screen house. However it was two colour transparencies ahead of Valencia Late.

G5

This selection was included in the programme as a large, seedless late maturing Valencia selection from the Malelane area. The fruit had good quality with an acceptable test and very high juice. The flesh colour is bright orange and rind colour could be delayed as the trees are in the psylla screen house. However it was one colour transparency ahead of Valencia Late.

McClellan Seedless SL

The fruit had fair quality and a good test, cores slightly open.

Delicia

This selection was included in the programme as a large, near seedless late maturing Valencia selection from the Kirkwood area. The tree size, yield, fruit size and quality are typical for the selection. The test just met Midnight requirements, few seeds, open cores and odd out of season fruit.

Kleinhans

This selection was included in the programme as a large, near seedless very late maturing Valencia selection from the Patensie area. The trees are planted next to a windbreak. The evaluation is typical for trees under stress and the results should be interpreted with caution.

Conclusion

Mouton Early

Older topworked and young trees were evaluated, the latter still recovering from severe frost damage the previous season. Production was good on the old trees with good fruit size, good quality and virtually seedless. Colour and maturity is early, around early July. The rind texture is much smoother than last season. The selection is early maturing and should rather be planted on a heavier soil or citrange rootstock to induce a smoother rind. The selection is protected and virus free material is available. Commercial plantings are not yet recommended and evaluations to continue.

Turkey

Production was good to excellent with good fruit size. Fruit quality was poor and did not meet export standards. The selection can be planted commercially but quality aspects must be taken into account and a better quality inducing rootstock be considered. The compatibility of Turkey on Rangpur Lime seems to be questionable as there is sucker and nodule growth on the rootstocks. Other rootstocks should rather be used. Evaluate for one more year.

Rietspruit

Production was poor with good fruit size, poor quality and low juice. Further evaluations are necessary.

Portsgate

Production was good with medium fruit size, fair quality just meeting minimum standards and seedless. Further evaluations are necessary.

Bend 8A2

Production was fair with large fruit size, an acceptable test and seedless. Colour was ahead of Valencia Late. Further evaluations are necessary.

G5

Production was good with medium fruit size, an acceptable test and seedless. Colour was ahead of Valencia Late. Further evaluations are necessary.

McClellan Seedless SL

Production was good with medium fruit size, fair quality and a good test, similar acid to Valencia Late, high juice and seedless. Further evaluations are necessary.

Delicia

Production was fair with good fruit size, just meeting Midnight standards. The characteristics are typical of the selection.

Kleinhans

As the trees are planted next to a windbreak, the results are indicative of a tree under stress and not too much emphasis should be laid on them.

Table 6.2.2.7.4. Internal fruit quality data for Valencia orange selections for the Eastern and Western Cape during the 2004 season.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Mouton Early Val ^{up}	RL	Sewe Oliene	20/07	1	72	53.2	10.2	1.16	8.8	0.2
Mouton Early Val ^{lo}	RL	Sewe Oliene	20/07	1	64	48.5	9.3	1.29	7.2	0.7
Midnight ^{up}	RL	Sewe Oliene	20/07	1	56	59.4	9.8	1.70	5.8	0
Valencia Late ^{up}	RL	Sewe Oliene	20/07	1-3	88	54.0	10.7	2.00	5.4	2.9
Turkey Valencia ^{lo}	RL	Sewe Oliene	20/07	1	56	50.8	9.2	1.43	6.4	4.6
Turkey Valencia	RPL	Hexrivier	30/06	1-2	64	47.9	11.1	1.80	6.2	5.0
Turkey Valencia	RPL	Hexrivier	20/07	1	64	51.7	9.9	1.62	6.1	5.6
Rietspruit	RL	CFB	30/08	1	56	42.1	9.0	1.17	7.7	0
Portsgate	TC	CFB	30/08	1	56	50.0	9.1	1.05	8.7	0
Bend 8A 2	TC	CFB	30/08	2-3	56	58.7	9.5	1.04	9.1	0
G5	TC	CFB	30/08	3-4	56	59.7	9.7	0.99	9.8	0
McClellan SL	SC	CFB	30/08	1	72	58.0	10.3	1.25	8.2	0
Delicia	TC	CFB	30/08	1	48	52.0	10.5	1.14	9.2	0.3
Kleinhans Valencia	TC	CFB	30/08	1	72	50.7	13.1	2.4	5.4	1.8
Midnight	TC	CFB	30/08	1	56	52.2	9.6	1.00	9.6	0
Valencia Late	TC	CFB	30/08	4-5	56	54.7	11.2	1.01	11.1	0.8
Valencia Late	SC	CFB	30/08	1	56	50.6	10.3	1.25	8.2	0

^{up} = upper orchard

^{lo} = lower orchard

Future research

Evaluate all selections and new ones coming into production at the CFB and evaluate commercial Turkey on Rangpur Lime for one more season.

6.2.2.8 Evaluation of Genoa Lemon on various rootstocks in Citrusdal

Experiment 588 by C J Alexander (Private)

Opsomming

Die doel van die proef is om die prestasie van die Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Die bome het hul derde, maar teleurstellende drag gehad. Boomgrootte was ongelyk tydens uitplant en net die beste bome is vir evaluasie doeleindes gebruik. Growweskiisuurlemoen en Volckameriana het die grootste bome en vruggrootte gehad, M X T, Tri x Sweet, Benton, Swingle en Carrizo die mees gewenste vruggrootte. Al die onderstamme het maklik die sap uitvoer standaard gehaal, Benton en Rangpur die hoogste, Growweskiisuurlemoen die laagste. Die meeste vrugte het soortgelyke vrugkleur gehad, M X T en Trifoliaat X effens vroeër. Enkele Carrizo en Growweskiisuurlemoen vrugte het weens hoër skouers nie uitvoer standaard behaal nie, Carrizo, Japanse sitroen, Rangpur en Tri x Sweet die meeste oleo. Growweskiisuurlemoen het die meeste windletsels gehad, die ander almal uitvoerbaar. Van die Growweskiisuurlemoen vrugte was skurf, Swingle, M X T en Trifoliate x Sweet die gladste. Die meeste onderstamme het redelik gladde entverbinding gehad behalwe Rangpur en Japanse Sitroen wat knoppies en laasgenoemde ook suiers gehad het. Evaluasies moet voortgaan.

Introduction

The Genoa is a newly acquired slightly earlier maturing lemon selection. The trial was established in Citrusdal to determine the performance of this selection in the area on ten different rootstocks and in so doing possibly provide an alternative to the currently planted lemon selections and on different rootstocks.

Materials and methods

The Genoa lemon was budded to ten different rootstocks and planted at Hexrivier, Citrusdal in January 2000 in adjacent rows. The trees were evaluated according to certain criteria, including production, sets, tree size, compatibility, fruit size, juice percentage, average seed, rind thickness, high shoulders, oleocellosis and wind scars. Commercial Genoa and Lisbon lemons on Rough lemon that form part of the orchard are included in the evaluations. The list of rootstocks and number of trees evaluated is presented in Table 6.2.2.8.1.

Results and discussion

The trees were evaluated on the 12 May and tree heights measured on 20 July 2004. There is some variation in the tree size and therefore only the better/larger trees have been used for evaluation purposes – refer Table 6.2.2.8.1. Overall the production was most disappointing, resulting in some of the evaluation samples having relatively few fruit. The results of all the data are presented in the various tables.

Table 6.2.2.8.1. List of rootstocks, rootstock selection, number of trees evaluated and average tree height and diameter with Genoa Lemon as scion at Hexrivier, Citrusdal during 2004.

Rootstock	Selection	No of trees evaluated	Ave. tree height (m)	Ave. tree * diameter (m)
Cairn Rough lemon	163	16	3.6	2.3
Volckameriana	575	13	3.4	2.7
Trifoliata X	1242	8	3.3	2.3
Benton citrange	980	5	3.2	2.6
Trifoliata x Sweet orange	1287	14	3.1	2.3
Japanese Citroen	184	13	3.1	2.6
Minneola x trifoliata	1238	13	3.1	2.4
Swingle citrumelo	715	11	3.0	2.4
Rangpur Lime	225	4	3.1	2.6
Carrizo citrange	608	4	3.1	2.5
Genoa/Rough lemon		19	3.1	2.6
Lisbon/Rough lemon		22	3.1	2.6

* Trees are pruned between the rows to allow tractors to pass through.

Table 6.2.2.8.2. Visual evaluation of tree production, number of sets, tree size and bud union. Rootstocks arranged in order of planting.

Rootstock	Production	No of sets	Tree size	Bud union
Cairn Rough lemon	Poor – fair, variable	Mainly one set	Large spread	Clean, smooth with occasional slight fluting
Volckameriana	Poor - fair variable	Also a later set T8	Large spread, paler leaves	Clean, smooth with occasional slight fluting
Trifoliata X	Poor	Variable?	Variable small - medium-large	Clean, almost smooth, occasional fluting
Benton citrange	Poor - fair	Mainly one set	Variable, medium - large	Clean, fairly smooth
Trifoliata X Sweet orange	Poor - fair	Few of a much later set T8	Medium-large - large	Clean, inverted bench
Japanese Citroen	Poor - fair	Three sets	Medium-large – large. Odd paler trees	Fairly smooth, lots of nodules and odd sucker growth
Minneola X trifoliata	Poor. Pick fairly easily	One set	Medium-large - large, fairly tall, green trees	Clean, fairly smooth, odd fluting
Swingle citrumelo	Poor	A small much later set	Medium-large, tall	Clean, slight bench, odd fluting
Rangpur Lime	Fair	A small later set	Medium-large	Some nodules to profuse. Yellow coloured rootstock
Carrizo citrange	Poor	One set	Medium - large, fairly dense	Clean, slightly fluted. Smooth to almost inverted?
Genoa/RL commercial	Fair - good	One set	Large, fairly spread	Smooth. Nodules variable from few to many
Lisbon/RL commercial	Fair - good	One set	Large spread	Smooth? Mostly with nodules

A very light harvest was done late April, which would have had little effect on the yields. Stem and scion diameters were not measured as the trees were allowed to branch just above the bud union in the nursery that also makes it difficult to always see the bud union.

There was little difference between in season fruit colour between the rootstocks and commercial orchards. Most fruit were colour T6 (66-85%) and the balance T5. MXT had 50% T5 and T6, Tri X 45% and 55% respectively.

Table 6.2.2.8.3. Average fruit size and percentage fruit per count of Genoa lemon on various rootstocks. Rootstocks arranged according to descending fruit size.

Rootstock	Ave fruit size (mm)			Percentage fruit per count 2004							% in counts 216-113
	2002	2003	2004	≤189	162	138	113	100	88	≥75	
Volckameriana	71.8	63.1	65.1				31	19	30	20	31
Rangpur Lime	71.6	63.1	63.9	1		11	25	25	21	17	37
Cairn RL	72.4	62.9	63.4	1	2	7	36	21	22	11	46
Japanese Citroen	70.8	63.7	61.6	1	2	16	44	23	9	5	63
Trifoliolate X	64.6	61.0	60.9			35	25	30	10		60
Benton citrange	69.8	61.9	60.5	1	2	21	58	13	5		82
Swingle citrumello	64.6	57.5	59.5	4	4	34	37	14	6	1	79
Carrizo citrange	64.0		59.1	8	8	36	26	14	6	2	78
Trifoliolate x Sweet Orange	67.6	58.8	59.1	6	10	33	35	9	6	1	84
Minneola X Trifoliolate	63.2	57.1	57.8	10	18	38	23	7	3	1	89
Genoa/RL commercial			64.9			2	27	26	27	18	29
Lisbon/RL commercial			61.5		6	14	43	26	9	2	63

Some of the rootstocks had a high percentage of fruit not desired by the export markets i.e. larger than count 113. These included Volckameriana, Rangpur Lime and Rough lemon (commercial orchard also), while the best were M X T, Tri x Sweet orange, Benton citrange, Swingle citrumelo and Carrizo citrange. Lisbon fell in the intermediate range.

Table 6.2.2.8.4. Test sample size and colour, juice percentage (tested 24 June 2002, 13 May 2003 and 12 May 2004), average seed counts and rind thickness of the Genoa lemon on various rootstocks. Rootstocks arranged according to descending juice percentage in 2004.

Rootstock	Sample Count	Sample colour	Juice %			Average seed per fruit	Rind thickness (mm)
	2004	2004	2002	2003	2004	2004	2004
Benton citrange	113	5-6	47.4	45.4	49.0	6.1	4.4
Rangpur Lime	100	6	46.7	47.0	48.3	5.3	4.8
Trifoliolate X	113	5-6	42.9	44.3	47.9	10.5	4.5
Volckameriana	100	6	45.2	43.0	47.6	9.2	5.1
Japanese Citroen	113	5-6	46.6	36.7	47.5	8.8	5.0
Minneola x trifol	138	5-6	45.0	43.0	47.3	11.3	4.2
Carrizo citrange	113	5-6	44.4		47.3	8.4	4.4
Swingle citrumel	138	5-6	45.0	43.8	46.5	9.2	3.9
Tri x Sweet oran	113	6	46.3	46.6	46.0	11.0	4.6
Cairn Rough lem	113	6	46.0	45.2	45.6	8.1	4.7
Genoa/RL comm	100	6			44.9	9.5	5.3
Lisbon/RL comm	113	5-6			46.0	11.2	4.5

All rootstocks easily met the minimum juice export standards of 40% with Rough lemon the lowest. There is overall a slight improvement on last season juice percentages.

Table 6.2.2.8.5. Analyses of high shoulders, Oleocellosis and wind scars of Genoa lemons on various rootstocks. Rootstocks arranged from least to most high shoulders.

Rootstock	High Shoulders					Oleocellosis					Wind scars						
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	5	6
Japanse Sit	45	33	17	5		71	18	9		2	87	10	1	1	1		
Volckamer	41	35	19	5		84	12	2	2		79	11	10				
Swingle citru	36	40	19	5		82	15	3			81	18	1				
Tri x Sweet	34	36	25	5		76	15	9			80	16	4				
Minn X trifol	33	42	18	7		83	16			1	80	17	3				
Rangpur Lim	33	37	26	4		66	24	7	3		80	18	2				
Benton citr	28	46	22	4		88	8	4			80	15	5				
Trifoliata X	15	55	25	5		95	5				80	10	10				
Carrizo citra	36	32	12	14	6	72	16	10		2	68	24	6	2			
Cairn RL	21	38	24	14	3	88	8	3	1		71	15	6	3	2	1	2
Genoa/RL	37	33	15	15		66	22	9	3		78	16	5	1			
Lisbon/RL	63	22	12	3		71	23	5	1		89	10	1				

There was little difference in high shoulders except for Carrizo and Rough lemon, nearly all selections all exportable. There was some oleocellosis (trees slightly wet on sampling) on all rootstocks, the worst Carrizo and the commercial Genoa, most rootstocks all exportable. Cairn RL had the highest incidence of wind scars, all the others all exportable.

Evaluations are based on Outspan Colour Prints for Blemish and Appearance standards. High Shoulders (Set 39) and oleocellosis (Set 28), prints 0 - 3 are exportable: Wind Scars (Set 8), prints 0 - 4 exportable. Botrytis (Set 43): There was a considerable amount of Botrytis on the fruit varying between 29-46%. Rough lemon had the coarsest fruit, the others with the occasional slightly coarse fruit. The smoothest were Swingle, M X T and Trifoliata x Sweet orange. The Lisbon was not as attractive with possibly slightly flatter stylar ends.

Conclusion

This was the third year of production, with an increase in yield over last season, although disappointing. Unfortunately the trees are variable in size and only the best used for evaluation purposes. Rough lemon and Volckameriana had the largest tree and fruit size. M X T, Tri x Sweet, Benton, Swingle and Carrizo had the most desirable fruit size. All rootstocks easily met the juice standards, a slight improvement on last season, Benton and Rangpur the highest, Rough lemon the lowest. Most of the fruit had similar rind colour, M X T and Trifoliata X slightly earlier. Carrizo and Rough lemon had a few non exportable fruit due to high shoulders, Carrizo, Japanse Citroen, Rangpur and Tri x Sweet the most oleocellosis. Rough lemon had the highest incidence of wind scars, all the others all exportable. Rough lemon had the coarsest fruit, Swingle, M X T and Trifoliata x Sweet orange the smoothest. Most had fairly smooth bud unions, except Rangpur and Japanse Citroen which had nodules and the latter also sucker growth. Due to the relatively poor yields probable differences in characteristics between the various rootstocks are not so apparent.

Future research

Continue evaluations for another two seasons.

6.2.2.9 Establishment of new and evaluation of existing cultivars at Lancewood, Knysna area Experiment CJA-1 by C J Alexander (Private)

Opsomming

Die doel van die proef is om nuwe, belowende kultivars in die Knysna area te vestig asook om die huidige proewe te evalueer. Geen nuwe kultivars was in die area gevestig nie weens besnoeiing van die begroting en dat die ITSG kultivars nie vrygestel was nie. Die proef op Karatara is beter bestuur en van die bome het begin dra maar ongelukkig weens ontydige reën, was die interne gehalte vrugte swak. Die Ueno word later as die Miho Wase ryp. Die Mor, Or en Nectar het geen of te min vrugte gehad om te evalueer. Die Afourer het min vrugte gedra met relatief klein vruggrootte en redelike gehalte, maar vrunk. Bay Gold se vruggrootte was goed, maar swak gehalte met bruin kolle op die skil. Sweet Spring op Rheenendal het goeie produksie, vruggrootte en toetse gehad, maar vaal skil kleur en min geur. Nouvelle en Temple het ook min geur gehad.

en laasgenoemde 'n gladder skil as ander gebiede. Die laat Clementines het geen vrugte gedra. Evaluasies moet vir nog een jaar voortgaan.

Introduction

The Knysna area produces mandarins for export, but due to climatic constraints the cultivar range is restricted. The aim is to find suitable, high quality, especially late maturing soft citrus cultivars for the area by evaluating existing trials and establishing new, potential selections.

Materials and methods

The trial trees are topworked within a commercial block of Miho Wase satsumas, (used as control) at Karatara and other trees at Rheenendal and Ruigtevlei. Field evaluations were conducted on the trees and fruit maturity based on subjective tasting. The selections and sites evaluated are presented in Table 6.2.2.9.1 and internal quality tests in Table 6.2.2.9.2.

Results and discussion

Evaluations

Some of the trees were evaluated on the 26 April and all on 23-24 June. The trees at Lancewood were in a poor condition but have since been better managed and are now coming into production.

Table 6.2.2.9.1. Cultivar trial sites evaluated during 2004.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Ueno	Karatara	Lancewood	1998	TC	2 topwork
Miho Wase (control)	Karatara	Lancewood	1996	TC	commercial
Mor 22	Karatara	Lancewood	1998	TC	4 topwork
Or 2	Karatara	Lancewood	1998	TC	4 topwork
Afourer B64	Karatara	Lancewood	1998	TC	2 topwork
Bay Gold	Karatara	Lancewood	1998	TC	4 topwork
Nectar	Karatara	Lancewood	1998	TC	5 topwork
Aoshima	Rheenendal	Candlewood	2003		5
Bay Gold	Rheenendal	Candlewood	2003	TC	5
Sweet Spring	Rheenendal	Candlewood	1995	TC	9
Nouvelle	Rheenendal	Candlewood		SC	7 topwork
CELL	Ruigtevlei	Rushmere	1996	X639	5
Clementarde	Ruigtevlei	Rushmere	1996	RL/X639	1, 4
Clemlate	Ruigtevlei	Rushmere	1988	X639	4
Thoro Temple	Ruigtevlei	Rushmere	1996	RL	15

The Miho Wase and trial trees at Lancewood received excessive water from January (irrigation and then rain) resulting in puffiness and poor quality fruit overall.

Ueno

The trees had a good crop of small to medium-large fruit size, colour T7 on 26 April, T2-4 for the remaining fruit on 22 June. The eating quality was poor on both dates, lacking sugar and acid, overmature and slightly puffy in June. Adjacent Miho Wase had a good crop (partially picked), medium fruit size and T1-4 in April, also poor quality and starting to go puffy.

Fruit shape was flat with a smooth rind, some seed, mainly seedless to up to 6 seeds/fruit (mixed block), easily peeled with open cores and excellent orange coloured flesh.

Mor 22

The trees are tall with odd fruit of very small to small fruit size, colour T5-6 on 22 June. The fruit had high acid (higher than Afourer) and probably high sugars and raggy. Maturity from about mid July. No test was done. The fruit had a flat shape, smooth but ribbed rind and odd navel end split. Peelability was difficult and oily, orange flesh and odd seed.

Or 2

The trees are tall with no fruit.

Afourer (B64)

There were only odd fruit of medium small fruit size, colour T4-5 on 22 June. The quality was fair with fairly good sugars but acid still a bit high. Maturity in about two weeks depending on the acid level. The fruit has a flat shape with a smooth rind, fairly easily peeled with orange flesh, closed cores and some seed.

Bay Gold

The trees at Lancewood had a poor to fair crop of medium to medium-large fruit size and some late set fruit. Fruit colour was T7 on 26 April, varied between trees from T2-4 on 22 June, developing a deep orange colour. The sugars were probably good but acid high and some fruit dry. Probably 2-3 weeks short of full maturity. Except for the excessive acid, the test was good. There were odd seed. In April the fruit had some brown blotches and or a brown ring on the styler end.

The very young trees at Candlewood had odd fruit of good fruit size, variable colour, T4-7 on 22 June. The taste was reasonable with relatively low acid, possibly 1-2 weeks short of peak maturity.

Fruit shape is flattish to obovoid, some large nipples and a smooth rind. There were the occasional split fruit in June. Peelability was difficult, slightly oily and the rind breaking up into pieces. The flesh colour was orange with slightly open to open cores.

Nectar

There were only a few out of season fruit.

Aoshima

Trees not yet in production.

Sweet Spring

The trees had a fair crop, far more fruit on the northern side, with medium-large to large fruit size. The fruit colour was mainly T4-5 and some T6 on 22 June but with a yellow rind. Quality was fair, not too sweet and lowish acid, lacking any real strong flavour and raggy. Maturity estimated in July. The test was good. The fruit shape is fairly flat to slightly obovoid with large pebbles on a smooth textured rind. Peelability was fair to difficult and very oily, good orange flesh, closed cores and few seeds to seedless.

Nouvelle

The young topwork trees had odd fruit of medium-large fruit size, colour T8 on 22 June. Quality was fair with fair sugars but low acid, lacking flavour. The fruit shape was round with a fine pebbly rind and an excellent orange, fine textured flesh.

Thoro Temple

The trees had a poor to good crop of good, medium to medium-large fruit size, colour T2-4 on 23 June. There were odd out of season fruit. The quality was fair, lacking flavour, sugars not so high but fairly high acid, probably mature in July. The test just met Scarlet standards (borderline TSS). There were some seed (on the edge of a Clementine orchard), adjacent Clementines 0-1 seed/fruit. The fruit shape was fairly flat with a relatively smooth rind, smoother than in other areas. This has been recorded previously. The fruit peels fairly easily with the thin rind breaking up and very oily. The flesh colour is orange with cores closed to just starting to open.

CELL, Clementarde and Clemlate

All three selections had dense trees, poor crops and out of season fruit and could not be evaluated.

Table 6.2.2.9.2. Internal fruit quality data for various satsuma and mandarin selections for the Knysna area during 2004.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed
Ueno	TC	Lancewood	22/06	3-4	2	46.7	8.2	0.68	12.1	1.5
Bay Gold	TC	Lancewood	22/06	3-4	1	57.8	11.7	1.91	6.1	4.7
Sweet Spring	TC	Candlewood	22/06	4-5	1XX	55.2	10.5	0.86	12.2	1.7
Thoro Temple	RL	Rushmere	23/06	1-3	1X	60.3	9.5	1.16	8.2	1.3

Establishment

No new selections were established in the area. Besides the fact that the budget for the area was cut drastically, the new selections to be established were primarily ITSC selections. None of these selections were released and could therefore not be established for trial purposes.

Conclusion

Ueno

The trees had a good crop of variable fruit size. Colour was much later than Miho Wase. The quality, like Miho Wase was poor, probably due to excess water and there was some seed. No conclusions can be drawn and further evaluations are necessary.

Mor 22

As there were too few fruit to evaluate properly, no conclusions could be drawn and further evaluations are necessary.

Or 2

There were no fruit to evaluate. Evaluate next season.

Afourer (B64)

There were only a few fruit of relatively small size and reasonable quality although still tart in late June. Evaluations to continue.

Bay Gold

Taking into account the tree condition, the yield was acceptable with good fruit size and high acid. There were brown marks on the fruit. Further evaluations necessary.

Nectar

The trees were not yet in production and need to be evaluated.

Aoshima

The trees were not yet in production and need to be evaluated.

Sweet Spring

The trees carried a fair crop of good fruit size but pale rind colour. Eating quality was poor but the test good. Although the fruit has some good characteristics the lack of flavour and poor colour are probably not acceptable to the overseas market.

Nouvelle

There were too few fruit to draw any conclusions. Further evaluations are necessary.

Thoro Temple

The trees had a reasonable crop of good fruit size, acceptable tests but lacking flavour. The rind was smoother than in other areas.

CELL, Clementarde and Clemlate

Not evaluated due to lack of fruit and out of season fruit.

No new selections were established in the area.

Future research

Evaluate cultivars at the existing sites for one more season.

6.2.2.10 **Evaluation of Turkey Valencias on different rootstocks**
Experiment CJA - 4 by C J Alexander (Private)

Opsomming

Die doel van die proef is om uit te vind wat die mees geskikte onderstam vir die Turkey Valencia in die Citrusdal gebied is. Die bome was verlede seisoen deur ryp beskadig en het nog nie volkome herstel nie. Interne gehalte was oor die algemeen nie so goed nie weens die relatief hoër suur vlakke. Van die onderstamme het suier lote op die onderstam gehad. Rangpurlemmetjie lyk nie baie belowend as 'n

onderstam nie en Growweskiisuurlemoen het onverenigbaarheidsimptome gewys. Hulle moet dus nie as 'n onderstam vir Turkey Valencia gebruik word nie. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate the early maturing Turkey Valencia on different rootstocks to establish which is the most suitable rootstock for the Citrusdal area as incompatibility problems have been encountered on Rough lemon.

Materials and methods

The trees were planted in a block on 7 different rootstocks in September 1999. Each block is in an adjacent block of four rows. Rootstocks (selections) used and number of trees of each selection are given in Table 6.2.2.10.1 below. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following Valencia standards previously considered most acceptable by the market: 48% juice; 9.0% TSS; 0.6 – 1.8% acid; 7.0:1 ratio; colour T3 + 20% T4 of set 34. Seed maximum average 9.0 seeds per fruit.

Table 6.2.2.10.1. Rootstocks (selections) and number of trees per selection of Turkey Valencias on 7 different rootstocks planted at Sewe Oliene, Citrusdal in September, 1999.

Rootstock	Selection	No of trees
Cairn Rough Lemon	163	25
Carrizo citrange	608	26
Citrango C32		10
Citrumelo F 80.0		6
Rangpur Lime	184	26
Swingle citrumelo	715	13
1209		22

Results and Discussion

The internal quality results are presented in Table 6.2.2.10.3 which need to be referred to when reading the text. The orchard suffered unusually severe frost during 2003 causing damage to the trees and fruit and light frost in June 2004. Tree size, yield, fruit size and bud union bench is of the various rootstocks is presented in Table 6.2.2.10.2 and internal quality in Table 6.2.2.10.3 below.

Table 6.2.2.10.2. Tree size, yield, fruit size and bud union bench of Turkey Valencias on seven different rootstocks evaluated on 20 July 2004 at Sewe Oliene, Citrusdal.

Rootstock	Tree size	Yield	Fruit size	Bud union bench
Cairn Rough Lemon	Medium-large	Poor – fair	Medium-large - large	Lump at bud union with line under bark. Sucker growth. Suspect
Carrizo citrange	Medium-medium-large, variable	Poor – fair	Medium-large	Typical, slight. Fluted. No incompatibility observed under bark
Citrango C32	Medium small	Fair	Medium – medium-large	Slight bench, smooth
Citrumelo F 80.8	Medium	Poor – good	Medium – medium-large	Fairly smooth, odd sucker
Rangpur Lime	Medium small	Poor - good, variable	Medium – medium-large	Pale colour bark, rough, shoots and pitted. Slightly inverted. No brown ring. Suspect.
Swingle citrumelo	Medium	Fair	Medium-large, slightly variable	Slight bench, odd suckers
1209	Small	Zero		Slight

Table 6.2.2.10.3. Internal fruit quality data for Turkey Valencia oranges on different rootstocks at Sewe Oliene, Citrusdal on 20 July 2004.

Rootstock	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Cairn Rough Lemon	1	56	50.8	9.2	1.43	6.4	4.6
Carrizo citrange	1	56	55.1	11.6	1.50	7.7	4.1
Citrango C32	1	64	52.8	11.7	1.75	6.7	1.8
Citrumelo F 80.8	1	72	51.7	11.5	1.73	6.6	5.7
Rangpur Lime	1	56	50.8	9.4	1.45	6.5	3.4
Swingle citrumelo	1	56	52.5	11.9	1.67	7.1	5.3

Only trees on Carrizo met Midnight standards (except for seed) and Swingle Valencia standards, the rest failing on ratio due to fairly high acid. Rough lemon and Rangpur had much lower TSS than the rest which were good.

Trees on Rough lemon had a softer fruit with a slightly thick to thick rind with tinges of green on the stem end, Rangpur a slightly thick and coarser rind, Carrizo slightly thick and C32 variable with F80.0 the smoothest. C32 had fine flesh while Rough lemon, C32 and F80.0 picked fairly easily. No oleo was observed in any of the fruit.

Cause for concern are Rough lemon and Rangpur Lime and observations of the latter are consistent with with older trees at Hexrivier.

Conclusion

The trees are still recovering from previous frost damage and are not yet in full production. Fruit quality on most of the various rootstocks was not yet good enough as the acid was still fairly high. Rough lemon, F 80.0, Rangpur Lime and Swingle citrumelo had some sucker growth on the rootstock. Rangpur Lime does not look good as a rootstock for Turkey and Rough lemon shows signs of incompatibility. These two rootstocks should be avoided as rootstocks for Turkey Valencia.

Future research

Continue evaluations to get more clarity as to the most suitable rootstock.

6.2.2.11 Establishment of new cultivar trials

Experiment CJA-3 by C J Alexander (Private)

Opsomming

Die doel van die proef is om nuwe, potensiële kultivars in die veld te vestig. Sodra die bome in drag is, evalueer, versamel en versprei die inligting. Persele in die Oos en Wes-Kaap is geïdentifiseer en dertig seleksies is tans besig om geënt of oorgewerk te word.

Introduction

The objective of the project is to establish new, potential cultivars in the field. Once in production, gather data and disseminate information to ensure that the local citrus industry keeps abreast with local performance of any new, superior or alternative cultivars compared to the existing cultivar range.

Materials and methods

Go through the lists of cultivars available in South Africa and determine what new cultivars/selections are suitable to be established in the Cape areas. Establish at suitable sites by means of planting budded trees or topworking existing trees. Five trees of each selection per cultivar to be budded or topworked next to each other with a suitable control.

Results and discussion

The following selections have been organised and are in the process of being budded or to be topworked at sites in the different areas.

Cultivar	Selection	Area
Mandarin hybrids	MH7 Clara Tacle	Fort Beaufort, Citrusdal Adelaide, Wolseley, Citrusdal, Clanwilliam Adelaide, Wolseley, Citrusdal, Clanwilliam
Early navels	Krajewski Early Letaba Early Dream Atwood Fukumoto Cliff Early	Adelaide, Heidelberg, Citrusdal Adelaide, Citrusdal Citrusdal Citrusdal Adelaide Adelaide
Midseason navels	Kirkwood Red Santa Catarina 1 Santa Catarina 2	Adelaide, Citrusdal Adelaide Adelaide
Late navels	Glen Ora Late Witkrans Cambria Royal Late Rhode Summer Coetzee Late	Sunland, Fort Beaufort Fort Beaufort Sunland, Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort
Midseasons	Tarocco Scire Tarocco Scire N Tarocco Tapi	Adelaide, Ashton Adelaide, Ashton Adelaide, Ashton
Early Valencias	Limpopo Seedless Benny 1 or Benny 2	Fort Beaufort Fort Beaufort
Midseason Valencias	Ruby Valencia Rietspruit (Alpha) Nouvelle Le Cotte Jassie Skilderkrans Portsgate G5	Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort
Late Valencias	Glenora Soetbas Henrietta Letaba Oranje Midknight (1) Moosrivier 1 Moosrivier 2 Bend 8A1 Bend 8A2	Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort Fort Beaufort

Conclusion

Trial sites have been determined and the trees are to be budded or topworked in 2005.

Future research

Continue with establishment of new selections that become available.

6.2.2.12 Navel Rootstock trial

Experiment CJA-2 by C J Alexander (Private)

Opsomming

Die doel is om 'n nawel onderstam proef te vestig om te bepaal watter onderstam die mees geskikte is vir optimale produksie en gehalte in 'n nawel area aangesien daar 'n toename in nawel aanplantings is. 'n Perseel is uiteindelik in Citrusdal gevind en saad sal in 2005 geplant word.

Introduction

The objective of the project is to establish a navel rootstock trial in order to determine which rootstock is the most suitable for optimum navel production and quality in a navel producing area. There is a resurgence in navel plantings in the Citrusdal area.

Materials and methods

Go through the lists of rootstocks available and suitable for navel production. Procure seed and have a nursery bud Washington navel to the rootstocks. Plant the trees in a random block design of 3 x 4 tree plots, including a commercially planted rootstock as a control.

Results and discussion

Research reports were scrutinised for potential rootstocks to be established. A list of suitable rootstocks has been drawn up and a grower in the Citrusdal area has agreed to plant the trial. Seed, depending on availability, will be planted out in 2005 and rootstocks budded in 2006 for later planting out.

Conclusion

A trial site has finally been found and seed should be planted out in 2005.

Future research

Continue with establishment and evaluation of the trial once in production.

6.2.3 Sub-Project: Cultivar evaluation in the Northern and inland region

By J. Joubert (CRI)

6.2.3.1 Sub-Project summary

The primary focus of the Cultivar evaluation project is to procure, evaluate, report on and make cultivar recommendations for the South African Citrus Industry.

In the 2004 season there were severe drought conditions in most of the main production areas causing problems. This scenario had a major influence on the internal quality of the fruit in regards to TSS and Acid percentages. Assumptions can be made regarding the tolerance of drought conditions to specific cultivars and rootstocks, as well as the best rootstock-scion combination. On average there were less fruit on the trees with large fruit size. Thank you to all the growers for their assistance with all the trials conducted on behalf of CRI.

Sub-Projekopsomming

Die hoofdoel van die Kultivar -evaluasiëprojek is om kultivars te vestig, te evalueer, verslag te lewer en aanbevelings vir die Suid-Afrikaanse Sitrusbedryf te maak.

Die 2004 seisoen het verskeie probleme opgelewer met die ernstige droogtes wat in die verskillende produksie areas voorgekom het. Hierdie senario het sekere interne kwaliteitse beïnvloed t.o.v. TOV en Suur persentasies. Goeie aflydings kan hieruit gemaak word aangaande kultivars en onderstamme wat moontlik meer weerstandbiedend is teen droogtes en verskillende bo-en onderstam kombinasies. Daar is oor die algemeen minder vrugte op die bome geproduseer wat die vruggroote in meeste gevalle laat vergroot het. Baie dankie vir al die produsente se ondersteuning namens die CRI waar die verskillende proewe aangetref word.

6.2.3.2 Evaluation of Clementine mandarins in Mpumalanga

Experiment 72 by J. Joubert (CRI)

Opsomming

'n Proef is saamgestel om te bepaal of sekere Clementine manderyne kommersieel vir uitvoer in die intermediaire en koel binnelandse sitrusproduserende streke van die land in reaksie op markbehoefte, geproduseer kan word. Daar word gesoek na uitstaande seleksies met betrekking tot interne vruggehalte, eksterne vrugkleur en vruggroote verspreiding.

Ain Toujdate en Sidi Aissa kan die vroegste ge-oes word, gevolg deur Nour. Clementarde en LL sal van die later kultivars wees om te oes, maar die eksterne kleur kan deur ontgroening aangehelp word. Die beste produksie op die bome het voorgekom by Ain Toujdate en Nour. Dit gee ook 'n moontlike aanduiding van meer weerstandbiedendheid teen Roodidopluis as die ander seleksies. Alle bome is blootgestel aan dieselfde hoeveelheid Roodidopluis besmetting in die boord. Die hoeveelheid sade per vrug het oor die algemeen heelwat afgeneem teenoor die 2003 seisoen.

Introduction

A trial was laid out to ascertain whether certain Clementine mandarins could be produced commercially for export in the intermediate and cool inland citrus production areas of the country in accordance with market needs, as well as finding superior selections in terms of internal fruit quality, colour and fruit size.

Materials and methods

Field evaluations and laboratory analysis were conducted on Ain toujdate, Clementarde, L.L., Nour and Sidi Aissa Clementine selections (Table 6.2.3.2.1).

The minimum export requirements for the internal fruit quality of Clementines (Capespan) was compared during the 2004 season: 48% Juice; 9.5% TSS; 0.7% Acid (Min); 1.5% Acid (Max) Ratio 8.0:1; Colour T2 and 20% T3 of set 36.

Table 6.2.3.2.1. List of Clementine trial sites evaluated in Burgersfort area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Ain Toujdate	Mpumalanga	Zalo Sitrus	CC, SC	1999	5 each
Clementarde	Mpumalanga	Zalo Sitrus	CC, SC	1999	5 each
LL	Mpumalanga	Zalo Sitrus	CC, SC	1999	5 each
Nour	Mpumalanga	Zalo Sitrus	SC	1999	5 each
Primasole	Mpumalanga	Zalo Sitrus	CC	1999	5 each
Sidi Aissa	Mpumalanga	Zalo Sitrus	CC, SC	1999	5 each

Results and discussion

Ain Toujdate - Trees were evaluated at L Lötter, Burgersfort during the 2004 season

The average number of seeds per fruit decreased in comparison to the 2003 season (number dropped by at least two thirds). The yield varied from good to very good, with average fruit size between count 1XX and 4 (slightly larger fruit on Swingle citrumelo). The TSS% decreased slightly on Carrizo citrange when compared to the 2003 season, but was still above the minimum requirements for export standards. The fruit had a good internal colour (orange) and a sweet taste. On average the internal quality with both rootstocks tested well and the Juice% was quite high. There was a Red Scale problem in the trial orchard. It will be treated properly with Confidor for the 2005 season. Maturity is at the end of April.

Clementarde - Trees were evaluated at L Lötter, Burgersfort during the 2004 season

Clementarde had the highest Acid% when tested for internal quality of all the Clementine mandarins in the trial. The external colour of the fruit started at colour plate T7. When the fruit was internally ready to harvest the external colour was still on the green side at T4-6. The TSS% on Swingle citrumelo tested slightly higher than the 2003 season. Number of seeds per fruit remained on average the same. Fruit production on Swingle citrumelo was very good, but on Carrizo citrange the yield varied between the different trees from poor to good. Ready to harvest internally middle to end of May, but would have to de-green for external colour.

L.L.

Trees were evaluated at L Lötter, Burgersfort during the 2004 season. Good yield on both Carrizo citrange and Swingle citrumelo. Small to medium fruit size on average, with the exception of the evaluation done on the 6th of April (1XX). External colour of the fruit was between that of Ain Toujdate and Clementarde when ready to harvest (colour plate T3-4). On both rootstocks the fruit had a sweet but watery taste. The external colour of the fruit on Swingle citrumelo was more yellow compared to the fruit on Carrizo citrange. Number of seeds per fruit was the highest for all the Clementines evaluated in this trial. TSS% on Carrizo citrange was higher when compared to the 2003 season. Maturity was estimated between the third and last week of May.

Nour

Trees were evaluated at L Lötter, Burgersfort during the 2004 season. Fruit peels easily and the fruit size varies between small to medium. The average number of seeds per fruit increased slightly from 2003. Production per tree was very good with good internal quality and sweet taste. The TSS% increased from 13,99% (2003) to 16,59% in the 2004 season. Acid% slightly on the high side, but still acceptable within the export standards. Maturity was estimated at the middle of May and ready to harvest between Ain Toujdate and Clementarde.

Primasole

Trees were evaluated at L Lötter, Burgersfort during the 2004 season. Not enough fruit to evaluate. Will continue in the 2005 season.

Sidi Aissa

Trees were evaluated at L Lötter, Burgersfort during the 2004 season. The yield on the trees varied from good on CC to poor on SC. The Red Scale problem was very severe on SC rootstock. TSS% decreased slightly on SC, but the average seeds per fruit decreased drastically from 20.7 to 3.73 seeds per fruit. Average fruit size between medium and large fruit (1XX – 3). The Acid% was slightly lower than in the 2003 season, but bear in mind the severe drought in the production areas. Maturity was estimated at the end of April and ready to harvest with Ain Toujdate.

Conclusion and recommendations

On average the number of seeds per fruit on all the cultivars decreased, with Sidi Aissa on SC the most phenomenal decrease. The Red Scale problem will be treated with Confidor for the 2005 season to prevent additional fruit waste on the trees. This will also affect the evaluation on the average yield produced on the trees. Ain Toujdate and Nour produced the best yield per tree with good internal quality. Ain Toujdate and Sidi Aissa can be harvested first followed by Nour and then Clementarde and LL. The highest Acid% for the season was produced with Clementarde on SC, but still acceptable.

Table 6.2.3.2.2. Internal fruit quality data for Clementine mandarin selections for the inland areas during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Ain Toujdate	CC	06/04/2004	Zalo Citrus	1X-3	52.8	12.44	0.83	14.99	5.3	T3-4
Ain Toujdate	CC	05/05/2004	Zalo Citrus	1X-3	53.4	13.40	0.89	15.06	2.3	T1-2
Ain Toujdate	CC	18/05/2004	Zalo Citrus	1-3	50.8	13.94	0.98	14.22	1.4	T1
Ain Toujdate	SC	06/04/2004	Zalo Citrus	1XX-3	52.7	13.06	0.96	13.60	2.8	T2-3
Ain Toujdate	SC	05/05/2004	Zalo Citrus	1-4	55.7	14.91	1.10	13.55	2.3	T1-2
Ain Toujdate	SC	18/05/2004	Zalo Citrus	1-3	54.3	15.98	1.20	13.32	1.5	T1-2
Clementarde	CC	06/04/2004	Zalo Citrus	2-5	53.2	13.26	1.41	9.40	1.2	T7-8
Clementarde	CC	05/05/2004	Zalo Citrus	1-3	55.8	14.44	0.97	14.89	3.1	T4-5
Clementarde	CC	18/05/2004	Zalo Citrus	2-3	57.0	14.91	1.01	14.76	0.5	T5-6
Clementarde	SC	06/04/2004	Zalo Citrus	3-5	52.0	14.22	1.44	9.88	1.8	T7
Clementarde	SC	05/05/2004	Zalo Citrus	2-4	57.8	15.31	1.07	14.31	0.0	T4-5
Clementarde	SC	18/05/2004	Zalo Citrus	2-4	57.8	16.58	1.19	13.93	1.0	T4-5
LL	CC	06/04/2004	Zalo Citrus	2-4	52.8	14.41	1.08	13.34	5.6	T5
LL	CC	05/05/2004	Zalo Citrus	1-3	51.7	15.61	1.05	14.87	6.2	T2-3
LL	CC	18/05/2004	Zalo Citrus	2-4	56.6	16.48	1.10	14.98	1.6	T2-4
LL	SC	06/04/2004	Zalo Citrus	1XX-3	49.6	14.11	0.99	14.25	11.6	T6
LL	SC	05/05/2004	Zalo Citrus	1-4	55.9	15.01	0.97	15.47	1.6	T4-5
LL	SC	18/05/2004	Zalo Citrus	2-4	57.9	15.61	1.10	14.19	0.8	T3-4
Nour	SC	06/04/2004	Zalo Citrus	3-5	52.2	14.61	1.23	11.88	4.0	T6-7
Nour	SC	05/05/2004	Zalo Citrus	1X-4	52.5	15.71	1.09	14.41	4.4	T1-2
Nour	SC	18/05/2004	Zalo Citrus	2-4	50.5	16.59	1.17	14.18	3.3	T3-4
Sidi Aissa	CC	06/04/2004	Zalo Citrus	1X-2	51.7	12.64	0.87	14.53	4.4	T3-4
Sidi Aissa	CC	05/05/2004	Zalo Citrus	1X-2	53.7	12.58	0.89	14.13	1.3	T1-2
Sidi Aissa	CC	18/05/2004	Zalo Citrus	1XX-2	52.1	13.29	0.96	13.84	2.8	T1-2
Sidi Aissa	SC	06/04/2004	Zalo Citrus	1X-2	46.4	13.46	0.94	14.32	7.8	T2-3

Sidi Aissa	SC	05/05/2004	Zalo Citrus	1X-3	54.2	14.04	0.99	14.18	1.8	T1-2
Sidi Aissa	SC	18/05/2004	Zalo Citrus	1-4	52.6	15.41	1.19	12.95	1.6	T1-2

6.2.3.3 Evaluation of late maturing Mandarins in the inland areas

Experiment 73 by J.Joubert (CRI)

Opsomming

Gesikhte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul.

Hou in gedagte dat Moosrivier Landgoed (Marble Hall) 'n baie ernstige droogte beleef het gedurende die 2003 seisoen. Die vruggrootte het egter toegeneem a.g.v. die ligter oes op die bome. By Zalo Citrus (Burgersfort) was daar nie so 'n groot droogte impak nie. Die bome is nog relatief jonk en behalwe vir Bay Gold en Hadas, dra al die ander seleksies vir die eerste seisoen vrugte vir evaluasies. Hadas toon baie hoë suur persentasies vanaf die eerste evaluasies tot en met oes. Die eksterne kleur van die vrugte kleur ook stadig op met 'n redelike vertraging (T5-7). Evaluasies sal herhaal word vir die 2005 seisoen.

Introduction

The objective is to find suitable mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

Materials and methods

Field evaluations were conducted. Internal fruit analysis was conducted for Burgersfort and Marble Hall area during the 2004 season (Table 6.2.3.3.1).

The minimum export requirements for the internal fruit quality of Mandaryns (Capespan) was compared during the 2004 season: 48% Juice; 9.5% TSS; 0.7% Acid (Min); 1.5% Acid (Max) Ratio 8.0:1; Colour T2 and 20% T3 of set 36.

Table 6.2.3.3.1. List of Mandarin trial sites evaluated during the 2004 season.

Selection	Area	Site	Rootstock	Tree age	No. of trees
A25	Mpumalanga	Moosrivier Estate	CC	2001	10
Bay Gold	Mpumalanga	Moosrivier Estate	CC	2001	9
Cami	Mpumalanga	Moosrivier Estate	CC	2001	5
C27	Mpumalanga	Moosrivier Estate	CC	2001	5
Hadas	Mpumalanga	Moosrivier Estate	CC	2001	9
M26	Mpumalanga	Moosrivier Estate	CC	2001	10
Primasole	Mpumalanga	Moosrivier Estate	CC	2001	9
Roma	Mpumalanga	Moosrivier Estate	CC	2001	10
A25	Mpumalanga	Zalo Citrus	CC	2001	5
Bay Gold	Mpumalanga	Zalo Citrus	CC	2001	5
Cami	Mpumalanga	Zalo Citrus	CC	2001	4
C27	Mpumalanga	Zalo Citrus	CC	2001	4
Hadas	Mpumalanga	Zalo Citrus	CC	2001	5
M26	Mpumalanga	Zalo Citrus	CC	2001	5
Primasole	Mpumalanga	Zalo Citrus	CC	2001	5

Results and discussion

A25

Trees were evaluated at Zalo Citrus, Burgersfort during the 2004 season. Trees bore enough fruit to evaluate for the first time since planting in 2001. Fruit size was medium to large (1XX-3) and the TSS% between 14.51% and 15.01% (high). The yield was poor. The Acid% was on the high side (1.52%) and remained high up to the third evaluation. However the external colour was ready by the time of harvest. Maturity seems to be middle to end of May depending on the Acid% of the fruit. Future evaluations will determine characteristics of the selection and possible future for commercial plantings.

Bay Gold

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. On average there was poor yield on the trees with large fruit size (1XXX-1X). Production at Zalo Citrus was slightly better than Moosrivier Estates, severe drought at the latter. Average seed per fruit produced decreased from 19.8 to 13.3 seeds per fruit at Moosrivier. When cutting the fruit there was a lemon smell arising from the skin oils. There was a lot of granulation. In appearance it is very similar to a Minneola type of fruit and peels easily. This seems like a later maturing selection probably ready to harvest at the end of May to beginning of June because of slightly higher Acid% and delay in external colour.

Cami

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. There was an average to good yield on most of the trees evaluated, but at Zalo Citrus there was alternative bearing between the trees. Fruit size varied from medium to large (1XXX-3) with a very smooth skin texture. The trees bear most of the fruit on the end of the branches, causing the branches to hang close to the ground. Acid% seems to be on the high side, but drops sufficiently closer to harvest time. The average seed count is high and varies between 14.3 and 25.8. Maturity seems to be at the end of May.

Hadas

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. The average fruit size varied from medium to large (1XXX-3). The internal quality was acceptable, besides the very high Acid% between 2.03% and 2.94%. The external colour was delayed (T5) and the number of seeds per fruit varied between 8 and 13.8. Production per tree was poor to average. Maturity might from be middle to end of June.

M26

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. The trees produced fruit between large to extra large size (1XXX-1) with a high Juice% of between 58.7% and 65.5%. The number of seeds per fruit at Moosrivier was higher (8.4-15.3) compared to Zalo Citrus (0.3-0.8). M26 produced good internal colour (orange) and acceptable quality. Maturity middle to end of May.

Roma

Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2004 season. Roma produced poor due to severe drought conditions. Peelability is slightly difficult due to the rather thin rind. The fruit size was medium and varied between 1XX and 2. The seed count was between 13.4 and 16.6. Maturity at the end of May.

Conclusion and recommendations

Most of the selections evaluated in this trial are young trees and bearing fruit for the first time. More evaluations must be completed before recommendations can be made. Bay Gold, Hadas and M26 produced fairly large fruit. Hadas was evaluated for the second time and the internal quality results again showed very high Acid% (2.03%-2.94%). M26 produced high Juice% on average with acceptable TSS% and Acid%.

Table 6.2.3.3.2. Internal fruit quality data for Mandarin selections for the cool inland areas during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
A25	CC	06/04/2004	Zalo Citrus	1X-3	51.2	14.51	1.86	7.8	7.7	T6
A25	CC	05/05/2004	Zalo citrus	1XX-2	54.2	14.54	1.32	11.0	9.2	T2-3
A25	CC	18/05/2004	Zalo citrus	1XX-3	56.3	15.01	1.39	10.8	2.9	T2-3
Bay Gold	CC	15/04/2004	Moosrivier	1XXX-1X	48.4	9.50	1.25	7.6	10.3	T8
Bay Gold	CC	06/05/2004	Moosrivier	1XX-1XXX	50.0	10.58	0.99	10.7	18.0	T6-7
Bay Gold	CC	20/05/2004	Moosrivier	1XXX-1XX	47.2	10.98	1.19	9.2	11.7	T3-5
Bay Gold	CC	06/04/2004	Zalo citrus	1XXX-1X	49.8	9.91	1.28	7.7	2.2	T6-7
Bay Gold	CC	05/05/2004	Zalo citrus	1XX-1XXX	48.1	10.25	1.21	8.5	1.5	T3,T5
Bay Gold	CC	18/05/2004	Zalo citrus	1XX-1XXX	47.6	10.58	1.19	8.9	1.1	T4-5
Cami	CC	15/04/2004	Moosrivier	1X-3	53.6	9.80	1.41	7.0	14.6	T8
Cami	CC	06/05/2004	Moosrivier	1XXX-2	57.1	11.18	1.29	8.7	18.4	T4-6

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Cami	CC	20/05/2004	Moosrivier	1X-2	54.6	12.08	1.22	9.9	15.3	T3
Cami	CC	06/04/2004	Zalo citrus	1-2	53.2	12.64	1.48	8.5	25.8	T7
Cami	CC	05/05/2004	Zalo citrus	1X-2	52.5	13.20	1.26	10.5	21.9	T3-5
Cami	CC	18/05/2004	Zalo citrus	1XX-2	53.0	13.64	1.19	11.5	14.3	T3
Hadas	CC	15/04/2004	Moosrivier	1XX-3	51.7	8.87	2.94	3.0	10.6	T8
Hadas	CC	06/05/2004	Moosrivier	1X-1	53.3	9.22	2.40	3.8	10.9	T7-8
Hadas	CC	20/05/2004	Moosrivier	1XX-2	53.0	6.65	2.42	2.7	8.0	T6-7
Hadas	CC	06/04/2004	Zalo citrus	1XX-2	54.0	9.27	2.38	3.9	12.3	T7
Hadas	CC	05/05/2004	Zalo citrus	1XXX-1	57.5	10.78	2.04	5.3	13.8	T5
Hadas	CC	18/05/2004	Zalo citrus	1XXX-1	56.9	10.58	2.03	5.2	12.8	T5
M26	CC	15/04/2004	Moosrivier	1XX-1	58.7	9.50	1.04	9.1	15.3	T8
M26	CC	06/05/2004	Moosrivier	1XXX-1	62.6	11.18	1.14	9.8	8.4	T3-6
M26	CC	20/05/2004	Moosrivier	1XXX-1X	62.1	11.68	1.07	10.9	11.2	T3
M26	CC	06/04/2004	Zalo citrus	1XX-2	60.4	10.11	1.15	8.8	0.5	T8
M26	CC	05/05/2004	Zalo citrus	1XX-1	65.5	12.48	1.11	11.2	0.8	T2-3
M26	CC	18/05/2004	Zalo citrus	1XX-1X	63.5	12.17	1.11	11.0	0.3	T3-4
Roma	CC	15/04/2004	Moosrivier	1-2	50.4	9.70	1.16	8.4	13.4	T8
Roma	CC	06/05/2004	Moosrivier	1X-2	49.1	11.28	0.93	12.1	15.8	T5-6
Roma	CC	20/05/2004	Moosrivier	1XX-1	52.1	12.08	0.94	12.9	16.6	T2-3

6.2.3.4 Evaluation of Navels in the intermediate and cool inland areas

Experiment 74 by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, en binnedrag) en vruggehalte (skilkleur vroeg in die seisoen, sappehalte, granulasie, lae suur) te verbeter, asook om die oes- en bemarkingseisoen (vroee-, en laatrypwordende seleksies) te verleng.

Cara Cara het van al die nawels wat in hierdie proef geëvalueer is die beste smaak opgelewer. Die vrugte se interne kleur was baie mooi eenvormig rooi, maar daar moet veral gekyk word na die verskillende klimaatstreke. Die vrugte het genoeg hitte eenhede nodig om die univorme rooi kleur te produseer.

Die Fukumoto bome wat by Zalo Citrus aangeplant is, toon moontlike tekens van onverenigbaarheid by die entlas. Die bome is effens verdwerg en die toestand (spanning) kon bygedra het tot die simptome by die entlas.

Introduction

Objectives are to optimise profitability by improving productivity (fruit set and size); packout percentage (creasing and oleo resistance, smaller navel ends to counter mealybug, *Alternaria* infection, less wind prone – time of flowering and inside bearing), fruit quality (rind colour early in the season, juice quality, granulation, low acidity) and extend the harvest and marketing season (early- and late maturing selections).

Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Autumn Gold, Cara Cara, Chislett, Dream, Fukumoto, Powel Summer and Tuligold selections at two sites in the inland areas.

Internal quality data was compared with the minimum average export requirements (Capespan) for navels : 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

Table 6.2.3.4.1. List of Navel trial sites evaluated during the 2004 season.

Selection	Area	Site	Rootstock	Tree age	No. of trees
Atwood	Mpumalanga	Moosrivier Estate	CC	2001	5
Autumn Gold	Mpumalanga	Moosrivier Estate	CC	1997	6
Cara Cara	Mpumalanga	Moosrivier Estate	CC	1997	Semi-Com.
Chislett	Mpumalanga	Moosrivier Estate	CC	1997	6
Dream	Mpumalanga	Moosrivier Estate	CC	2001	9
Fukumoto	Mpumalanga	Moosrivier Estate	CC	2001	4
Tuligold	Mpumalanga	Moosrivier Estate	CC	2001	9
Atwood	Mpumalanga	Zalo Citrus		2001	5
Autumn Gold	Mpumalanga	Zalo Citrus	CC, SC	1999	10
Bahianinha	Mpumalanga	Zalo Citrus	SC	2001	6
Cara Cara	Mpumalanga	Zalo Citrus		2001	9
Dream	Mpumalanga	Zalo Citrus		2001	9
Fukumoto	Mpumalanga	Zalo Citrus		2001	5
Powel Summer	Mpumalanga	Zalo Citrus	CC, SC	1999	10
Tuligold	Mpumalanga	Zalo Citrus		2001	9

Results and discussion

Atwood

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Trees at Zalo Citrus bore fruit for the first time in 2003. Atwood produced a poor to average yield. The trees planted at Zalo Citrus appear to have a colour advantage (T2 with second evaluation) compared to Moosrivier Estates (T3 with third evaluation). Atwood at Zalo Citrus had no seed compared to Moosrivier Estates with 2.7 seeds per fruit. The Zalo Citrus TSS% (9.65% -11.38%) was higher and Acid% (0.68% - 0.76%) lower compared to Moosrivier Estates TSS% (8.77% - 9.85%) and Acid% (0.83% - 0.87%). The fruit size varied from medium to large (drought conditions at Moosrivier Estates). Maturity estimated at the end of May to the first week in June.

Autumn Gold (Late maturing Navel)

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. The following mainly represents the Autumn Gold at Zalo Citrus (drought at Moosrivier). The yields on the trees were good and the fruit size varied from medium to large (count 40-88). Autumn Gold produced good internal quality with high TSS% and no seed. There was some granulation and a fairly high fibre content. Maturity seems to be in the first week of June.

Bahianinha

Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Bahianinha was top-worked at Zalo Citrus onto SC rootstocks. The yield varied from poor to average with alternative bearing between the trees (first crop) and fruit size varied from small to large. Two evaluations were done with insufficient fruit for a third evaluation. The internal quality was acceptable and no seed found. Maturity might be end of May.

Cara Cara

A semi-commercial orchard was evaluated at Moosrivier Estates (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Cara Cara produced a medium to good yield (drought affected yield at Moosrivier Estate). The fruit started colouring early in the season and matured by the middle to end of May. The internal quality was good with the Acid% slightly on the low side, but still acceptable for export standards. This might be the best tasting navel of all the selections evaluated in the trials. The internal colour pigmentation was an even deep red colour. Cara Cara needs heat units to improve the internal red colour pigmentation, whereas Blood oranges require sufficient cold units to produce their reddish internal colour pigmentation.

Chislett

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2004 season. Chislett produced a very poor yield for the 2003 season because of the drought conditions. Evaluations continued and the internal quality did not meet the export standards with low Acid% and Juice%.

Dream

Trees were evaluated at Moosrivier Estates (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Compared to the 2003 season at Zalo Citrus, Dream produced fruit with slightly low Juice% (46.0-51.6%) and Acid% (0.87%-1.00%), but a high TSS% (11.28%-12.47%). There was a slight delay in rind colour at Moosrivier Estates, but at Zalo Citrus the rind colour met the standards. The opposite happened during 2003. Fruit production per tree varied from poor (Moosrivier) to good (Zalo) bearing in mind the drought conditions. The fruit size at Moosrivier Estate varied between count 36 and 72 (very large fruit) with a thick rind. Maturity seems to be from middle to end of May.

Fukumoto

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. The trees are still too young with insufficient fruit for internal quality analysis. Trees look semi-dwarfed. In California incompatibility on citrange rootstock has been recorded. At Zalo Citrus similar incompatibility symptoms appeared, but it could be the stress conditions enhancing these symptoms.

Powel Summer (Late maturing Navel)

Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Powel Summer produced a good (SC) to very good (CC) yield on the trees with medium to large (count 40-88) fruit size. Internally quality was acceptable with slightly low Juice% (46.0%-51.4%) and a high TSS% (11.79%-12.74%). The internal texture was coarse with fibre but definitely less than Autumn Gold. The fruit had a sweet taste (yellow colour internally) from the first evaluation onwards and was ready to harvest the first week in June.

Tuligold

Trees were evaluated at Moosrivier Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2004 season. Moosrivier Estate was severely affected by the drought and is therefore not discussed. Tule Gold at Zalo Citrus produced a good yield with a medium to large fruit size (count 48-88). The internal quality was good with slightly low Acid% (0.67%) when ready to harvest. There were no seeds. Maturity might be middle to the end of May.

Conclusion and recommendations

The implications of the drought conditions on the evaluation results at Moosrivier Estates must be borne in mind. Cara Cara seems to be one of the best navel selections evaluated this season. The specific climatic area for Cara Cara with enough heat units for the internal red colour pigment to develop uniform internally is very important. The slightly low Acid% at harvest might cause some decay problems – reduce shelf life. The Powel Summer is a late maturing navel with good internal and external qualities. The Fukumoto is still an experimental selection and bear in mind the incompatibility scenario before considering this cultivar for new plantings. This trial was evaluated for only two seasons (young trees planted at trial site in 2001) and with more information becoming available in the future, more recommendations will be made.

Table 6.2.3.4.2. Internal fruit quality data for Navel selections in the intermediate and cool inland areas during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	15/04/2004	Moosrivier	40-72	47.8	8.77	0.83	10.57	0.0	T6-7
Atwood	CC	06/05/2004	Moosrivier	40-64	50.9	9.22	0.87	10.60	3.3	T5-6
Atwood	CC	20/05/2004	Moosrivier	40-72	52.2	9.85	0.85	11.59	2.1	T4-6
Atwood	CC	06/04/2004	Zalo Citrus	40-72	49.2	9.65	0.76	12.70	0.0	T6
Atwood	CC	05/05/2004	Zalo Citrus	40-64	53.6	11.13	0.76	14.64	0.0	T2-4
Atwood	CC	18/05/2004	Zalo Citrus	40-72	53.4	11.38	0.68	16.74	0.0	T1-2
Autum Gold	CC	15/04/2004	Moosrivier	40-72	37.6	10.10	0.97	10.41	0.0	T7
Autum Gold	CC	06/05/2004	Moosrivier	48-72	44.6	10.88	0.91	11.96	0.0	T7
Autum Gold	CC	20/05/2004	Moosrivier	40-64	47.3	10.88	0.80	13.60	0.0	T5-6
Autum Gold	CC	05/05/2004	Zalo Citrus	48-72	51.6	11.62	1.01	11.50	0.0	T4-5
Autum Gold	CC	18/05/2004	Zalo Citrus	48-88	51.4	11.77	0.85	13.85	0.0	T3-5
Autum Gold	CC	07/06/2004	Zalo Citrus	40-72	52.8	11.00	0.93	11.83	0.0	T2-4
Autum Gold	SC	05/05/2004	Zalo Citrus	48-72	47.9	12.42	1.19	10.44	0.0	T5-7
Autum Gold	SC	18/05/2004	Zalo Citrus	48-72	50.5	12.59	1.19	10.58	0.0	T3-5

Autum Gold	SC	07/06/2004	Zalo Sitrus	48-64	50.6	12.30	1.15	10.70	0.1	T2-4
Bahianinha	SC	06/04/2004	Zalo Sitrus	72-125	49.3	10.68	1.12	9.54	0.0	T7
Bahianinha	SC	05/05/2004	Zalo Sitrus	48-72	55.9	10.73	0.87	12.33	0.0	T5-6
CaraCara	CC	15/04/2004	Moosrivier	48-88	48.8	9.50	0.71	13.38	0.0	T1-2
CaraCara	CC	06/05/2004	Moosrivier	48-72	49.1	10.78	0.69	15.62	0.0	T2
CaraCara	CC	20/05/2004	Moosrivier	48-72	52.4	10.88	0.69	15.77	0.0	T1
CaraCara	CC	06/04/2004	Zalo Sitrus	56-72	49.8	9.95	0.84	11.85	1.0	T2
CaraCara	CC	05/05/2004	Zalo Sitrus	48-72	52.5	9.90	0.80	12.38	0.3	T1-2
CaraCara	CC	18/05/2004	Zalo Sitrus	56-88	52.0	11.08	0.71	15.61	0.0	T1-2
Chislett	CC	15/04/2004	Moosrivier	40-72	34.0	9.80	1.80	5.44	0.0	T7
Chislett	CC	06/05/2004	Moosrivier	36-56	36.1	9.85	0.69	14.28	0.0	T6-8
Dream	CC	15/04/2004	Moosrivier	40-56	46.0	8.97	0.93	9.65	0.3	T7
Dream	CC	06/05/2004	Moosrivier	36-72	48.5	9.55	0.78	12.24	0.2	T5-7
Dream	CC	20/05/2004	Moosrivier	40-56	48.8	9.75	0.76	12.83	0.4	T5
Dream	CC	06/04/2004	Zalo Sitrus	56-105	49.8	11.28	1.00	11.28	0.0	T6
Dream	CC	05/05/2004	Zalo Sitrus	48-72	51.6	12.02	0.87	13.82	0.0	T2-4
Dream	CC	18/05/2004	Zalo Sitrus	48-88	51.5	12.47	0.91	13.70	0.0	T2-3
Powel Summer	CC	05/05/2004	Zalo Sitrus	48-72	49.8	12.22	1.00	12.22	0.0	T4-5
Powel Summer	CC	18/05/2004	Zalo Sitrus	48-72	49.0	12.47	0.92	13.55	0.0	T3-5
Powel Summer	CC	07/06/2004	Zalo Sitrus	48-72	51.4	12.19	1.03	11.83	0.2	T2-3
Powel Summer	SC	05/05/2004	Zalo Sitrus	48-88	46.0	12.79	1.01	12.66	0.0	T4-6
Powel Summer	SC	18/05/2004	Zalo Sitrus	56-72	49.2	12.74	0.95	13.41	0.0	T3-5
Powel Summer	SC	07/06/2004	Zalo Sitrus	40-64	48.4	11.79	1.02	11.56	0.0	T2-4
Tuligold	CC	15/04/2004	Moosrivier	36-64	48.7	9.07	0.67	13.54	0.0	T6-7
Tuligold	CC	06/05/2004	Moosrivier	40-64	52.7	10.15	0.73	13.90	0.0	T5-6
Tuligold	CC	20/05/2004	Moosrivier	40-56	48.0	9.85	0.66	14.92	0.0	T3-5
Tuligold	CC	06/04/2004	Zalo Sitrus	48-88	51.4	10.35	0.93	11.13	0.0	T4-5
Tuligold	CC	05/05/2004	Zalo Sitrus	48-72	55.1	10.83	0.80	13.54	0.0	T2-4
Tuligold	CC	18/05/2004	Zalo Sitrus	64-88	54.6	10.58	0.67	15.79	0.0	T2-3

6.2.3.5 Evaluation of Valencia selections in the inland areas

Experiment 75 by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sappehalte) te verbeter; beter boomproduksie en vruggrootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skikbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke).

Delicia lyk belowend, maar die alternatiewe drag tussen seisoene mag problematies wees. Die interne kwaliteit het nie aan al die uitvoer spesifikasies voldoen nie. Die ernstige droogte wat in die 2004 seisoen in hierdie produksie areas voorgekom het, kan verseker 'n groot invloed uitoefen op die produksie en kwaliteit van die vrugte. Die Benny seleksie toon ook goeie potensiaal t.o.v produksie, vruggrootte en interne kwaliteit met moontlike weerstandbiedendheid teen die droogte toestande.

Introduction

Objectives are to optimise profitability by improving internal fruit quality (seedlessness, fibre toughness, juice quality); improving productivity and fruit size distribution (count 48, 56 and 72); improving rind colour, external appearance and peelability; extending the picking period by developing early (June / mid July) and late maturing selections (for hot areas).

Materials and methods

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Benny (control), Delicia, EEL-T, G5, Glen Ora, McClean SL, Midnight, Portsgate, Ruby Valencia, Turkey (control) at various inland sites/areas.

Table 6.2.3.5.1. Internal fruit quality data was compared with the minimum export requirements (Capespan) for Valencia types.

Variety	% Juice	% TSS	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
Midnight	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
Delta Seedless	52	10.5	0.85	1.5%	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 6.2.3.5.2. List of Valencia selections trial sites evaluated during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	Mpumalanga	Esselen Nursery	CC	1996	1
Delicia (Delpport)	Mpumalanga	Esselen Nursery	CC	1996	1
EEL-T	Mpumalanga	Esselen Nursery	Troyer	1998	1
Glen Ora	Mpumalanga	Esselen Nursery	CC	2000(Top)	3
McClean SL	Mpumalanga	Esselen Nursery	TB		1
Midnight	Mpumalanga	Esselen Nursery	C35	1998	1
Portsgate	Mpumalanga	Esselen Nursery	CC	1996	1
Ruby Val	Mpumalanga	Esselen Nursery	RL	2001	2
Turkey	Mpumalanga	Esselen Nursery	CC	1996	1
Alpha	Limpopo	Group 91	CC	1996	24
Benny (control)	Limpopo	Group 91	CC	1996	23
Delicia (Delpport)	Limpopo	Group 91	CC	1996	21
G5	Limpopo	Group 91	CC	1996	21
Glen Ora	Limpopo	Group 91	CC	1996	2
Portsgate	Limpopo	Group 91		1996	23
Turkey (control)	Limpopo	Group 91		1996	18

Results and discussion

Alpha

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2004 season.

Alpha produced a high Juice% (60.0%-62.2%) at Esselen and fairly high Acid% (1.57%-1.62%) compared to the 2003 season at Group 91. Bearing in mind the Juice% and Acid%, the internal quality complied to the export packing specifications. The yield produced on the trees varied between poor and average with alternate bearing. The fruit size measured between medium to large (count 40 to 88) fruit. Fruit evaluated at Esselen produced low fibre content with good internal colour in the fruit. There were no seeds. Maturity seems to be middle to end of June.

Benny

Trees were evaluated at Group 91, Limpopo Province during the 2004 season.

Benny produced a good yield with medium to large fruit (count 56-72). The internal quality analysed met all export requirements. There were 2.2 seeds per fruit. Maturity of the fruit will be the middle of June.

Delicia

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2004 season.

There seems to be a pattern of alternate bearing between the production years evaluated. In 2003 the trees at Esselen produced a very good yield and a good yield at Group 91. In the 2004 season at Leon Esselen the trees produced barely any fruit and at Group 91 a poor yield was produced on the trees. There was also a remarkable variation in fruit size between the trees evaluated at Group 91. The TSS% tested below the

minimum of 9.75% for the packing specifications provided. Bear in mind this was one of the regions (Letsitele) with severe drought conditions. Maturity at the end of June.

EEL-T

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2004 season.

EEL-T produced a medium yield on the trees and the fruit size varied from small to medium (64-88). The fruit evaluated tasted watery with slightly high acids. On average there were between 4.9 and 5.2 seeds per fruit. The TSS% (9.65%) was below the minimum requirement (9.75%) for export fruit, but all the other internal quality factors were acceptable. Matures end of June.

G5

Trees were evaluated at Group 91, Limpopo Province during the 2004 season.

There were no seeds counted in the fruit evaluated. G5 produced an average yield on the trees and the internal quality met export specifications. Fruit size varied between the trees evaluated, but measured between count 64 and 88. Maturity seems to be end of June.

Glen Ora

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2004 season.

The yield produced on the Glen Ora trees were average with medium to large fruit size (count 40-72). Internally the quality meets the packing specifications, except for the TSS% (9.55%) that seems to be on the low side at Group 91 (9.75% specification). Virtually no seeds were counted in the fruit (0.1 seed per fruit). Glen Ora produced slightly coarse rinded fruit. This selection with a delay in external colour, will mature the latest of the Valencia's evaluated in this trial. Maturity first week to middle of July.

Mc Clean SL

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2004 season.

Mc Clean SL is an improved selection on the current Mc Clean Valencia used in the industry. Mc Clean SL produced a poor yield with medium to large fruit size on the trees. A good yield was produced in the 2003 season in compared to 2004. There was a possibility of alternate bearing between the different production seasons. Dark yellow internal colour and exceptional external colour on the fruit with no seeds counted. This selection was selected because of better yield produced with no seeds in the fruit. Further evaluations will be conducted. Maturity seems to be end of June.

Midknight

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2004 season.

Midknight produced a good yield with fairly large fruit size (count 40-64). The internal quality complied with the packing specifications, except for the TSS% (8.23%) that tested too low. The fruit shape was slightly elongated and no seeds were counted. Maturity middle to end of June.

Portsgate

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2004 season.

The internal colour of the fruit was deep yellow. Portsgate produced an average yield on the trees with alternate bearing patterns. The fruit size varied from small to medium fruit (count 88-105). There was a low fibre content internally and no seeds were present when the fruit was evaluated. In comparison with all the other Valencia's evaluated, Portsgate had the sweetest taste. Maturity end of June.

Ruby

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2004 season.

The Ruby Valencia trees evaluated are the original trees planted on Rough Lemon before they received radiation treatment. In future the radiated trees will be evaluated (no of seeds counted).

Ruby produced an average yield on the trees with a medium fruit size. The internal colour of the fruit was red and the colour pigment was distributed uniformly. The TSS% (7.81%) tested on the low side and the fruit had a sweet, but watery taste. Maturity might be middle of July.

Conclusions and recommendations

The Ruby Valencia seems to be a promising selection if you take the red internal colour and seedlessness. The overall taste of the fruit and the larger fruit size will improve the qualities of the selection. Midnight produced 81kg on the tree, followed by Delicia with 72 kg and Turkey (C35) with 70 kg for the 2003 season. This season Delicia produced virtually no fruit and alternate bearing patterns seems to be a problem between the seasons for this selection. Turkey produced an average to good yield. Evaluations will continue for the 2005 season.

Table 6.2.3.5.3. Internal fruit quality data for Valencia orange selections for the inland areas during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Alpha	CC	04/06/2004	Esselen	56-72	60.0	10.31	1.09	9.5	0.0	T3-4
Alpha	CC	17/06/2004	Esselen	40-72	62.2	9.85	1.11	8.9	0.0	T1-2
Alpha	CC	09/06/2004	Groep 91	56-88	56.9	10.08	1.57	6.4	0.0	T1-2
Alpha	CC	24/06/2004	Groep 91	64-88	57.9	10.37	1.62	6.4	0.0	T1
Benny	CC	09/06/2004	Groep 91	56-72	57.0	9.88	1.32	7.5	2.2	T1-2
Delicia	CC	09/06/2004	Groep 91	48-64	61.4	9.38	1.23	7.6	0.3	T1-2
Delicia	CC	24/06/2004	Groep 91	40-64	57.0	9.15	1.06	8.6	0.0	T1-2
EEL-T	Troyer	04/06/2004	Esselen	64-88	56.8	9.78	1.07	9.1	5.2	T5
EEL-T	Troyer	17/06/2004	Esselen	64-88	59.8	9.65	1.10	8.8	4.9	T2-3
G5	CC	09/06/2004	Groep 91	64-72	54.6	9.98	1.27	7.9	0.0	T1-3
G5	CC	24/06/2004	Groep 91	64-88	56.1	10.27	1.40	7.3	0.0	T1-2
Glenora Late	CC	04/06/2004	Esselen	56-72	59.2	10.08	1.54	6.5	0.0	T6-7
Glenora Late	CC	17/06/2004	Esselen	48-72	59.4	10.47	1.51	6.9	0.0	T5-6
Glenora Late	CC	09/06/2004	Groep 91	40-64	55.2	9.28	1.47	6.3	0.0	T1-3
Glenora Late	CC	24/06/2004	Groep 91	40-72	57.6	9.55	1.50	6.4	0.1	T1-2
McClellan SL	CC	04/06/2004	Esselen	48-64	58.6	9.58	1.17	8.2	0.3	T3-4
McClellan SL	CC	17/06/2004	Esselen	40-72	60.7	10.05	1.10	9.1	0.5	T1-2
Midnight	C35	04/06/2004	Esselen	40-64	59.0	8.36	0.97	8.6	0.0	T3-4
Midnight	C35	17/06/2004	Esselen	40-56	59.5	8.23	0.94	8.8	0.0	T1-4
Portsgate	CC	04/06/2004	Esselen	56-105	60.8	10.31	1.12	9.2	0.0	T4
Portsgate	CC	17/06/2004	Esselen	48-88	61.1	9.75	0.98	9.9	0.0	T3-4
Portsgate	CC	09/06/2004	Groep 91	56-88	55.2	9.88	1.29	7.7	0.0	T1-2
Portsgate	CC	24/06/2004	Groep 91	56-88	56.6	10.47	1.12	9.3	0.0	T1
Ruby	RL	04/06/2004	Esselen	56-72	55.7	8.26	0.95	8.7	2.9	T1-2
Ruby	RL	17/06/2004	Esselen	56-72	57.4	7.81	0.93	8.4	3.5	T2
Turkey	CC	04/06/2004	Esselen	40-72	59.2	11.01	1.04	10.6	4.7	T2-3
Turkey	CC	17/06/2004	Esselen	40-72	58.6	10.77	1.02	10.6	5.5	T1
Turkey	CC	09/06/2004	Groep 91	56-72	56.3	10.91	1.20	9.1	7.0	T1-2

6.2.3.6 Evaluation of Midseason oranges in the inland areas Experiment 77 by J. Joubert (CRI)

Opsomming

Geskikte middelseisoen lemoene moet gevind word om die belangrike middelseisoenleemte (Junie/Julie) in die warmer binnelandse streke te vul. Vind 'n bron van gepigmenteerde lemoene en bepaal hul aanpasbaarheid vir 'n wye reeks klimaatstoestande. Markaanvaarbaarheid van die kultivars sal ook vasgestel word.

Hierdie proef word gestaak en sal nie verder geëvalueer word vir die 2005 seisoen nie. Die perseel is besoek en daar was slegs 3 van die 7 seleksies wat 'n ligte oes geproduseer het. Daar is ook probleme ondervind om die vrugte te akkommodeer in die pakhuis program tussen die ander kultivars. Die perseel is weer besoek deur Hennie le Roux, Faan v Vuuren en Johan Joubert aan die einde van die seisoen. Daar was moontlike tekens van chemiese brandskade (bespuitings) op die bome en sekere kultivars (veral

Tarocco seleksies) was die meeste beïnvloed. Hierdie proef sal vervang word deur 'n nuwe Pomelo aanplanting met verskillende seleksies op 'n reeks onderstamme.

Introduction

Objectives are to find suitable midseason orange cultivars to fill the important "midseason" gap (June/July) in the warmer inland areas. To source pigmented oranges and test their adaptability to a broad range of climatic conditions. Market acceptance of these cultivars will also be assessed.

Materials and methods

Field and laboratory analyses were conducted on Barlerin and various Tunisian Maltese Orange selections, Crookes Shamouti, Grosse Sanquine, Raratonga, Sanquinella, Tarocco Gallo and Tarocco 57 this season.

Table 6.2.3.6.1. Internal quality export requirements (Capespan) for midseason oranges.

Variety	% Juice	% TSS	% Acid Min	% Acid Max	Ratio	Colour
Tomango	52.0	9.0	0.7	1.8	7.0:1	Set 34 no. 3
Shamouti	44.0	9.0	0.6	1.8	7.0:1	Set 34 no. 3
Sanguinello	48.0	9.0	0.6	1.8	7.0:1	Set 34 no. 3
Salustiana	52.0	9.0	0.7	1.8	7.0:1	Set 34 no. 3

Table 6.2.3.6.2. List of midseason orange trial sites evaluated during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Barlerin Maltese (MMBA)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4,4,4.
Crookes Shamouti	Kwazulu Natal	Riversbend	CC, C35, X639	1999	22, 23, 7.
Grosse Sanquinne (MGS)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	3, 3, 3, 3, 3.
Raratonga (MRAR)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4, 4, 4.
Sanquinella (MSAN)	Kwazulu Natal	Riversbend	CC, C35, SC.		12, 14, 16.
Tarocco Gallo (MTG)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 3, 4, 4, 4.
Tarocco 57 (MT57)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4, 3, 4.
Tunisian Maltese (BKM)	Mpumalanga	Moosriver Estates	MxT, CC	1997	5 each
Tunisian Maltese (HMM ²)	Mpumalanga	Moosriver Estates	CC, SC	1997	5 each
Tunisian Maltese (MLM)	Mpumalanga	Moosriver Estates	CC, SC, MxT	1997	5 each

Results and discussion

No results.

Conclusion and recommendations

The trees were evaluated early in the season and only three out of the seven cultivars produced some fruit. The trial site was visited by Hennie le Roux, Faan van Vuuren and myself to evaluate the trees and determine the cause of the problem. We decided that the area is probably too warm for mid-season oranges for optimum production. It seems as if the trees were burnt by some chemicals sprayed on the rest of the orchard when they were under water stress conditions. The trial was stopped and will be replaced by a new grapefruit trial planted on various rootstock selections.

6.2.3.7 Evaluation of Lemons in the inland areas

Experiment 79 by J. Joubert (CRI)

Opsomming

Kouegeharde, doring- en saadlose suurlemoenseleksies (met aanvaarbare vruggrootte), wat met 'n wye reeks onderstamme verenigbaar is, moet ontwikkel word. Die oesseisoen moet verleng word om

aaneenlopende produksie van Maart tot September te verseker, aangesien dit vroeë en laatrypwordende seleksies insluit. Probleme wat met lang blomtyd geassosieer kan word, moet verminder word. Goeie vrugkwaliteit (kleur, skildikte, sapinhoud) moet behou word.

Die boomeienskappe en prestasie van nuwe kultivars moet met die kommersieel gekweekte Eureka vergelyk word om te bepaal of hulle aan bogenoemde doelwitte voldoen.

Villafranca produseer steeds die minste sade per vrug, gevolg deur Verna. Die res van die seleksies het heelwat sade per vrug opgelewer en vergelyk nie goed met Eureka saadloos (LNR) nie. Die Sap% tussen die verskillende seleksies was redelik hoog, maar verskil nie dramaties baie nie.

Introduction

Objectives are to develop cold hardy, thornless, seedless (with acceptable fruit size) lemon selections which are compatible with a wider rootstock range; to extend the picking period to ensure continuity of supply from March to September, i.e. early and late maturing selections; to reduce the problems associated with protracted flowering; to maintain high fruit quality (colour, rind thickness, juice content).

To compare the tree characteristics and performance of new cultivars with the commercially grown Eureka SL to establish if they fulfil the above objectives.

Materials and methods

Field evaluations were conducted on Eureka SL (control), Eureka (Israel), Fino 49 & 95, Genoa, Limoneira 8A, Lisbon (control), Verna and Villafranca SL on various rootstocks.

Table 6.2.3.7.1. List of lemon trial sites evaluated during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Eureka SL (ARC)	Mpumalanga	Esselen	RL	2000	5
Eureka (Israel)	Mpumalanga	Tekwane	RL	1998	4
Fino 49	Mpumalanga	Tekwane	RL	1998	4
Fino 95	Mpumalanga	Tekwane	RL	1998	4
Genoa	Mpumalanga	Tekwane	RL	1998	4
Limoneira 8A	Mpumalanga	Tekwane	RL	1998	3
Lisbon	Mpumalanga	Tekwane	RL,SO	1998	2,2
Verna	Mpumalanga	Tekwane	RL	1998	4
Villafranca	Mpumalanga	Tekwane	RL	1998	3

Results and discussion

Eureka SL (ARC)

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2004 season. Trees at Esselen Nursery are still seedless and produced good quality fruit. Ensure that the bud wood is sourced from the Citrus Foundation Block only.

Tekwane Estates

Trees were evaluated at Tekwane Estates, Mpumalanga during the 2004 season.

Villafranca produced fruit with 0.3 seeds per fruit, followed by Verna with 5 seeds per fruit. These figures were slightly higher when compared to the 2003 season. Limoneira, Lisbon (SO) and Eureka (IR) produced fruit with more or less the same number of seeds per fruit (12.9-15.5 seeds). The highest number of seeds produced per fruit was in Fino49, Fino 95, Genoa and Lisbon (RL) selections (20.5-23.3 seeds).

Limoneira produced the highest Juice% (37.8%) and Fino 49 the lowest (23.9%).

Conclusions and recommendations

Villafranca develops virtually no seeds per fruit and produces a high juice % followed by Verna with slightly more seeds per fruit and a lower juice %. These two selections perform the closest to Eureka Seedless (ARC) with more or less the same qualities. Evaluations to continue.

Table 6.2.3.7.2. Internal fruit quality data for Lemons during the 2004 season at Tekwane Estates.

Selection	Root-stock	Date harvested	Site	Juice %	Ave. seed
Eureka seedless (ARC)	No fruit was available for internal quality tests.				
Eureka (Israel)	RL	20/04/2004	Tekwane	25.7	12.9
Fino 49	RL	20/04/2004	Tekwane	23.9	23.3
Fino 95	RL	20/04/2004	Tekwane	33.3	20.5
Genoa	RL	20/04/2004	Tekwane	32.4	21.4
Lisbon	RL	20/04/2004	Tekwane	28.2	21.1
Lisbon	SO	20/04/2004	Tekwane	29.1	13.3
Limineira	RL	20/04/2004	Tekwane	37.8	15.5
Verna	RL	20/04/2004	Tekwane	29.4	5.0
Villafranca	RL	20/04/2004	Tekwane	33.6	0.3

6.2.4 Sub Project: Evaluation of citrus rootstocks in the Northern and inland region

By J. Joubert (CRI)

6.2.4.1 Sub-Projekopsomming

Die hoofdoel van die Onderstam-evaluasieprojek is om onderstamme te vestig, te evalueer, verslag te lewer en aanbevelings vir die Suid-Afrikaanse Sitrusbedryf te maak.

Die 2004 seisoen het verskeie probleme opgelewer met die ernstige droogtes wat in die verskillende produksie areas voorgekom het. Hierdie senario het sekere interne kwaliteitse beïnvloed t.o.v. TOV en Suur persentasies. Goeie aflydings kan hieruit gemaak word aangaande kultivars en onderstamme wat moontlik meer weerstandbiedend is teen droogtes en verskillende bo-en onderstam kombinasies. Daar is oor die algemeen minder vrugte op die bome geproduseer wat die vruggroote in meeste gevalle laat vergroot het. Baie dankie vir al die produsente se ondersteuning namens die CRI waar die verskillende proewe aangetref word.

Sub-Project summary

The primary focus of the Rootstock evaluation project is to procure, evaluate, report on and make rootstock recommendations for the South African Citrus Industry.

In the 2004 season there were severe drought conditions in most of the main production areas causing problems. This had a major influence on the internal quality of the fruit with regards to TSS and Acid percentages. Assumptions can be made from these circumstances, which provide information on the tolerance to drought conditions of specific cultivars and rootstocks, as well as the best rootstock-scion combination. On average there were less fruit on the trees with large fruit size. Thank you to all the growers for their assistance with all the trials conducted on behalf of CRI.

Abbreviations used in text:

SYMBOL	ROOTSTOCK
1. AT	Australian trifoliolate
2. BC	Benton citrange
3. C	Calamandarin
4. CA	C. amblycarpa
5. CC	Carrizo citrange
6. CM	C. macrophylla
7. ChM	Changsa mandarin
8. CLM	Cleopatra mandarin
9. CO	C. obovoideae

SYMBOL		ROOTSTOCK
10.	C32	citrange (trifoliolate orange x Ruby sweet orange)
11.	C35	citrange (trifoliolate orange x Ruby sweet orange)
12.	C61	Sunki x macrophylla
13.	FD	Flying Dragon
14.	FF6	Sunki x MTO trifoliolate orange
15.	F80/3	citrumelo
16.	F80/9	citrumelo
17.	GT	Gou Tou
18.	HRS 802	Siamese pummelo x trifoliolate orange
19.	HRS 809	Changsa x English large flowered trifoliolate orange
20.	HRS 812	Sunki mandarin x Beneke trifoliolate orange
21.	IRL	Indian rough lemon
22.	JC	Japanese citron
23.	JT	Jacobsen trifoliolate
24.	K	Konejime
25.	KC	Koethen citrange
26.	ML	Milan Lemon
27.	MXT	Minneola x trifoliolate
28.	N	Natsudaikai
29.	O	Orlando tangelo
30.	PT	Pomeroy trifoliolate
31.	RC	Rusk citrange
32.	RL-C	Rough lemon Cairn
33.	RL-S	Rough lemon Schaub
34.	RL-W	Rough lemon Wallace
35.	RP	Rangpur lime
36.	RT	Rubidoux trifoliolate
37.	RXT	Rangpur x troyer
38.	SC	Swingle citrumelo
39.	SCS	Sun chu sha
40.	SFS	Smooth flat Seville
41.	SM	Shekwasha mandarin
42.	SO	Sour orange
43.	ST	Sampson tangelo
44.	Sunki 1112	Flying dragon x Sunki (1112)
45.	Sunki 1113	Flying dragon x Sunki (1113)
46.	Sunki 1116	Flying dragon x Sunki (1116)
47.	TB	Terrabella
48.	TC	Troyer citrange
49.	Volk	Volckameriana
50.	X639	Cleopatra mandarin x trifoliolate

SYMBOL		ROOTSTOCK
51.	YC	Yuma citrange
52.	61 AA3	Cleopatra mandarin x <i>P. trifoliata</i>
53.	75 AB 12/13	Mc Carthy grapefruit x <i>P. trifoliata</i>
54.	79 AC 6/2	Cleo x Swingle citrumelo

6.2.4.2 Evaluation of Delta Valencia rootstocks at Moosrivier Estates, Groblersdal, Mpumalanga Experiment 94 by J. Joubert (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word.

In die 2004 seisoen het Moosrivier Estate 'n ernstige droogte ondervind. Die produksie van die bome en die algemene vruggroote geproduseer word die meeste geraak. Die inligting wat uit die evaluasie verkry word, gee 'n aanduiding van watter onderstam kombinasie meer weerstandbiedend is teen droogte toestande en watter meer gevoelig is. Let daarop dat hierdie senario slegs in uiterste gevalle van droogte spanning by die bome sal voorkom. Hierdie inligting toon ook die belangrikheid van besproeiings skedulering en die impak van onder-besproeiing op produksie. Die algemene tendens by Sitrus produksie is oor-besproeiing eerder as onder-besproeiing. Die vruggroote op die bome het toegeneem van telling 105/125 tot telling 72. Hierdie is 'n algemene tendens wat voorkom as daar minder vrugte op die bome geproduseer word (ligter oes).

Introduction

The objective is to evaluate the performance of Delta Valencia on 42 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area.

Materials and methods

A randomised block comprising of 22 data rootstocks of two replicates of five trees each, the remainder (20) were planted in a non-randomised design comprising of 10 trees per stock. Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, AT, X639, RL-C, RL-S, RL-W, PT, HRS812, K, ChM, N, RxT, CLM, Sunki 1113, CM, C, SCS, GT, CO, CC, TC, Volk, KC, TB, ML, OT, CA, RC, JT, RT, JC, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116.

Table 6.2.4.2.1. List of rootstocks for Delta Valencias evaluated in the Marble Hall area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Delta Valencia	Mpumalanga	Moosriver Estate	F80/8	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	PT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	C32	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	AT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	HRS812	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	K	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	CM	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	C35	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	C	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	SCS	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	X639	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	GT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	ML	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	OT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	CA	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	RC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	JT	1998	2
Delta Valencia	Mpumalanga	Moosriver Estate	RL-S	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	SC	1998	5

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Delta Valencia	Mpumalanga	Moosriver Estate	RP	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	SM	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	ChM	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	N	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	RxT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	RL-C	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	CLM	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	Sunki 1113	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	CO	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	CC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	TC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	Volk	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	KC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	TB	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	RT	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	JC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	BC	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	F80/3	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	Sunki 1112	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	ST	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	SFS	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	Sunki 1116	1998	5
Delta Valencia	Mpumalanga	Moosriver Estate	RL-W	1998	5

Results and discussion

Trees were evaluated at Moosriver Estates (Marble Hall), in Mpumalanga during the 2004 season.

The internal fruit quality analysis contains the following parameters:

- Juice %

F80/8 rootstock produced the highest Juice% (59.9%) followed by BC (58.8%) and RC (57.1%). The Juice% on all the rootstocks complies with the packing specifications with K measuring 52.0% (lowest).

- TSS %

The highest TSS % was produced by CO (10.99 %) followed by RC (10.75 %), C35 (10.69 %) and F80/8 (10.55 %). The rest of the rootstocks evaluated produced lower than 10.5 % TSS value and did not comply to the packing specifications, with RL-C the lowest (8.2 %).

- Acid %

GT had the highest Acid % (1.49 %) and BC second (1.45 %) followed by SCS (1.44 %) and CO (1.42 %). The lowest Acid % measured was 0.97 % and complies with the minimum export specifications of 0.85 %.

The fruit size distribution per rootstock:

- The fruit size evaluation shows the largest peak at count 72 on 23 of the 42 rootstocks. The next highest count in fruit size was count 56 with 14 rootstocks. The third highest count evaluated in fruit size was count 88 with 26 rootstocks. Considering that count 72, followed by count 56, and then count 88, was ranked from the highest percentage fruit per rootstock to the lowest percentage.
- Due to the drought conditions the fruit size was larger this season. In the 2003 season the fruit size peak was at count 105/125 and 144, but for the 2004 season the peak shifted to count 72 and 56 because of less fruit on the trees.

Production per tree:

- CM rootstock produced the best yield per tree (51.0 kg). BC was the second highest producer with 35.7 kg per tree, followed by RP (45.7 kg) and SFS (40.1 kg). K and C produced the lowest yield for the 2004 season with 3.7 kg per tree.
- There is a decrease in yield production between the 2003 and 2004 season, with only one rootstock that increased in production. The trend shows the severe impact of the water problems that occurred in this area on the different rootstocks.
- The table shows the decrease in yield between the rootstocks from the smallest impact to the largest impact.

Table 6.2.4.2.2 Decrease in yield between the rootstocks from the smallest impact to the largest impact.

Cultivar	Rootstock Selection	Kg/tree 2003	Kg/tree 2004	Decrease in kg (%)
Delta Valencia	JT	26.3	30.8	-17.11
Delta Valencia	RT	36.8	35.7	2.99
Delta Valencia	CM	57.4	51.0	11.15
Delta Valencia	KC	39.0	32.7	16.15
Delta Valencia	Sunki 1116	32.5	25.8	20.62
Delta Valencia	SFS	51.4	40.1	21.98
Delta Valencia	BC	61.7	47.7	22.69
Delta Valencia	RP	59.4	45.7	23.06
Delta Valencia	PT	43.1	32.5	24.59
Delta Valencia	RC	50.1	37.4	25.35
Delta Valencia	F80/8	48.4	33.4	30.99
Delta Valencia	RxT	36.2	22.5	37.85
Delta Valencia	CC	37.0	22.0	40.54
Delta Valencia	Sunki 1113	48.4	28.7	40.70
Delta Valencia	ML	60.5	35.4	41.49
Delta Valencia	ST	18.5	10.6	42.70
Delta Valencia	X639	47.2	26.3	44.28
Delta Valencia	F80/3	49.9	27.1	45.69
Delta Valencia	CA	21.1	11.2	46.92
Delta Valencia	Sunki 1112	43.9	22.9	47.84
Delta Valencia	JC	23.4	11.9	49.15
Delta Valencia	Volk	56.7	28.1	50.44
Delta Valencia	RL-C	55.7	26.8	51.89
Delta Valencia	HRS 812	50.7	24.0	52.66
Delta Valencia	C35	58.4	27.4	53.08
Delta Valencia	AT	25.3	11.8	53.36
Delta Valencia	C32	54.9	25.4	53.73
Delta Valencia	RL-S	54.3	25.1	53.78
Delta Valencia	SM	28.8	13.3	53.82
Delta Valencia	RL-W	34.8	14.2	59.20
Delta Valencia	CO	31.1	11.3	63.67
Delta Valencia	TB	49.5	16.9	65.86
Delta Valencia	CLM	31.7	9.6	69.72
Delta Valencia	OT	23.4	6.9	70.51
Delta Valencia	SC	40.0	10.9	72.75
Delta Valencia	ChM	34.9	9.0	74.21
Delta Valencia	N	46.2	11.3	75.54
Delta Valencia	GT	26.3	5.8	77.95
Delta Valencia	TC	50.6	11.1	78.06

Delta Valencia	C	18.7	3.7	80.21
Delta Valencia	SCS	34.9	5.5	84.24
Delta Valencia	K	25.1	3.7	85.26

Conclusion and recommendations

The drought in this production area led to irrigation problems. Only the most important trees were irrigated (those with best export possibilities) to keep them alive for the season and not to produce optimum yield. The trees had bigger fruit size because the trees bore less fruit.

The production decreased (kg per tree) on most of the selections except for JT. This shows that some of the rootstock selections are more tolerant to dry conditions than others. This emphasises the importance of optimum irrigation scheduling to prevent the trees from going into any stress situation which might impact on the yield.

Table 6.2.4.2.3. Internal fruit quality of Delta Valencia on different rootstocks at Moosriver Estate, (Marble Hall) during the 2004 season.

Root-stock	Date harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
F80/8	19/07/2004	56-88	59.9	10.55	1.29	8.18	0.0	T1
PT	19/07/2004	56-88	53.2	10.12	1.01	10.02	0.0	T1
C32	19/07/2004	64-72	54.2	10.45	1.20	8.71	0.0	T1
AT	19/07/2004	56-72	52.9	9.82	0.97	10.12	0.0	T1
HRS 812	19/07/2004	56-72	54.3	10.25	1.21	8.47	0.0	T1
K	19/07/2004	48-88	52.0	9.55	1.11	8.60	0.0	T1
CM	19/07/2004	56-88	55.6	9.35	1.15	8.13	0.0	T1
C35	19/07/2004	64-88	56.5	10.69	1.26	8.48	0.0	T1
C	19/07/2004	56-88	54.9	10.02	1.27	7.89	0.0	T1
SCS	19/07/2004	56-72	53.4	10.06	1.44	6.99	0.0	T1
X639	19/07/2004	64-88	54.4	10.25	1.29	7.95	0.0	T1
GT	19/07/2004	56-88	56.1	9.76	1.49	6.55	0.0	T1
ML	19/07/2004	56-72	54.3	8.84	1.17	7.56	0.0	T1
OT	19/07/2004	56-105	55.6	9.96	1.39	7.17	0.0	T1
CA	19/07/2004	56-88	55.8	10.02	1.31	7.65	0.0	T1
RC	19/07/2004	56-72	57.1	10.75	1.22	8.81	0.0	T1
JT	19/07/2004	56-88	52.6	10.12	1.04	9.73	0.0	T1
RL-S	19/07/2004	64-88	54.7	9.55	1.34	7.13	0.0	T1
SC	19/07/2004	64-105	55.6	10.45	1.35	7.74	0.0	T1
RP	19/07/2004	64-88	56.3	8.84	1.06	8.34	0.0	T1
SM	19/07/2004	64-72	53.4	9.95	1.26	7.90	0.0	T1
ChM	19/07/2004	64-72	53.2	9.75	1.28	7.62	0.0	T1
N	19/07/2004	64-88	53.1	9.76	1.38	7.07	0.0	T1
RxT	19/07/2004	56-88	56.1	9.86	1.18	8.36	0.0	T1
RL-C	19/07/2004	56-72	56.9	8.20	1.12	7.32	0.0	T1-2
CLM	19/07/2004	56-88	55.3	9.76	1.31	7.45	0.0	T1
Sunki 1113	19/07/2004	48-88	55.1	9.85	1.12	8.79	0.0	T1
CO	19/07/2004	64-88	54.9	10.99	1.42	7.74	0.0	T1
CC	19/07/2004	64-88	55.5	10.02	1.12	8.95	0.0	T1
TC	19/07/2004	56-88	54.9	9.55	1.37	6.97	0.0	T1
Volk	19/07/2004	56-88	54.4	9.04	1.11	8.14	0.0	T1
KC	19/07/2004	56-72	53.5	9.85	1.23	8.01	0.0	T1
TB	19/07/2004	56-88	54.6	10.02	1.36	7.37	0.0	T1
RT	19/07/2004	56-72	56.3	10.39	1.14	9.11	0.0	T1
JC	19/07/2004	48-72	54.7	8.90	1.20	7.42	0.0	T1
BC	19/07/2004	64-88	58.8	10.39	1.45	7.17	0.0	T1

Root-stock	Date harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
F80/3	19/07/2004	64-72	56.3	9.96	1.37	7.27	0.0	T1
Sunki 1112	19/07/2004	56-88	55.1	9.95	1.32	7.54	0.0	T1
ST	19/07/2004	64-88	54.3	9.45	1.11	8.51	0.0	T1
SFS	19/07/2004	56-88	56.5	9.66	1.29	7.49	0.0	T1
Sunki 1116	19/07/2004	56-72	55.9	10.12	1.25	8.10	0.0	T1
RL-W	19/07/2004	48-72	53.2	10.45	1.22	8.57	0.0	T1

Table 6.2.4.2.4. Fruit Size distribution per rootstock of Delta Valencia trees on different rootstocks at Moosriver Estate (Marble Hall) during the 2004 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	F80/8	48	1.07	Delta	ChM	48	6.88
Delta	F80/8	56	15.68	Delta	ChM	56	38.75
Delta	F80/8	72	32.72	Delta	ChM	72	31.88
Delta	F80/8	88	27.09	Delta	ChM	88	14.38
Delta	F80/8	105/125	20.85	Delta	ChM	105/125	7.50
Delta	F80/8	144	2.59	Delta	ChM	144	0.63
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	PT	48	3.30	Delta	N	48	3.57
Delta	PT	56	29.51	Delta	N	56	26.53
Delta	PT	72	36.11	Delta	N	72	33.16
Delta	PT	88	18.40	Delta	N	88	21.43
Delta	PT	105/125	10.42	Delta	N	105/125	12.24
Delta	PT	144	2.26	Delta	N	144	3.06
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C32	48	10.17	Delta	RxT	48	5.53
Delta	C32	56	36.88	Delta	RxT	56	35.26
Delta	C32	72	29.55	Delta	RxT	72	36.32
Delta	C32	88	15.60	Delta	RxT	88	14.74
Delta	C32	105/125	6.15	Delta	RxT	105/125	6.84
Delta	C32	144	1.65	Delta	RxT	144	1.32
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	AT	48	10.45	Delta	RL-C	48	1.79
Delta	AT	56	34.33	Delta	RL-C	56	27.49
Delta	AT	72	36.82	Delta	RL-C	72	39.24
Delta	AT	88	11.94	Delta	RL-C	88	19.12
Delta	AT	105/125	4.98	Delta	RL-C	105/125	10.56
Delta	AT	144	1.49	Delta	RL-C	144	1.79
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	HRS812	48	18.77	Delta	CLM	48	3.93
Delta	HRS812	56	50.13	Delta	CLM	56	24.72
Delta	HRS812	72	19.03	Delta	CLM	72	32.58
Delta	HRS812	88	7.77	Delta	CLM	88	20.22
Delta	HRS812	105/125	4.02	Delta	CLM	105/125	15.73
Delta	HRS812	144	0.27	Delta	CLM	144	2.81
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	K	48	3.95	Delta	Sunki 1113	48	21.99
Delta	K	56	11.84	Delta	Sunki 1113	56	43.50
Delta	K	72	26.32	Delta	Sunki 1113	72	22.93
Delta	K	88	30.26	Delta	Sunki 1113	88	7.80
Delta	K	105/125	23.68	Delta	Sunki 1113	105/125	3.31

Delta	K	144	3.95	Delta	Sunki 1113	144	0.47
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	CM	48	0.47	Delta	CO	48	0.00
Delta	CM	56	13.64	Delta	CO	56	9.82
Delta	CM	72	30.97	Delta	CO	72	28.57
Delta	CM	88	30.02	Delta	CO	88	29.02
Delta	CM	105/125	22.73	Delta	CO	105/125	30.36
Delta	CM	144	2.18	Delta	CO	144	2.23
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	3.58	Delta	CC	48	4.37
Delta	C35	56	31.37	Delta	CC	56	29.82
Delta	C35	72	29.47	Delta	CC	72	32.90
Delta	C35	88	16.42	Delta	CC	88	22.11
Delta	C35	105/125	15.37	Delta	CC	105/125	10.28
Delta	C35	144	3.79	Delta	CC	144	0.51
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C	48	1.59	Delta	TC	48	7.22
Delta	C	56	15.87	Delta	TC	56	33.51
Delta	C	72	23.81	Delta	TC	72	26.29
Delta	C	88	31.75	Delta	TC	88	19.59
Delta	C	105/125	25.40	Delta	TC	105/125	8.25
Delta	C	144	1.59	Delta	TC	144	5.15
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SCS	48	1.00	Delta	Volk	48	7.03
Delta	SCS	56	13.00	Delta	Volk	56	36.48
Delta	SCS	72	37.00	Delta	Volk	72	34.07
Delta	SCS	88	20.00	Delta	Volk	88	14.95
Delta	SCS	105/125	28.00	Delta	Volk	105/125	5.93
Delta	SCS	144	1.00	Delta	Volk	144	1.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	4.06	Delta	KC	48	4.63
Delta	X639	56	19.68	Delta	KC	56	36.83
Delta	X639	72	31.24	Delta	KC	72	28.11
Delta	X639	88	27.18	Delta	KC	88	17.08
Delta	X639	105/125	16.63	Delta	KC	105/125	12.28
Delta	X639	144	1.22	Delta	KC	144	1.07
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	GT	48	0.00	Delta	TB	48	9.89
Delta	GT	56	10.96	Delta	TB	56	41.34
Delta	GT	72	26.03	Delta	TB	72	25.09
Delta	GT	88	31.51	Delta	TB	88	11.66
Delta	GT	105/125	24.66	Delta	TB	105/125	11.66
Delta	GT	144	6.85	Delta	TB	144	0.35
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	ML	48	6.24	Delta	RT	48	1.88
Delta	ML	56	30.76	Delta	RT	56	27.00
Delta	ML	72	31.95	Delta	RT	72	40.66
Delta	ML	88	18.28	Delta	RT	88	20.25
Delta	ML	105/125	11.00	Delta	RT	105/125	9.11
Delta	ML	144	1.78	Delta	RT	144	1.10
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	OT	48	1.42	Delta	JC	48	13.22

Delta	OT	56	12.77	Delta	JC	56	54.02
Delta	OT	72	24.11	Delta	JC	72	22.99
Delta	OT	88	26.95	Delta	JC	88	6.32
Delta	OT	105/125	32.62	Delta	JC	105/125	3.45
Delta	OT	144	2.13	Delta	JC	144	0.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	CA	48	5.53	Delta	BC	48	1.07
Delta	CA	56	16.58	Delta	BC	56	17.70
Delta	CA	72	29.65	Delta	BC	72	29.67
Delta	CA	88	28.14	Delta	BC	88	23.35
Delta	CA	105/125	17.59	Delta	BC	105/125	24.71
Delta	CA	144	2.51	Delta	BC	144	3.50
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RC	48	14.88	Delta	F80/3	48	3.93
Delta	RC	56	39.50	Delta	F80/3	56	31.20
Delta	RC	72	27.93	Delta	F80/3	72	34.50
Delta	RC	88	7.93	Delta	F80/3	88	16.94
Delta	RC	105/125	7.77	Delta	F80/3	105/125	10.95
Delta	RC	144	1.98	Delta	F80/3	144	2.48
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	JT	48	0.41	Delta	Sunki 1112	48	4.05
Delta	JT	56	14.23	Delta	Sunki 1112	56	31.14
Delta	JT	72	32.52	Delta	Sunki 1112	72	29.11
Delta	JT	88	23.58	Delta	Sunki 1112	88	18.99
Delta	JT	105/125	26.42	Delta	Sunki 1112	105/125	12.91
Delta	JT	144	2.85	Delta	Sunki 1112	144	3.80
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RL-S	48	3.37	Delta	ST	48	2.01
Delta	RL-S	56	24.00	Delta	ST	56	22.11
Delta	RL-S	72	30.11	Delta	ST	72	30.15
Delta	RL-S	88	23.16	Delta	ST	88	25.13
Delta	RL-S	105/125	18.32	Delta	ST	105/125	19.10
Delta	RL-S	144	1.05	Delta	ST	144	1.51
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SC	48	0.46	Delta	SFS	48	2.77
Delta	SC	56	15.74	Delta	SFS	56	28.22
Delta	SC	72	27.78	Delta	SFS	72	35.27
Delta	SC	88	25.00	Delta	SFS	88	19.09
Delta	SC	105/125	23.61	Delta	SFS	105/125	12.72
Delta	SC	144	7.41	Delta	SFS	144	1.94
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RP	48	4.15	Delta	Sunki 1116	48	9.51
Delta	RP	56	15.07	Delta	Sunki 1116	56	50.24
Delta	RP	72	26.42	Delta	Sunki 1116	72	27.07
Delta	RP	88	20.63	Delta	Sunki 1116	88	9.27
Delta	RP	105/125	28.28	Delta	Sunki 1116	105/125	2.93
Delta	RP	144	5.46	Delta	Sunki 1116	144	0.98
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SM	48	3.56	Delta	RL-W	48	8.73
Delta	SM	56	24.11	Delta	RL-W	56	35.37
Delta	SM	72	41.11	Delta	RL-W	72	37.55
Delta	SM	88	18.18	Delta	RL-W	88	12.23
Delta	SM	105/125	11.86	Delta	RL-W	105/125	6.11

Delta	SM	144	1.19	Delta	RL-W	144	0.00
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Table 6.2.4.2.5. Production per tree of Delta Valencia's on different rootstocks at Moosriver Estate (Marble Hall) during the 2004 season.

Cultivar	Rootstock	Kg/tree
Delta Valencia	F80/8	33.4
Delta Valencia	PT	32.5
Delta Valencia	C32	25.4
Delta Valencia	AT	11.8
Delta Valencia	HRS 812	24.0
Delta Valencia	K	3.7
Delta Valencia	CM	51.0
Delta Valencia	C35	27.4
Delta Valencia	C	3.7
Delta Valencia	SCS	5.5
Delta Valencia	X639	26.3
Delta Valencia	GT	5.8
Delta Valencia	ML	35.4
Delta Valencia	OT	6.9
Delta Valencia	CA	11.2
Delta Valencia	RC	37.4
Delta Valencia	JT	30.8
Delta Valencia	RL-S	25.1
Delta Valencia	SC	10.9
Delta Valencia	RP	45.7
Delta Valencia	SM	13.3
Delta Valencia	ChM	9.0
Delta Valencia	N	11.3
Delta Valencia	RxT	22.5
Delta Valencia	RL-C	26.8
Delta Valencia	CLM	9.6
Delta Valencia	Sunki 1113	28.7
Delta Valencia	CO	11.3
Delta Valencia	CC	22.0
Delta Valencia	TC	11.1
Delta Valencia	Volk	28.1
Delta Valencia	KC	32.7
Delta Valencia	TB	16.9
Delta Valencia	RT	35.7
Delta Valencia	JC	11.9
Delta Valencia	BC	47.7
Delta Valencia	F80/3	27.1
Delta Valencia	Sunki 1112	22.9
Delta Valencia	ST	10.6
Delta Valencia	SFS	40.1
Delta Valencia	Sunki 1116	25.8
Delta Valencia	RL-W	14.2

6.2.4.3 Evaluation of Star Ruby grapefruit, Midnight and Delta Valencias in the Limpopo Province Experiment 137 by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer.

Die Star Ruby seleksies het 'n beter oes geproduseer met dieselfde vruggrootte in vergelyking met die 2003 seisoen. Die Delta Valencia seleksies het gemiddeld 'n swakker oes geproduseer as in die 2003 seisoen en die groter meerderheid bome se vruggrootte was telling 105/125 gewees. Die Midnight Valencia seleksies wat nie in 2003 geëvalueer kon word nie, het 'n goeie oes geproduseer (neem droogte in ag). Die meerderheid bome het 'n vruggrootte tussen telling 72 en 88 geproduseer. Die TOV persentasie by al drie varieteite is laer en in meeste gevalle voldoen dit nie aan die minimum verpakkings spesifikasies nie. Die Sap- en Suur persentasies voldoen aan die spesifikasies en lyk baie belowend. Die ernstige droogte en water probleme wat ook in hierdie produksie area voorgekom het, speel 'n baie groot rol op produksie en interne kwaliteit van die vrugte.

Introduction

The objective is to evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Star Ruby grapefruit, Midnight Valencia and Delta Valencia. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Star Ruby grapefruit, Midnight Valencia and Delta Valencia were grafted onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates in Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per rootstock (total of 20 trees per rootstock).

Table 6.2.4.3.1. List of scion/rootstock combinations evaluated in the Letsitele area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Delta Valencia	Limpopo	Letaba Estate	F80/9	1997	10
Delta Valencia	Limpopo	Letaba Estate	SC	1997	10
Delta Valencia	Limpopo	Letaba Estate	CC	1997	10
Delta Valencia	Limpopo	Letaba Estate	F80/3	1997	10
Delta Valencia	Limpopo	Letaba Estate	C35	1997	10
Delta Valencia	Limpopo	Letaba Estate	KC	1997	10
Delta Valencia	Limpopo	Letaba Estate	MxT	1997	10
Delta Valencia	Limpopo	Letaba Estate	BC	1997	10
Delta Valencia	Limpopo	Letaba Estate	X639	1997	10
Delta Valencia	Limpopo	Letaba Estate	RL-C	1997	8
Midnight Valencia	Limpopo	Letaba Estate	RL-C	1997	10
Midnight Valencia	Limpopo	Letaba Estate	F80/9	1997	10
Midnight Valencia	Limpopo	Letaba Estate	BC	1997	10
Midnight Valencia	Limpopo	Letaba Estate	MxT	1997	10
Midnight Valencia	Limpopo	Letaba Estate	SC	1997	10
Midnight Valencia	Limpopo	Letaba Estate	F80/3	1997	10
Midnight Valencia	Limpopo	Letaba Estate	CC	1997	10
Midnight Valencia	Limpopo	Letaba Estate	C35	1997	10
Midnight Valencia	Limpopo	Letaba Estate	KC	1997	10
Midnight Valencia	Limpopo	Letaba Estate	X639	1997	10
Midnight Valencia	Limpopo	Letaba Estate	61AA 3	1997	10
Midnight Valencia	Limpopo	Letaba Estate	79AC 6/2	1997	10
Midnight Valencia	Limpopo	Letaba Estate	75AB 12/3	1997	8
Star Ruby	Limpopo	Letaba Estate	BC	1997	10
Star Ruby	Limpopo	Letaba Estate	CC	1997	10
Star Ruby	Limpopo	Letaba Estate	C35	1997	10

Star Ruby	Limpopo	Letaba Estate	F80/3	1997	10
Star Ruby	Limpopo	Letaba Estate	F80/9	1997	7
Star Ruby	Limpopo	Letaba Estate	KC	1997	10
Star Ruby	Limpopo	Letaba Estate	MxT	1997	10
Star Ruby	Limpopo	Letaba Estate	RL-C	1997	9
Star Ruby	Limpopo	Letaba Estate	SC	1997	10
Star Ruby	Limpopo	Letaba Estate	X639	1997	9

Results and discussion

Star Ruby

Trees were evaluated at Letaba Estates (Letsitele), in Limpopo during the 2004 season.

The production on the trees increased compared to the 2003 season. SC produced the highest yield (150.3 kg per tree) followed by CC (149.2 kg per tree). F80/3 and MxT produced the third highest yield (141.2 kg - 142.6 kg per tree) with RL-C producing the lowest yield for this season (78.4 kg per tree).

The fruit size produced on the different rootstock combinations with Star Ruby remained more or less the same and peaked at count 48, followed by count 64 and 40.

The internal quality test showed a decrease in TSS % on average from 10.84 % to 8.52 %. The explanation might be due to the drought and water problems in the area or the fact that the fruit had been harvested one month later than in the 2003 season. All the other internal contents met the packing specifications. Maturity seems to be end of April.

Delta Valencia

Trees were evaluated at Letaba Estates (Letsitele), in Limpopo during the 2004 season.

The internal quality of the fruit evaluated decreased in comparison to the 2003 season. F80/9 was the only rootstock that met the packing specifications. The TSS % of all the other rootstocks was below the specifications of 10.5 %. CC, C35 and BC did not comply with the minimum Acid % (0.85 %). RL-C (9.1 seeds) had the most seed.

C35 and MxT had the best fruit size distribution between count 72 and 88, followed by F80/9, CC, F80/3 and KC. The smallest fruit size was on X639 and RL-C, between counts 105/125 and 144.

RL-C produced the highest yield per tree (68.3 kg) despite the small fruit size, followed by SC (53.1 kg) and KC (48.9 kg). X639 produced the lowest yield per tree with 6.8 kg. Maturity seems to be at the end of July.

Midnight Valencia

Trees were evaluated at Letaba Estates (Letsitele), in Limpopo during the 2004 season.

The internal quality of the fruit was acceptable with regards to juice % and acid %, but not one of the selections met the packing specifications for the minimum TSS % of 52 %. F80/9 produced the highest TSS % of 10.12 % and C35 the highest juice % (60.9 %).

The best fruit size distribution was produced by BC, SC, F80/3 and KC which peaked between count 72 and 88. The smallest fruit size was produced by 79AC 6/2 and 75AB 12/3 peaking at count 105/125.

SC produced the best production per tree of 82 kg, followed by CC with 74.4 kg and MxT with 70.5 kg. F80/9 produced the lowest yield per tree with 46.9 kg. Maturity seems to be middle of July.

Conclusion and recommendations

The Star Ruby, Delta- and Midnight Valencia selections produced fruit with low TSS percentages, but all the other internal quality factors complied with the specifications. Star Ruby increased on their average yield and export fruit size between count 72 and 88.

Evaluations will continue for the next season. When compared with the trial conducted at Moosriver Estates (Marble Hall), there was a similar reaction with the TSS % lower because of the drought and water problems.

Table 6.2.4.3.2a. Internal fruit quality of Star Ruby grapefruit trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
TSR	RL-C	1/06/2004	Letaba	23-48	55.4	7.48	1.37	5.46	0.1	T1-2
TSR	CC	1/06/2004	Letaba	23-56	58.9	8.20	1.29	6.36	0.6	T1-2
TSR	SC	1/06/2004	Letaba	23-56	57.3	7.18	1.39	5.17	0.2	T1-2
TSR	F80/9	1/06/2004	Letaba	23-40	53.2	9.42	1.61	5.85	1.0	T1-2
TSR	MxT	1/06/2004	Letaba	23-56	53.8	9.22	1.67	5.52	0.3	T1-2
TSR	BC	1/06/2004	Letaba	23-40	59.9	8.50	1.26	6.75	0.7	T1-2
TSR	X 639	1/06/2004	Letaba	23-48	57.9	8.80	1.38	6.38	0.4	T1-2
TSR	KC	1/06/2004	Letaba	27-64	59.7	8.70	1.24	7.02	0.3	T1-2
TSR	C-35	1/06/2004	Letaba	23-48	52.9	8.80	1.58	5.57	0.5	T1-2
TSR	F80/3	1/06/2004	Letaba	32-64	56.3	8.90	1.61	5.53	1.5	T1-2

Table 6.2.4.3.2b. Fruit Size distribution per rootstock of Star Ruby grapefruit trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Size	% Fruit
Star Ruby	RL-C	27	6.07
Star Ruby	RL-C	32	4.50
Star Ruby	RL-C	36	11.37
Star Ruby	RL-C	40	19.89
Star Ruby	RL-C	48	34.79
Star Ruby	RL-C	64	23.37
Cultivar	Rootstock	Size	% Fruit
Star Ruby	SC	27	2.13
Star Ruby	SC	32	3.19
Star Ruby	SC	36	11.93
Star Ruby	SC	40	23.93
Star Ruby	SC	48	40.62
Star Ruby	SC	64	18.19
Cultivar	Rootstock	Size	% Fruit
Star Ruby	F80/3	27	3.18
Star Ruby	F80/3	32	2.43
Star Ruby	F80/3	36	8.13
Star Ruby	F80/3	40	17.11
Star Ruby	F80/3	48	35.73
Star Ruby	F80/3	64	33.42
Cultivar	Rootstock	Size	% Fruit
Star Ruby	CC	27	2.22
Star Ruby	CC	32	2.94
Star Ruby	CC	36	9.35
Star Ruby	CC	40	18.64
Star Ruby	CC	48	40.14
Star Ruby	CC	64	26.71
Cultivar	Rootstock	Size	% Fruit
Star Ruby	KC	27	3.96
Star Ruby	KC	32	3.16
Star Ruby	KC	36	11.99
Star Ruby	KC	40	21.30
Star Ruby	KC	48	39.05
Star Ruby	KC	64	20.54
Cultivar	Rootstock	Size	% Fruit

Star Ruby	BC	27	2.46
Star Ruby	BC	32	3.23
Star Ruby	BC	36	10.28
Star Ruby	BC	40	19.66
Star Ruby	BC	48	40.16
Star Ruby	BC	64	24.21
Cultivar	Rootstock	Size	% Fruit
Star Ruby	MxT	27	2.42
Star Ruby	MxT	32	2.33
Star Ruby	MxT	36	7.91
Star Ruby	MxT	40	16.99
Star Ruby	MxT	48	38.60
Star Ruby	MxT	64	31.75
Cultivar	Rootstock	Size	% Fruit
Star Ruby	C35	27	1.11
Star Ruby	C35	32	1.58
Star Ruby	C35	36	6.15
Star Ruby	C35	40	18.55
Star Ruby	C35	48	44.64
Star Ruby	C35	64	27.98
Cultivar	Rootstock	Size	% Fruit
Star Ruby	X639	27	1.28
Star Ruby	X639	32	1.82
Star Ruby	X639	36	8.76
Star Ruby	X639	40	21.50
Star Ruby	X639	48	47.17
Star Ruby	X639	64	19.47
Cultivar	Rootstock	Size	% Fruit
Star Ruby	F80/9	27	2.74
Star Ruby	F80/9	32	4.14
Star Ruby	F80/9	36	17.89
Star Ruby	F80/9	40	29.35
Star Ruby	F80/9	48	36.27
Star Ruby	F80/9	64	9.62

Table 6.2.4.3.2c. Production per tree of Star Ruby Grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Kg/tree
Star Ruby	RL-C	78.4
Star Ruby	SC	150.3
Star Ruby	F80/3	142.6
Star Ruby	CC	149.2
Star Ruby	KC	87.1
Star Ruby	BC	118.7
Star Ruby	MxT	141.2
Star Ruby	C35	117.7
Star Ruby	X639	99.3
Star Ruby	F80/9	94.9

Table 6.2.4.3.3a. Internal fruit quality data for Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
DV	F80/9	4/08/2004	Letaba	48-105	54.6	10.83	1.00	10.83	0.0	T1
DV	SC	4/08/2004	Letaba	56-88	56.1	9.60	0.91	10.55	0.0	T1-3
DV	CC	4/08/2004	Letaba	40-88	55.8	8.87	0.80	11.09	0.3	T1-2
DV	F80/3	4/08/2004	Letaba	48-88	53.2	9.80	0.88	11.14	0.2	T1-2
DV	C-35	4/08/2004	Letaba	56-88	57.6	10.30	1.04	9.90	0.0	T1-4
DV	KC	4/08/2004	Letaba	56-88	54.1	9.07	0.83	10.93	0.0	T2-3
DV	MxT	4/08/2004	Letaba	40-72	54.6	9.40	0.90	10.44	0.2	T2
DV	BC	4/08/2004	Letaba	48-88	55.7	9.50	0.80	11.88	0.0	T1-3
DV	X639	4/08/2004	Letaba	64-125	55.4	9.50	0.90	10.56	0.9	T1
DV	RL-C	4/08/2004	Letaba	72-125	55.2	8.37	1.40	5.98	9.1	T4-5

Table 6.2.4.3.3b. Fruit Size distribution per rootstock of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Size	% Fruit
Delta	F80/9	48	0.62
Delta	F80/9	56	9.98
Delta	F80/9	72	23.19
Delta	F80/9	88	20.39
Delta	F80/9	105/125	32.54
Delta	F80/9	144	13.28
Cultivar	Rootstock	Size	% Fruit
Delta	SC	48	0.20
Delta	SC	56	10.99
Delta	SC	72	26.98
Delta	SC	88	27.34
Delta	SC	105/125	28.88
Delta	SC	144	5.60
Cultivar	Rootstock	Size	% Fruit
Delta	CC	48	0.22
Delta	CC	56	4.72
Delta	CC	72	18.34
Delta	CC	88	23.85
Delta	CC	105/125	37.46
Delta	CC	144	15.41
Cultivar	Rootstock	Size	% Fruit
Delta	F80/3	48	0.15
Delta	F80/3	56	5.59
Delta	F80/3	72	18.32
Delta	F80/3	88	23.47
Delta	F80/3	105/125	39.59
Delta	F80/3	144	12.88
Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	0.57
Delta	C35	56	7.18
Delta	C35	72	24.43
Delta	C35	88	25.57
Delta	C35	105/125	25.29
Delta	C35	144	16.95
Cultivar	Rootstock	Size	% Fruit

Delta	KC	48	0.09
Delta	KC	56	3.83
Delta	KC	72	18.93
Delta	KC	88	27.02
Delta	KC	105/125	38.90
Delta	KC	144	11.23
Cultivar	Rootstock	Size	% Fruit
Delta	MxT	48	1.08
Delta	MxT	56	14.31
Delta	MxT	72	27.72
Delta	MxT	88	24.75
Delta	MxT	105/125	25.20
Delta	MxT	144	6.93
Cultivar	Rootstock	Size	% Fruit
Delta	BC	48	0.25
Delta	BC	56	10.07
Delta	BC	72	22.50
Delta	BC	88	23.10
Delta	BC	105/125	31.35
Delta	BC	144	12.73
Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	0.00
Delta	X639	56	1.11
Delta	X639	72	6.09
Delta	X639	88	13.30
Delta	X639	105/125	46.81
Delta	X639	144	32.69
Cultivar	Rootstock	Size	% Fruit
Delta	RL-C	48	0.03
Delta	RL-C	56	0.06
Delta	RL-C	72	0.58
Delta	RL-C	88	5.52
Delta	RL-C	105/125	55.18
Delta	RL-C	144	38.64

Table 6.2.4.3.3c. Production per tree of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Kg/tree
Delta	F80/9	32.9
Delta	SC	53.1
Delta	CC	18.2
Delta	F80/3	30.0
Delta	C35	7.5
Delta	KC	48.9
Delta	MxT	26.6
Delta	BC	43.5
Delta	X639	6.8
Delta	RL-C	68.3

Table 6.2.4.3.4a. Internal fruit quality data for Midnight Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
MM	RL-C	12/07/2004	Letaba	48-72	56.5	8.10	1.16	6.98	0.7	T2-3
MM	F80/9	12/07/2004	Letaba	56-105	57.7	10.12	1.27	7.97	0.5	T1-2
MM	BC	12/07/2004	Letaba	48-72	58.5	9.00	1.01	8.91	0.2	T1-2
MM	MxT	12/07/2004	Letaba	48-88	59.2	9.52	1.26	7.56	0.8	T1-2
MM	SC	12/07/2004	Letaba	48-72	59.1	9.22	1.19	7.75	0.7	T1-3
MM	F80/3	12/07/2004	Letaba	48-72	57.2	9.42	1.03	9.15	0.4	T1-2
MM	CC	12/07/2004	Letaba	48-72	56.7	8.90	1.21	7.36	0.8	T1-2
MM	C35	12/07/2004	Letaba	48-72	60.9	9.82	1.22	8.05	0.3	T1-2
MM	KC	12/07/2004	Letaba	48-72	56.7	8.90	0.97	9.18	0.5	T1-2
MM	X639	12/07/2004	Letaba	64-72	58.0	8.60	1.05	8.19	0.4	T1-2
MM	61AA 3	12/07/2004	Letaba	56-72	50.6	8.60	1.16	7.41	0.0	T4
MM	79AC 6/2	12/07/2004	Letaba	56-88	56.7	9.32	1.07	8.71	0.0	T3
MM	75AB 12/3	12/07/2004	Letaba	56-105	53.6	9.72	1.05	9.26	0.0	T2-3

Table 6.2.4.3.4b. Fruit size distribution per rootstock of Midnight Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Size	% Fruit
Midnight	RL-C	48	0.00
Midnight	RL-C	56	7.36
Midnight	RL-C	72	19.91
Midnight	RL-C	88	27.38
Midnight	RL-C	105/125	36.94
Midnight	RL-C	144	8.41
Cultivar	Rootstock	Size	% Fruit
Midnight	F80/9	48	0.67
Midnight	F80/9	56	6.20
Midnight	F80/9	72	17.11
Midnight	F80/9	88	24.10
Midnight	F80/9	105/125	38.57
Midnight	F80/9	144	13.34
Cultivar	Rootstock	Size	% Fruit
Midnight	BC	48	0.94
Midnight	BC	56	18.58
Midnight	BC	72	31.01
Midnight	BC	88	26.48
Midnight	BC	105/125	21.01
Midnight	BC	144	1.97
Cultivar	Rootstock	Size	% Fruit
Midnight	MxT	48	0.31
Midnight	MxT	56	6.13
Midnight	MxT	72	17.82
Midnight	MxT	88	26.24
Midnight	MxT	105/125	39.08
Midnight	MxT	144	10.43
Cultivar	Rootstock	Size	% Fruit
Midnight	SC	48	0.56
Midnight	SC	56	13.48
Midnight	SC	72	30.65
Midnight	SC	88	28.63

Midnight	SC	105/125	23.37
Midnight	SC	144	3.31
Cultivar	Rootstock	Size	% Fruit
Midnight	F80/3	48	1.44
Midnight	F80/3	56	18.13
Midnight	F80/3	72	30.22
Midnight	F80/3	88	25.24
Midnight	F80/3	105/125	21.78
Midnight	F80/3	144	3.19
Cultivar	Rootstock	Size	% Fruit
Midnight	CC	48	0.81
Midnight	CC	56	10.20
Midnight	CC	72	23.91
Midnight	CC	88	24.33
Midnight	CC	105/125	32.70
Midnight	CC	144	8.04
Cultivar	Rootstock	Size	% Fruit
Midnight	C35	48	1.46
Midnight	C35	56	13.79
Midnight	C35	72	23.65
Midnight	C35	88	19.05
Midnight	C35	105/125	25.74
Midnight	C35	144	16.31
Cultivar	Rootstock	Size	% Fruit
Midnight	KC	48	0.16
Midnight	KC	56	10.64
Midnight	KC	72	29.10
Midnight	KC	88	30.07
Midnight	KC	105/125	27.45
Midnight	KC	144	2.58
Cultivar	Rootstock	Size	% Fruit
Midnight	X639	48	0.19
Midnight	X639	56	3.68
Midnight	X639	72	17.52
Midnight	X639	88	28.43
Midnight	X639	105/125	38.56
Midnight	X639	144	11.63
Cultivar	Rootstock	Size	% Fruit
Midnight	61AA 3	48	0.48
Midnight	61AA 3	56	10.52
Midnight	61AA 3	72	22.75
Midnight	61AA 3	88	24.93
Midnight	61AA 3	105/125	31.40
Midnight	61AA 3	144	9.91
Cultivar	Rootstock	Size	% Fruit
Midnight	79AC 6/2	48	0.06
Midnight	79AC 6/2	56	1.76
Midnight	79AC 6/2	72	11.44
Midnight	79AC 6/2	88	23.90
Midnight	79AC 6/2	105/125	46.01
Midnight	79AC 6/2	144	16.84
Cultivar	Rootstock	Size	% Fruit
Midnight	75AB 12/3	48	0.12
Midnight	75AB 12/3	56	1.69

Midnight	75AB 12/3	72	11.42
Midnight	75AB 12/3	88	23.94
Midnight	75AB 12/3	105/125	48.40
Midnight	75AB 12/3	144	14.44

Table 6.2.4.3.4c. Production per tree of Midnight Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2004 season.

Cultivar	Rootstock	Kg/tree
Midnight	RL-C	62.7
Midnight	F80/9	46.9
Midnight	BC	56.3
Midnight	MxT	70.5
Midnight	SC	82.0
Midnight	F80/3	65.3
Midnight	CC	74.4
Midnight	C35	66.5
Midnight	KC	62.7
Midnight	X639	58.9
Midnight	61AA 3	64.8
Midnight	79AC 6/2	64.7
Midnight	75AB 12/3	44.1

6.2.4.4 Evaluation of Valencia and Navel varieties on different rootstocks in the Vaalharts area Experiment 146 by J.Joubert (CRI)

Opsomming

Die prestasie van Valencia en Navel variëteite op verskillende onderstamme te ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die beste produksie, vruggrootte, interne kwaliteit en eksterne kleur moet verkry word vir uitvoere na bv. die VSA markte.

Die perseel in Vaalharts waar die proef aangeplant is, het ernstige koue laat in Augustusmaand ervaar met temperature so laag as -7°C . Die Navelina bome is die meeste deur die koue beskadig en enkele bome is selfs dood. Die C35 onderstam toon die mees gevoeligste vir koue skade. Die Delta Valencia's het die minste koue skade opgedoen en van die seleksies het selfs 'n ligte oes geproduseer. Die bome is gesnoei en goed bemes om spoedige herstel te verseker.

Introduction

The progress of Valencia and Navel varieties on different rootstocks must be investigated and the best rootstock determined for the Vaalharts area in order to optimise production, fruit size, internal quality and external fruit colour for export to markets such as the USA.

Materials and methods

Field evaluations and laboratory analysis were conducted on Bahianinha navels on the following rootstocks: C32, C35, F80, IRL, KC, MxT, RL-W, RxT, SFS, TB, X639; Delta Valencias on BT, C35, CC, F80, SFS, X639; Midnight Valencias on C35, CC, X639 and Royal Late on C32, C35, CC, GT, RC.

Table 6.2.4.4.1. List of Valencia and Navel selections on different rootstocks evaluated in the Vaalharts area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Bahianinha	Northern Cape	Vaalharts	C32	1998	6
Bahianinha	Northern Cape	Vaalharts	C35	1998	6
Bahianinha	Northern Cape	Vaalharts	F80	1998	6
Bahianinha	Northern Cape	Vaalharts	IRL	1998	5

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Bahianinha	Northen Cape	Vaalharts	KC	1998	6
Bahianinha	Northen Cape	Vaalharts	MxT	1998	6
Bahianinha	Northen Cape	Vaalharts	RL-W	1998	3
Bahianinha	Northen Cape	Vaalharts	RxT	1998	6
Bahianinha	Northen Cape	Vaalharts	SFS	1998	5
Bahianinha	Northen Cape	Vaalharts	TB	1998	6
Bahianinha	Northen Cape	Vaalharts	X639	1998	6
Delta Valencia	Northen Cape	Vaalharts	BC	1998	6
Delta Valencia	Northen Cape	Vaalharts	C35	1998	6
Delta Valencia	Northen Cape	Vaalharts	CC	1998	6
Delta Valencia	Northen Cape	Vaalharts	F80	1998	6
Delta Valencia	Northen Cape	Vaalharts	SFS	1998	6
Delta Valencia	Northen Cape	Vaalharts	X639	1998	6
Midknight	Northen Cape	Vaalharts	C35	1998	6
Midknight	Northen Cape	Vaalharts	CC	1998	6
Midknight	Northen Cape	Vaalharts	X639	1998	6
Royal Late	Northen Cape	Vaalharts	BC	1998	6
Royal Late	Northen Cape	Vaalharts	C32	1998	6
Royal Late	Northen Cape	Vaalharts	C35	1998	6
Royal Late	Northen Cape	Vaalharts	CC	1998	6
Royal Late	Northen Cape	Vaalharts	GT	1998	6
Royal Late	Northen Cape	Vaalharts	RC	1998	6

Results and discussion

This area received severe cold weather late in August 2003. Bahianinha on all the rootstock selections produced no fruit to be evaluated and fortunately the trees were not damaged by the cold. The Navelina selections were not tolerant to the cold and on C35/RxT rootstocks the trees died back. Royal Late reacted similar to the Bahianinha selections and produced no fruit to evaluate, fortunately no trees died. All the Delta selections seems tolerant to the cold weather with X639 and CC bearing some fruit. There was less cold damage to the trees compared to the other selections. The Midnight Valencias bore no fruit to evaluate and once again C35 showed more cold damage in comparison to the other rootstocks.

The trees were pruned to remove all the dead branches and fertilised to optimise the recovery process. Hopefully the trees will set fruit to evaluate for the next production season.

6.2.4.5 Evaluation of Valencia and Grapefruit varieties on new imported rootstocks in the Malelane and Swaziland area

Experiment 416 by J.Joubert (CRI)

Opsomming

Die prestasie van Valencia- en Pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Die produksie, vruggrootte, interne gehalte en skilkleur moet verbeter word, terwyl vruggrootte verminder moet word.

Die Star Ruby en Marsh bome het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate. Die gemiddelde vruggrootte het op telling 48 gestabiliseer en die interne kwaliteit lyk belowend, behalwe vir die effense lae TOV persentasies.

By die Delta's het TOV gedaal onder die uitvoer standaard en produksie met 50% afgeneem. Die Midnight Valencia bome het ook probleme ondervind met TOV% en produksie wat ernstig gedaal het. Hierdie tendens kan moontlik toegeskryf word aan onvoldoende besproeiing (droogte) en 'n ligte oes aan die bome.

Introduction

The progress of Valencia and Grapefruit varieties on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved and at the same time fruit size must be decreased.

Materials and methods

Identical methods were used for both sites. Seed of HRS 802, HRS 812, HRS 809 and C61 were imported and propagated in 1996 by Esselen Nursery, Malelane.

Star Ruby grapefruit was grafted onto four rootstock hybrids, Marsh grapefruit onto three rootstocks, and Oroblanco onto one rootstock in 1997. The newly imported rootstock hybrids include: Pummelo x trifoliolate orange 802, Changsa x English large flowered trifoliolate orange, Sunki x Beneke HRS 812 and Sunki x macrophylla C61. The trees were planted at Tambankulu Estates, Swaziland, in 1999. Experimental trees at Tambankulu Estates were transplanted at Tambuti Estates in Swaziland during November 2000 as certain orchards were to be removed from Tambankulu Estates. The trees were cut back and painted with PVA. Making use of a back-actor, the trees were uprooted and transplanted immediately at the new site. The trees were well watered and in good condition at the time of transplanting.

Delta Valencia was grafted onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: Sunki x Beneke HRS 802, Sunki x Beneke HRS 812 and Sunki x MTO trifoliolate orange (FF6). Midnight Valencia was grafted onto Sunki x Beneke HRS 812. The trees were planted at Esselen Nursery in March 1999.

Table 6.2.4.5.1. List of grapefruit, Pummelo and Valencia selections on various rootstocks evaluated in the Swaziland and Malelane area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No.of trees
Marsh	Swaziland	Tambuti	812	1999	1
Marsh	Swaziland	Tambuti	809	1999	2
Marsh	Swaziland	Tambuti	C61	1999	4
Oroblanco	Swaziland	Tambuti	C61	1999	5
Star Ruby	Swaziland	Tambuti	C32	1999	4
Star Ruby	Swaziland	Tambuti	802	1999	2
Star Ruby	Swaziland	Tambuti	809	1999	1
Star Ruby	Swaziland	Tambuti	812	1999	2
Star Ruby	Swaziland	Tambuti	C61	1999	5
Star Ruby	Swaziland	Tambuti	C35	1999	4
Midnight	Malelane	Esselen	Sunki 812	1999	5
Delta	Malelane	Esselen	Sunki 812	1999	5
Delta	Malelane	Esselen	Sunki 802	1999	5
Delta	Malelane	Esselen	FF-6	1999	5

Results and discussions

Star Ruby

Trees were evaluated at Tambuti Estates (Big Bend) in Swaziland during the 2004 season.

Star Ruby produced fruit on all the rootstocks selections with an acceptable internal quality with high Juice%, but the TSS % lower than the 2003 season (8.05 %-9.50 %). The minimum TSS % for export Star Ruby grapefruit in the 2004 season was 8.5 %. The following rootstock selections (C32, C35, Sunki 802, Sunki 809 and Sunki 812) complied with the specifications. The average fruit size peaked at count 48, followed by count 40 and count 36. Star Ruby on C32 produced the highest yield per tree (128.5 kg), followed by C35 (104.1 kg) and SC (101.6 kg). The lowest fruit producer was on C61 with 69.6 kg.

Marsh

Trees were evaluated at Tambuti Estates (Big Bend), in Swaziland during the 2004 season.

The export specifications for Marsh grapefruit TSS is 8.0 %. Sunki 812 (8.37 %) and C61 (8.15 %) exceeded 8.0 %, but Sunki 809 was below the minimum at 7.65 %. Fruit size distribution peaked at count 40, followed by count 36 and count 48. The highest yield was 80.5 kg fruit per tree on C61, followed by Sunki 809 (78.5 kg) and Sunki 812 (56.0 kg).

Midnight Valencia

Trees were evaluated at Esselen Nursery (Malelane), in Mpumalanga during the 2004 season.

The internal quality test of 9.85 % TSS was below the Capespan packing specifications of 10.5%. The juice % and acid % met the specifications. Compared to 2003 there was a severe decrease in production dropping from 46.3 kg to 17.3 kg. This caused the fruit size to increase and peak at count 48 (41.85 %), followed by count 56 (37.5 %) and count 72 (16.30 %).

Delta Valencia

Trees were evaluated at Esselen Nursery (Malelane) in Mpumalanga during the 2004 season.

There was also a decrease of about 50 % in yield on average compared to 2003. The fruit evaluated didn't comply with the packing specifications with low TSS and Acid %. Compared to the 2003 season the fruit size increased on all three selections evaluated.

Conclusion and recommendations

The Star Ruby and Marsh trees were evaluated for the second season and there was a promising increase in production this season. The TSS values decreased slightly, but could have been influenced by the drought and water problems. Evaluations will continue.

Table 6.2.4.5.2a. Internal fruit quality data for Grapefruit and Pummelo on different rootstocks at Tambuti Estates during the 2004 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Marsh	812	11/05/2004	Tambuti	27-56	57.4	8.37	1.06	7.90	3.4	T4-5
Marsh	809	11/05/2004	Tambuti	23-56	55.1	7.65	1.07	7.15	3.0	T4-5
Marsh	C61	11/05/2004	Tambuti	27-48	56.3	8.15	1.17	6.97	2.8	T4-5
Oroblanco	C61	11/05/2004	Tambuti	23-40	36.6	7.85	0.64	12.27	0.0	T5-6
Star Ruby	SC-C1	11/05/2004	Tambuti	32-56	59.2	8.15	1.20	6.79	0.3	T1-2
Star Ruby	SC-C2	11/05/2004	Tambuti	27-40	58.8	8.15	1.17	6.97	0.1	T1-2
Star Ruby	C32	11/05/2004	Tambuti	27-56	58.8	9.17	1.13	8.12	0.2	T1-2
Star Ruby	802	11/05/2004	Tambuti	32-48	59.1	9.50	1.27	7.48	0.4	T1-2
Star Ruby	809	11/05/2004	Tambuti	32-56	58.5	8.47	1.12	7.56	0.4	T1-2
Star Ruby	812	11/05/2004	Tambuti	27-56	59.3	9.07	1.06	8.56	0.3	T1-2
Star Ruby	C61	11/05/2004	Tambuti	27-48	58.4	8.05	1.12	7.19	0.0	T1-2
Star Ruby	C35	11/05/2004	Tambuti	27-56	58.5	8.97	1.20	7.48	0.5	T1-2

Table 6.2.4.5.2b. Fruit size distribution per rootstock at Tambuti Estate during the 2004 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 61	27	1.27	Marsh	C 61	27	3.16
Star Ruby	C 61	32	5.19	Marsh	C 61	32	5.58
Star Ruby	C 61	36	17.14	Marsh	C 61	36	17.70
Star Ruby	C 61	40	30.66	Marsh	C 61	40	35.62
Star Ruby	C 61	48	39.28	Marsh	C 61	48	31.72
Star Ruby	C 61	64	6.46	Marsh	C 61	64	6.22
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	3.19	Marsh	Sunki 812	27	3.25
Star Ruby	Sunki 812	32	1.77	Marsh	Sunki 812	32	10.39
Star Ruby	Sunki 812	36	11.33	Marsh	Sunki 812	36	24.03
Star Ruby	Sunki 812	40	34.34	Marsh	Sunki 812	40	37.01
Star Ruby	Sunki 812	48	42.30	Marsh	Sunki 812	48	18.83
Star Ruby	Sunki 812	64	7.08	Marsh	Sunki 812	64	6.49
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 809	27	1.35	Marsh	Sunki 809	27	5.30
Star Ruby	Sunki 809	32	2.69	Marsh	Sunki 809	32	8.69
Star Ruby	Sunki 809	36	17.04	Marsh	Sunki 809	36	23.94
Star Ruby	Sunki 809	40	33.63	Marsh	Sunki 809	40	34.96

Star Ruby	Sunki 809	48	42.15	Marsh	Sunki 809	48	23.52
Star Ruby	Sunki 809	64	3.14	Marsh	Sunki 809	64	3.60
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 802	27	1.73	Oroblanco	C 61	27	45.45
Star Ruby	Sunki 802	32	4.32	Oroblanco	C 61	32	18.69
Star Ruby	Sunki 802	36	15.89	Oroblanco	C 61	36	14.90
Star Ruby	Sunki 802	40	31.95	Oroblanco	C 61	40	10.61
Star Ruby	Sunki 802	48	40.41	Oroblanco	C 61	48	7.58
Star Ruby	Sunki 802	64	5.70	Oroblanco	C 61	64	2.78
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 32	27	1.67	Star Ruby	SC-C1	27	0.25
Star Ruby	C 32	32	3.14	Star Ruby	SC-C1	32	1.84
Star Ruby	C 32	36	16.09	Star Ruby	SC-C1	36	18.81
Star Ruby	C 32	40	30.64	Star Ruby	SC-C1	40	36.62
Star Ruby	C 32	48	40.59	Star Ruby	SC-C1	48	36.37
Star Ruby	C 32	64	7.88	Star Ruby	SC-C1	64	6.10
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 35	27	1.53	Star Ruby	SC-C2	27	0.72
Star Ruby	C 35	32	2.82	Star Ruby	SC-C2	32	2.48
Star Ruby	C 35	36	14.66	Star Ruby	SC-C2	36	12.63
Star Ruby	C 35	40	26.56	Star Ruby	SC-C2	40	29.34
Star Ruby	C 35	48	41.68	Star Ruby	SC-C2	48	43.57
Star Ruby	C 35	64	12.75	Star Ruby	SC-C2	64	11.27

Table 6.2.4.5.2c. Production per tree of Grapefruit and Pummelo on different rootstocks at Tambuti Estates during the 2004 season.

Cultivar	Rootstock	Kg/tree
Star Ruby	C 61	69.6
Star Ruby	Sunki 812	93.8
Star Ruby	Sunki 809	78.5
Star Ruby	Sunki 802	100.3
Star Ruby	SC-C1	100.9
Star Ruby	SC-C2	101.6
Star Ruby	C 32	128.5
Star Ruby	C 35	104.1
Marsh	C 61	80.5
Marsh	Sunki 812	56.0
Marsh	Sunki 809	78.5
Oroblanco	C 61	40.5

Table 6.2.4.5.3a. Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) during the 2004 season.

Selection	Root-stock	Date Harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Midnight	Sunki 812	12/08/2004	Esselen	40-64	56.0	9.85	0.92	10.71	0.2	T1
Delta	Sunki 812	12/08/2004	Esselen	40-64	56.7	10.47	0.89	11.76	0.0	T1
Delta	Sunki 802	12/08/2004	Esselen	48-105	57.7	9.95	0.95	10.47	0.0	T1
Delta	FF-6	12/08/2004	Esselen	48-88	55.7	9.85	0.82	12.01	0.0	T1

Table 6.2.4.5.3b. Fruit size distribution per rootstock at Esselen nursery during the 2004 season.

Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	41.85
Midknight	Sunki 812	56	37.50
Midknight	Sunki 812	72	16.30
Midknight	Sunki 812	88	2.72
Midknight	Sunki 812	105/125	1.63
Midknight	Sunki 812	144	0.00
Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 812	48	6.73
Delta	Sunki 812	56	34.42
Delta	Sunki 812	72	26.54
Delta	Sunki 812	88	18.65
Delta	Sunki 812	105/125	13.08
Delta	Sunki 812	144	0.58
Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 802	48	2.08
Delta	Sunki 802	56	17.82
Delta	Sunki 802	72	24.07
Delta	Sunki 802	88	26.16
Delta	Sunki 802	105/125	25.93
Delta	Sunki 802	144	3.94
Cultivar	Rootstock	Size	% Fruit
Delta	FF-6	48	0.70
Delta	FF-6	56	13.11
Delta	FF-6	72	32.87
Delta	FF-6	88	24.30
Delta	FF-6	105/125	25.17
Delta	FF-6	144	3.85

Table 6.2.4.5.3c. Production per tree of Midknight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) during the 2004 season.

Cultivar	Rootstock	Kg/tree
Midknight	Sunki 812	17.3
Delta	Sunki 812	37.0
Delta	Sunki 802	26.9
Delta	FF-6	35.1

6.2.4.6 Evaluation of various new Valencia and navel varieties on different rootstocks at Marble Hall and Komatipoort

Experiment 590 by J.Joubert (CRI)

Opsomming

Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia en nawel varieteite op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe varieteite. Maak betekenisvolle kommersiële aanbevelings vir die produsente.

Bome sal moontlik eerste oes produseer vir evaluasies in die 2005 seisoen.

Introduction

The objective is to evaluate and assess the horticultural performance and capability of various new Valencia and Navel varieties on different rootstocks. Determine the superior rootstock combinations for these new varieties and be able to make credible commercial recommendations.

Materials and methods

Evaluate visually to determine production per tree, trueness to type and compatibility with scion, and harvest each tree with the sizer to determine production and fruit size distribution. Samples will be taken and internal quality tested and analysed. Fruit colour will be evaluated and analysed.

Table 6.2.4.6.1. List of Valencias on various rootstocks evaluated in the Komatipoort area during the 2004 season.

Selection	Area	Site	Rootstock	Tree Age	No.of trees
Delta (Control)	Komatipoort	TSB Hectorspruit	C35	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	CC	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	KC	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	MxT	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	SC	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	Terrabella	2002	5
Delta (Control)	Komatipoort	TSB Hectorspruit	X639	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	C35	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	CC	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	KC	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	MxT	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	SC	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	Terrabella	2002	5
McClellan SL	Komatipoort	TSB Hectorspruit	X639	2002	5
Midnight	Komatipoort	TSB Hectorspruit	C35	2002	5
Midnight	Komatipoort	TSB Hectorspruit	CC	2002	5
Midnight	Komatipoort	TSB Hectorspruit	KC	2002	4
Midnight	Komatipoort	TSB Hectorspruit	MxT	2002	5
Midnight	Komatipoort	TSB Hectorspruit	SC	2002	5
Midnight	Komatipoort	TSB Hectorspruit	Terrabella	2002	5
Midnight	Komatipoort	TSB Hectorspruit	X639	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	C35	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	CC	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	KC	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	MxT	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	SC	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	Terrabella	2002	5
Portsgate	Komatipoort	TSB Hectorspruit	X639	2002	5

Results and discussion

No results – trees not in production.

Conclusion and recommendations

Trees were evaluated visually and will be producing fruit for the first season in 2005. Evaluations will continue.

7 CITRUS IMPROVEMENT PROGRAMME (CIP)

By Thys du Toit and Louise Jackson (CRI)

7.1 PROGRAMME SUMMARY

Citrus Foundation Block (CFB): A total of 2 314 730 buds were supplied by the Citrus Foundation Block during 2004, which is 64 645 less than in 2003. Midnight Valencia and Star Ruby grapefruit were the most popular cultivars. Seed sales increased dramatically in 2004 totalling 4033 litres compared to 2606 litre in 2003. This is mainly due to a large seed order from China for the third consecutive year. In addition a bumper seed harvest was experienced, resulting in a surplus of 907 litres, despite increased sales. Only one new cultivar was received from the ITSC for establishing, evaluating and increasing, compared to 28 scion and 5 rootstock cultivars in 2003. Two-thirds of the new insect-controlled greenhouse has been filled with rootstocks, of which one third has already been budded.

CFB evaluations: Two evaluations of the CFB were conducted during 2004. The CIP Evaluation committee granted the CFB a clean bill of health for both the horticultural trueness-to-type of the propagation material and freedom from phytosanitary diseases.

Nursery Accreditation: Twenty-four nurseries were visited twice during 2004, of which seventeen were accredited, one provisionally accredited and four were not accredited. In general, the standard in nurseries is high.

Tree Certification: During 2004, 554 certificates were processed, representing 1 198 373 trees compared to 593 certificates for 2003, which represented 1 454 996 trees.

Statutory Improvement Programme: No progress has yet been made and an urgent meeting with the Registrar, Plant Improvement, is planned for 2005.

Protected zone around CFB: The application to legislate a citrus-free protected zone around the CFB has been drafted but has not progressed to the point of promulgation by the Department of Agriculture. This will be pursued in 2005.

Shoot-tip-grafting: STG, virus indexing and pre-immunisation have been initiated at CRI's Nelspruit laboratories in support of the CIP.

Gene bank: The industry citrus gene bank at the ARC has been backed up with a duplicate virus-free gene bank at CRI's Nelspruit facilities.

Cultivar Development: Further negotiations took place with the ARC in respect of joint ownership of ITSC cultivars emanating from their breeding programme, but remained unsuccessful. On request from the CGA, CRI created the post of industry Cultivar Development Manager and Dr Graham Barry was appointed to this post as from 1 January 2005.

An overview of operations in the CIP follows.

Citrus Foundation Block

Budwood supply during 2004 compared to 2 preceding years, 10 most popular cultivar selections.

2004			2003			2002		
Selection	Buds	%	Selection	Buds	%	Selection	Buds	%
TOTAL	2314730		TOTAL	2379375		TOTAL	2283982	
Midnight	284815	12.3%	Star Ruby	212450	9.2%	Turkey	383970	16.1%
Star Ruby	275550	11.9%	Bahianinha	210220	9.1%	Bahianinha	262490	11.0%
Bahianinha	183180	7.9%	Turkey	203200	8.8%	Palmer	175410	7.4%
Du Roi	122870	5.3%	Palmer	173730	7.5%	Star Ruby	164810	6.9%

2004	2003					2002		
Selection	Buds	%	Selection	Buds	%	Selection	Buds	%
TOTAL	2314730		TOTAL	2379375		TOTAL	2283982	
Eureka	116350	5.0%	Nadorcott 1	169500	7.3%	Midnight	145450	6.1%
Palmer	113680	4.9%	Midnight	127450	5.5%	Nadorcott 1	91300	3.8%
Delta	109140	4.7%	Eureka	117450	5.1%	Delta	84230	3.5%
Turkey	102440	4.4%	Newhall	102460	4.4%	Miho Wase	69400	2.9%
Nadorcott 1	67070	2.9%	Miho Wase	102100	4.4%	Lane Late Cal.	69015	2.9%
Eureka SL	59432	2.6%	Lina	82050	3.5%	Eureka SL	66400	2.8%

Budwood supply per area during 2004 compared to 2 preceding years.

Area	2004	%	2003	%	2002	%
Eastern Cape	427080	18.5%	546660	23.0%	441902	19.3%
Western Cape	368255	15.9%	365665	15.4%	272850	11.9%
Northern Cape	68720	3.0%	116990	4.9%	156905	6.9%
Kwazulu Natal	16500	0.7%	36030	1.5%	20600	0.9%
Limpopo	1090435	47.1%	930650	39.1%	804120	35.2%
Mpumulanga	228750	9.9%	244590	10.3%	376205	16.5%
North West	86990	3.8%	101100	4.2%	121350	5.3%
Mozambique	0	0.0%	5400	0.2%	0	0.0%
Swaziland	0	0.0%	10600	0.4%	49400	2.2%
Other African States	0	0.0%	0	0.0%	1400	0.1%
Zimbabwe	28000	1.2%	21700	0.9%	39250	1.7%
Total	2314730		2379385		2283982	

Seed supplied per rootstock selection, local and export during 2004.

Area Name	C35	CC	MXT	RL	RLS	SC	TC	VA	X639	YC	Total	Local	Export
Australia/NZ		60		20	11		105				196	0	196
Carribbean						7					7	0	7
China		1500									1500	0	1500
Eastern Cape	20	98		41		20	1	3	23		206	206	0
KwaZulu Natal						2			2		4	4	0
Limpopo	60	450	73	121		437	110	14	52		1317	1317	0
Mozambique		1				7	2		1		11	0	11
Mpumalanga	5	24		14		22	3		4		72	72	0
North West Province	14	45		7		7			2		75	75	0
Northern Cape				5					17		22	22	0
Other African States	2					66	3	118			189	0	189
USA		110									110	0	110
Western Cape	40	118		30	20	45	63		5		321	321	0
Zimbabwe				1			2				3	3	0

Area Name	C35	CC	MXT	RL	RLS	SC	TC	VA	X639	YC	Total	Local	Export
TOTAL	141	2406	73	239	31	613	289	135	106	0	4033	2020	2013

Seed supplied, local and export 2002-2004.

	2004	2003	2002
South Africa	2020	1240.5	1461.5
Export	2013	1365.5	1675
Total	4033	2606	3136.5

CFB evaluations

During the first evaluations on 28 April 2004 mother trees of 28 early maturing cultivars were evaluated by the CIP Evaluation Committee specialists. The second evaluations took place on 28 July and mother trees of 30 late maturing cultivars were evaluated. Of the 253 mother trees examined, 25 were suspended. No material will be cut from these suspended trees for the purpose of producing increase trees. During the second evaluation the entire facility was scrutinized by plant pathologists and virologists of the Evaluation Committee. No phytosanitary diseases were found.

Nursery Accreditation

In total 24 citrus nurseries applied for accreditation and were audited in May and November 2004. Of these, 17 were accredited, one was provisionally accredited and 4 were rejected. In general the standard in the nurseries was very good. Growers should visit nurseries and ensure that they are satisfied with the standard in the nursery before selecting which nursery to order from. Any problems with nursery trees should first be discussed with the nursery in an effort to resolve the conflict. If no satisfaction is obtained through this route then contact the Citrus Improvement Programme Manager, Thys du Toit at: Tel: 041-9925366, Fax: 041-9911300, Cell: 0828892363

The following nurseries were accredited during 2004:

Nursery	Address	Telephone	Accreditation
Apapanzi	PO Box 147, Kirkwood, 6120	042 2300790	Full
B F Joubert	PO Box 193, Kirkwood, 6120	042 2300309	Full
Casmar	PO Box 3 Mooinooi, 0325	014 5743152	Full
Du Roi	PO Box 66, Letsitele, 0885	015 3451650	Full
Esselen	PO Box 100, Malelane, 1320	013 7900160	Full
H J Joubert	PO Box 207, Montagu, 6720	023 6142237	Full
Letsitele	PO Box 1, Letsitele, 0885	015 3451600	Full
Mistkraal	PO Box 16, Kirkwood, 6120	042 2301461	Full
Ngwenya	PO Box 36, Malelane, 1320	013 7903004	Full
Paksaam	PO Box 16, Patensie, 6335	042 2830201	Full
Sondagsrivier	PO Box 304, Kirkwood, 6120	042 2300349	Full
Stargrow	PO Box 189, Citrusdal, 7340	022 9212232	Full
Tweeling	PO Box 190, Kirkwood, 6120	042 230 1408	Full
Vaalharts	PO Box 317, Hartswater, 8570	053 4740565	Full
Waterfall	PO Box 339, Adelaide, 5760	046 6840738	Full
Westfalia	PO Box 14, Modadjiskloof, 0835	015 3090050	Full
Witkrans	PO Box 17, Boshhoek, 0301	014 5733036	Full

New Cultivars

Only one new cultivar was received from the ITSG, namely the Witkrans navel compared to 28 scion and 5 rootstock cultivars in 2003.

New Developments

The second insect-controlled greenhouse was filled with 30000, 10 ℓ plant bags, of which two thirds have been planted with Carrizo seedlings. One third of these seedlings have been budded to category one cultivars to serve as increase trees.

Tree Certification

Area	2004		2003		2002	
	Trees	%	Trees	%	Trees	%
Eastern Cape	530587	44.3%	504974	34.7%	1247347	39.7%
Limpopo	310968	25.9%	591012	40.6%	1068247	34.0%
Mpumalanga	181501	15.1%	54259	3.7%	279296	8.9%
North West Province	5250	0.4%	4835	0.3%	27816	0.9%
Northern Cape	0	0.0%	167552	11.5%	167552	5.3%
Western Cape	170067	14.2%	132364	9.1%	348855	11.1%
Total	1198373		1454996		3139113	

The demand for tree certificates is still high and 554 certificates were processed and issued, representing 1 198 373 trees, compared to 593 certificates representing 1 454 996 trees in 2003. The number of trees registered per annum is still substantially lower than the average quantity of buds supplied. Tree certification is still required by EurepGAP accreditation as a “minor must” and can only be upgraded to a “major must” by international agreement. A producer must achieve an overall compliance rate of 95% on all “minor must” categories, therefore tree certification remains an important factor.

Statutory Improvement Programme

The Registrar, Plant Improvement acknowledged receipt of the draft Procedural Guide of the Southern African Citrus Improvement Programme and recommended that a meeting be arranged with a delegation from the Department, in order that more information can be obtained regarding this matter. To date no meeting has taken place, but this issue will be treated as a matter of high priority early in 2005.

Protected zone around the CFB

An application was submitted to the Department of Agriculture to have a 5 km radius around the CFB declared citrus free. The Department has prepared the necessary documentation for notification, which now has to be approved by the Minister before it can be circulated to the owners surrounding the CFB. This matter will be expedited in 2005.

STG

A facility has been established at the CRI laboratories in Nelspruit to undertake shoot tip grafting, virus indexing and pre-immunization of all locally selected cultivars. Kobus Breytenbach is responsible for this service and he is advised by Dr Faan van Vuuren. They are currently working with 5 cultivars.

Gene bank

The citrus industry's gene bank which was historically only housed at the ARCs facilities in Nelspruit is being duplicated at CRI's facilities in Nelspruit and 215 virus-free cultivars have already been re-established. All outstanding cultivars will be re-established in 2005.

Cultivar Development

Further negotiations took place between CGA / CRI and the ARC in respect of joint ownership of ITSC cultivars emanating from their breeding programme, but remained unsuccessful. Funding by the industry of projects undertaken by the ARC has ceased.

In order to protect the interests of citrus producers in the process of cultivar commercialisation, the CGA requested CRI to establish the position of industry Cultivar Development Manager and Dr Graham Barry was appointed to this post as from 1 January 2005. Some of the key functions of this post will include negotiating for rights to new cultivars both locally and internationally, and launching a campaign to search for promising local cultivar mutations. These cultivars will then be thoroughly evaluated in the various production areas by CRI Cultivar Evaluators. The cultivars will simultaneously be put through shoot tip grafting and pre-immunisation at the CRI laboratory in Nelspruit, after which material will be released to the Citrus Foundation Block in Uitenhage, for increase and supply to the Industry via accredited nurseries. The owners of these cultivars will also be assisted in order to patent and commercialise these cultivars.

PROGRAMOPSOMMING

Sitrus Grondvesblok(SGB): 'n Totaal van 2 314 730 okuleerhout is deur die Sitrus Grondvesblok verskaf wat 64 645 minder is as in 2003 met Midnight Valencia en Star Ruby Pomelo die twee mees populêre kultivars. 'n Toename in saadverkope met 'n totaal van 4033 liter vir 2004 in vergelyking met 2606 liter in 2003 wat toegeskryf kan word aan 'n derde agtereenvolgende jaar se groot bestelling uit China. 'n Groot saadoes is ondervind met 'n surplus van 907 liter. Vanaf die ITSG is een nuwe bostamkultivar ontvang vir vestiging, evaluering en vermeerdering in vergelyking met 28 bostam en 5 onderstam kultivars in 2003. Twee derdes van die nuwe insekbeheerde kweekhuis is geplant met onderstamme waarvan 'n derde reeds geokuleer is.

SGB evaluasies: Twee evaluasies is in 2004 uitgevoer. Die SVP Evaluasie Komitee het die SGB se moederbome tuinboukundig vir tiepe-egtheid geëvalueer en die perseel is ook vry verklaar van fitosanitêre siektes.

Kwekery Akkreditasie: 24 Kwekerye is twee keer besoek waarvan 17 geakkrediteer is, een voorwaardelik en vier is afgekeur. Oor die algemeen is die standaard goed.

Boomsertifikate: In die tydperk is 554 sertifikate vir 1 198 373 bome geprosesseer en uitgereik in vergelyking met 593 sertifikate vir 1 454 996 bome in 2003.

Statutêre Verbeteringsprogram: Geen vordering is gemaak en 'n dringende vergadering met die Registrateur, Plantverbetering word vir 2005 beplan.

Beskermdede sone rondom die SGB: 'n Aansoek vir wetgewing vir 'n sitrusvrye area rondom die SGB is deur die Departement Landbou saamgestel, maar is nog nie geïmplementeer nie en sal in 2005 aandag geniet.

Groeipuntenting: Groeipuntenting, virus indeksering en pre-immunisering is by die CRI se laboratorium op Nelspruit geïmplementeer ter ondersteuning van die SVP

Genebron: Die bedryf se genebron by die LNR is gedupliseer deur die vestiging van 'n virusvrye genebron by die CRI se fasiliteit op Nelspruit.

Kultivarontwikkeling: Verdere onderhandeling met die LNR oor mede eienaarskap van kultivars uit die ITSG se telingsprogram het plaasgevind, maar geen oplossing is gevind nie. Op versoek van die CGA het die CRI die pos van Kultivar Ontwikkeling bestuurder geskep en dr Graham Barry is vanaf 1 Januarie 2005 aangestel.

8 INTERNATIONAL VISITS

8.1 T.G. GROUT

International Society of Citriculture's 10th Congress in Morocco - 15-20 February 2004

Introduction and overview

I had planned to participate in the International Plant Protection Congress in China in 2003 but this was cancelled due to the SARS virus. As the ISC congress occurred in the same financial year, I was able to use the money budgeted for the Chinese congress to attend the ISC congress in Morocco.

I presented two papers in two different sessions. For the first time the congress had a session on research management and communication in which I presented the paper "Multi-Institutional Task Teams: The CRI Group Approach To Citrus Research" that I co-authored with Vaughan Hattingh. The paper was received well and showed similarities to research management and funding processes in California and Australia presented in the same session. During the IPM session I presented the paper "Indigenous Vectors Of Citrus Pathogens In Southern Africa Now More Challenging" on citrus psylla and citrus grey mite.

More than 800 people were reported to have attended the congress but at least 10% of the scheduled papers were not presented. Abstracts that were supposed to have been withdrawn when registration payments were not received were not. This led to confusion within the sessions. In addition, sessions were held at different hotels so trying to catch specific talks at different sessions was impractical.

The University of Florida was strongly represented and many sessions were chaired by UF researchers. In the final plenary session, all papers except one were presented by UF researchers. This may have been largely due to the previous congress being in Florida and the Moroccan organizers requesting assistance from those playing major roles previously.

Apart from my formal talks I also talked about the M3 bait station in the Fruit Fly workshop. Graham Barry presented three talks and Piet van Rensburg presented one talk in which he gave credit to CRI. On the whole, CRI received good publicity. Several South African and Swaziland citrus farmers who attended the congress were impressed with the standard of CRI research, relative to other research presented.

As usual, the conference provided excellent opportunities to renew worldwide research acquaintances and make new ones. Discussions may result in further entomological collaboration between South Africa, California and Australia. There is interest in the movement of cultivars between South Africa and the USA so policies on rights and intellectual property need to be finalized.

Highlights from sessions attended (numbers refer to abstract numbers in the proceedings)

1. Opening plenary session and marketing and production trends
 - Morocco has managed to increase local consumption from 15 to 23 kg/person/year and the government is providing subsidies for the establishment of new orchards. Morocco has planted 7000 ha in the last four years and is aiming to produce 1.83 million tonnes by 2010. Their cultivar split is 70% oranges and 30% easy peelers. Of their exports, 55% go to the EU and 30% to Russia. Exports represent about 40% of the crop and sour orange is still the most popular rootstock. (Talks 1-3)
 - Mediterranean (CLAM) trends include declines in Shamoutis and blood oranges (except for Italy where 48% of the public still prefer blood oranges to others). Many of the region's countries are exporting more fruit to Russia and Poland. Greece and Cyprus are both exporting more easy peelers than Israel. Turkey's exports of oranges have increased dramatically and there has been a growth in the late orange production in the Mediterranean in general. Turkey now has approximately 30 million trees on 93 000 ha. Spain's production of 5.75 million tons is still increasing by 3000 ha/year, mostly comprising oranges and mandarins. The use of Plant Growth Regulators in Spain is widespread. Total citrus production in the EU is about 10 million tons per annum from 560 000 ha. (Talks 5, 34, 38, 43, 47, 48)
 - In Argentina, lemon production is still increasing and is by far the most important crop with approximately 260 million tons exported per year. Argentina recently started their own fruit certification system which has made the transition to Eurepgap simpler. Only fruit from orchards free of canker may be exported to the EU. (Talk 31)

- China is now producing 5 million tonnes of citrus per year, 70% of which is easy-peelers. Citrus covers 1.3 million ha. Newhall navel has become their most common navel cultivar and Carrizo is the favoured rootstock. The amount of fruit actually packed (exported?) has increased from 2% five years ago to 10%. Ninety percent of the exports are easy peelers. The main citrus imports are navel oranges from USA and Australia. (Talks 33, 39)
- In Japan, more than 50% of the citrus is grown on slopes exceeding 15° so techniques such as contour paths and monorails going up the slope are used. The use of plastic mulching and fertigation via drippers has allowed for better Brix levels and colour. (Talk 42)
- In the USA, citrus consumption per capita has remained unchanged for 20 years, despite an increase in the variety of fruit available. There has been a large swing towards “not from concentrate” juice which creates logistical problems in shipping pasteurized juice across the USA. Grapefruit consumption has declined, partly due to bad press about complications with naringin and high blood pressure medication. Urbanisation is pressurizing grapefruit growers in Texas and other citrus production in the northern citrus regions of Florida and in southern California. Florida has approximately 800 000 acres of citrus and only about 10% is used for the fresh fruit market. California has 300 000 acres, all of which is for fresh fruit. Both Texas and Arizona each have 30 000 acres of citrus, and Louisiana now has 5 000 acres of mandarins. (Talk 35)

2. Sudden death of citrus in Brazil by JM Bové

In the last three years approximately 2 million trees in the Sao Paulo state of Brazil have died or become infected with Citrus Sudden Death (CSD) syndrome. Ninety percent of citrus in this area is not irrigated so Rangpur lime rootstock is very popular as it is considered drought resistant. Orange or mandarin cultivars on this rootstock are susceptible to CSD whereas orange on Cleopatra mandarin appears unaffected (but contains the pathogen). Rangpur lime rootstocks can be inarched with Cleopatra or Swingle before symptoms appear and they remain healthy. This costs about \$1 per tree. It has been proven that CSD is not caused by a viroid or bacterium so is most likely a virus. The disease incidence and spread with time is identical to the spread of Tristeza in the Dominican Republic. The aphid *Toxoptera citricida* is present in Sao Paulo and is suspected of being a vector of CSD. (Talk 106)

3. Effect of climate and other environmental factors

- High temperatures on clear days limit photosynthesis but the vapour pressure deficit (VPD) is more important than temperature. Under 50% silver shade cloth the VPD is lowered 20% and photosynthesis increases. “Surround” kaolin sprays are equivalent to about 30% shade. (Talk 99)
- In Uruguay, Nova mandarins may have very high flowering levels but have a very low fruit set. It has been found that this high flowering intensity is caused by warm weather in winter. Girdling 15 days after petal fall improves fruit set. In Brazil it has also been shown that the winter and spring climate determines fruit set. Cool winter temperatures induce flowering. The effect of drought stress is still being investigated. In Chile, hot temperatures result in more elongated Navelinas. (Talks 100, 101, 103)
- Since 1949, the acidity of oranges in Florida has decreased by 27% and that of grapefruit by 30%, while the Brix levels have remained constant. This is due to a number of reasons including the loss of northern citrus production areas where acidity was higher to urbanization, new seedless grapefruit cultivars being used after the freeze having lower acidity and changes in rootstock from Sour Orange to Carrizo. (Talk 102)
- Ida Paul presented CLIMEX modeling results to show the possible spread of citrus black spot and Queensland fruit fly in the world. The usefulness of such models to raise questions about phytosanitary barriers to trade was discussed. (Talk 104)

4. Areawide suppression of fruit flies

- It is now very evident that whenever the Sterile Insect Technique (SIT) is mentioned for fruit fly it is referring to a control or suppression method and not eradication. Commitments by governments are essential for SIT projects and there is currently a move for all Mediterranean countries to develop SIT control systems for Medfly rather than baiting. The objective is that by 2010, SIT must have been adopted by 25% of the region (see <http://www.cleanfruitsit.org>). This will require the production of more sterile flies in the region. An SIT project near Valencia in Spain is getting flies from Argentina. There is a pilot SIT project in Tunisia and Morocco is planning to develop SIT. There is a joint project on SIT with Medfly in Israel and Jordan along

the Jordan River near Eilat and the Dead Sea. Releases did not result in complete control and bait sprays were also required. Road signs in four languages are used in Israel to notify the public. In Croatia, without fruit fly control, 50 to 60% of the fruit drop and nature reserves surround the fruit-growing areas so SIT offers a non-disruptive solution. (Talks 175, 176, 180, 183, 184, 187)

- Some plants are reported to be hosts of Medfly in Europe that are not considered hosts in South Africa. These include figs, *Solanum nigrum* and *Opuntia ficus-indica*. (Talks 176, 180, 181)
- The benefit of using gibberellic acid sprays to reduce fruit fly infestation by delaying colour break until the weather was cold was again proposed. However, control was by no means complete and there was no consideration of the effects of this practice on the following year's crop. (Talk 179)
- The peach fruit fly *Bactrocera zonata* has been present in Egypt for many years and in Reunion and Mauritius for a few years. It is probably the most serious fruit fly threat to the fruit industry in southern Africa. The worldwide trend is that *Bactrocera* species displace *Ceratitidis* species. Research in Reunion by S. Quilici has shown that *B. zonata* pupae are much more tolerant of low relative humidities than Natal fruit fly *C. rosa* and this is the main reason for the peach fruit fly displacing Natal fruit fly. Medfly is more tolerant of low relative humidities and this may be why Natal fruit fly is more common during summer in areas of summer rainfall and its numbers decline in autumn as the relative humidity drops. (Talk 182)
- Several papers were presented on comparisons of traps and the Israeli Tephri trap seems to be one of the preferred options. This is a dry bucket type trap with a clear top and yellow base and sides. Wet traps such as the McPhail trap were considered more sensitive for low populations but were more detrimental to beneficial insects. Research in Reunion on trap colour showed that flat coloured sticky traps attracted most Medfly if they were fluorescent yellow but if the shape was changed to a sphere, a red sphere was the most attractive. If a lure or attractant was placed in the sphere, the red sphere was better than yellow when both contained orange but if the attractant was *Torula* yeast, the yellow sphere was more attractant. Protein-deprived female flies will only go to protein-baited traps more than citrus when they are young (vitellogenesis). (Talks 175, 182, 183, 184)
- The parasitoid *Aganspis daci* causes 60 to 65% pupal mortality in Medfly on Chios Island near Greece. The parasitoid *Opius concolor* is only responsible for 14% parasitism of Medfly in Morocco and does not respond to population increases. (Talks 180, 181)
- Hendrik Hofmeyr's research on SIT of FCM was presented by one of his two collaborators, Stephanie Bloem. She gave an excellent presentation on the mechanics of lepidopteran F1 sterility. In SIT, fully sterile females and partially sterile males would be released. Partially sterile males mated with wild females produce mostly sterile male offspring. When these males cross with wild females, more sterile offspring are produced. A dose of between 150 and 200 Gy will be used for treatment of FCM in a pilot SIT project on 35 ha citrus near Citrusdal. Parasitism of sterile FCM eggs is also possible so numbers of parasitoids can be increased without any threat to the fruit. (Talks 177, 178)

5. Citrus research and development

- The downsizing of horticultural and related research departments is a worldwide trend. In North America, there were 61 university departments of Horticulture in 1970 but by 2000, 25 of these universities no longer had a department containing the name "Horticulture". Forces driving this include demographic and economic trends, university politics and other areas being emphasized for careers. Veterinary Science is highly respected because vets work with people's pets. People-plant relationships need to be publicized. We need to be able to say that "Life without horticulture would be no life at all". (Talk 190)
- Research is increasingly being funded by private groups. (Talk 189) The system we use for research prioritizing and funding has a lot of similarities with systems used in California and Australia. In California the California Research Board consists of 11 grower board members with 11 alternates. Funds are provided for the Californian Citrus Quality Council, Citrus Clonal Protection Program and the Citrus Research programme. The research comprises six elements or programmes: Plant breeding; Plant management; Plant diseases; Entomology; Exotic & invasive pests; Post-harvest technologies & robotic harvesting. The priority setting programme uses six annual grower education seminars and a five-year strategic plan system. The call for proposals is posted on their website in May. The CRB priorities committee makes the final decisions on funding. Approximately \$2.3 million is available for research per year. The new area of gene-chip technology may be of value to us for identifying pests or diseases. The idea is to develop a portable PCR test kit. (Talk 191)

- In Australia, growers pay A\$2/ton of all citrus for research and a further A\$0.75/ton of oranges for marketing. These funds can be matched by the government if the research benefits environmental, social and economic issues. Other voluntary funds may also be available. The citrus industry's five-year investment plan provides for Market development (28%), Quality and safety systems (13%), Supply and demand (15%), Production (32%) and Communication (12%). There is potential for aligning horticulture with Environmental Sciences and strengthening international ties. (Talk 193)
- Good literature sources are an important part of technology transfer. The Citrus Research & Education Centre in Florida has a literature database created in ENDNOTE (similar to our PROCITE). They also have an index that is searchable and should have most articles on citrus. The commercial Agricola database is also a good database to search for citrus but CAB is still considered the best general database for natural sciences and international research. CREC has all ISC proceedings and can provide papers from these. The CREC website is www.crec.ifas.ufl.edu and the librarian's email is pkrc@crec.ifas.ufl.edu. (Talk 195)
- Internet usage by citrus farmers in Florida has increased from 28% in 1995 to 42% in 2002. The average user is considered to spend 30 seconds per page. Fifty five percent of grower internet time in Florida is spent on weather, 18% on commodities and 8% on production interests. (Talk 196)

6. Insects/mites/nematodes and their control

- Since 1995, two million commercial citrus trees have been removed in Florida in an effort to eradicate canker. Canker doesn't make spores but is moved by windborne rain and cultural practices. Any cuticular damage allows easy access for canker and this is why citrus leafminer is important. Canker increased dramatically in Brazil after CLM arrived in 1996. The CLM parasitoid *Ageniaspis citricola* does not seem to be effective in a Mediterranean climate. Florida plans to import two more parasitoids: *Semilacher petiolatus* and *Citrostichus phylloclnoides*. (Talk 266)
- New Zealand has 1.6 million citrus trees, 3.8 million people and 40 million sheep. Most navels are grown near Gisborne where 900 mm rain falls per annum. Satsuma mandarins are mostly grown in the Bay of Islands where rainfall is approximately 1900 mm/annum. Integrated Fruit Production has been promoted since 2002 with a major objective to replace the organophosphates. *Saissetia oleae* is the main scale pest. *Pezothrips kellyanus* is probably the most important pest for which soft control options are required. The greenhouse thrips *Heliothrips haemorrhoidalis* is sometimes problematic where fruit touch and its parasitoid *Thripobius semiluteus* has become established after being introduced, but is not very effective. Rust mite is problematic on oranges and tangelos and scab is a major problem due to the high rainfall. Non-target effect tests have shown that the steel-blue ladybird beetle is very susceptible to thiamethoxam and thiacloprid. Diazinon (very common treatment in NZ) and acephate were more detrimental than fipronil. (Talk 249)
- Damage from the *Diaprepes* weevil in Florida citrus costs \$100 million per year. The entomopathogenic nematode *Steinernema riobrave* (Biovector 355) is being used on a large scale and applied via irrigation systems but is not very effective. A new nematode species *S. diaprepesi* has been found that is more effective and is being compared with Biovector. This species is covered with bacteria that are also pathogenic to *Diaprepes*. (Talks 250, 261)
- Garcia-Mari has been developing economic thresholds for scale insects based on infestation of fruit or cull at harvest. The main scale insect pests are *Parlatoria pergandii*, *Lepidosaphes beckii* and *Aonidiella aurantii*. Red scale only appeared in Spain in 1986 and is now in all citrus production regions of eastern Spain. Their results have been more consistent than our attempts at this with red scale, although they found red scale the most variable. No disruptive sprays were applied to orchards they used which probably reduced variability in parasitism. A cull of 1-3% fruit at harvest (with >10 scales) was the approximate economic threshold used for red scale. (Talk 252)
- *Aphytis melinus* is the only *Aphytis* species parasitising red scale in Morocco and it is being mass reared for augmentative releases by several large citrus estates in the Souss and Rabat areas. The main pests in citrus are red scale and fruit fly but scale numbers are low as summer temperatures above 40°C reduce populations. Three Moroccan entomologists agreed that FCM does not occur in Morocco at all. *Helicoverpa armigera* was a pest on tomatoes but not on citrus. They have no thrips pests and only sporadic mite problems. The whitefly *Aleurothrixus floccosus* is present but not important. Citrus mealybug is important near Marrakech and introductions of *Leptomastix dactylopii* have been made and this parasitoid is being reared. Moroccans believe that *Tetranychus evansi* populations on weeds between the citrus benefits

predator populations in orchards (*T. evansi* is not a citrus pest). Rearing of *A. melinus* depends on potatoes or a large, round, fluted, green pumpkin. Substrate is placed on racks unless there is a problem with *Encarsia* when the substrate is placed in clear plastic buckets with a gauze lid. To collect adult *Aphytis*, the parasitised scale are placed on the floor of a large walk-in cage surrounded by lights. The strong *Aphytis* fly onto the wall of the cage from which they are brushed into containers for release in orchards. This simple system ensures that only good quality parasitoids are collected and avoids the use of carbon dioxide. Releases of between 40 000 to 100 000 are made per hectare between March and July. Scouting involves five fruit per tree and 10-12 trees per hectare. Releases were made of *Ageniaspis citricola* for citrus leafminer but it did not become established. *Semilacher petiolatus* was found to be more effective and *Citrostichus phyllocnistoides* was the best where high temperatures occurred. (Talks 253, 265)

- A new product called Agri 50 and sold by Cal-Agri Products was claimed to be as effective as Ultracide or 1% oil against scale insects when used at 0.3%, but without any non-target effects. This product contains monopotassium phosphate (KPO₄) and polysaccharides and is said to have a physical mode of action. Some information on spinosad was provided. It was said to have originated from a soil sample in a disused rum factory in the Caribbean in 1982. (Talks 254, 262)
- Chile also has few problems with citrus pests, their primary pests being citrus red mite and the whitefly *Aleurothrixus floccosus*. Oil or detergent sprays are used for control. Argentine ants can increase red scale and spider mites but not whitefly. (Talk 257)
- In Italy, the thrips species *Heliethrips haemorrhoidalis*, *Thrips tabaci*, *Pezothrips kellyanus* and *Frankliniella occidentalis* are all considered pestiferous. Attempts have been made to use white or blue traps for monitoring but correlations between trap catches and damage were poor. Correlations between fruit infestation and damage are much better so fruit are inspected. A similar conclusion was drawn in New Zealand. (Talk 260)
- In California, between 1997 and 2002 the following chemicals were registered on citrus: cyfluthrin, fenpropathrin, spinosad, pyriproxyfen, buprofezin, imidacloprid and acetamiprid. In the past, one of the favoured scalicides was methidathion and it has virtually no effect on the natural enemy *Rodolia cardinalis*. On the other hand, pyriproxyfen (Nemesis) residues still cause 100% mortality after five months. Repercussions of Australian bug have occurred in California as was the case in South Africa in the early 1990s but in California the climate also plays an important role. Very few beetles are around in spring when Australian bug is on the increase and the high temperatures in summer reduce beetle fecundity. If harsh thripicides are also used in spring there is no increase in beetle numbers. (Talk 255)

7. Post-harvest physiology and pathology

A few presentations were attended in this session. An evaluation of the product Messenger containing harpin proteins showed that it was effective in reducing green mould and brown rot when sprayed as a pre-harvest application at 300 l/acre. It had no effect on sour rot and was not effective as a post-harvest treatment. It was not known whether Messenger would compromise pre-immunisation. (Talk 285) Another product in the form of a fungicide fumigant was presented called Fruitfog. It comprises imazalil and thiophanate methyl. One 600 g canister is used for 25 tons of fruit. The canister is lit and the smoke acts as a carrier for the fungicide. There is no effect on taste and residues are inside EU MRLs. (Talk 290) Work on yeast isolates continues and one researcher (bouzerdal@yahoo.fr) had 21 isolates that reduced *Penicillium digitatum* by more than 50%. (Talk 286) A product called Eurofit was reported to be more effective as a preharvest treatment against *Phytophthora* than fosetyl-Al or potassium phosphite. (Talk 251)

8. Closing plenary session

- E. Rabe gave a talk on tree spacing and shape. Considers the resource of light to be more important than any other. His objectives are to get the earliest possible economic yields of high quality fruit and never to use more than a three-step ladder. The old idea of dwarfing rootstocks is no good for early high yields. Irrigation and pruning are the most important cultural practices. Growth will be limited by high yields. Grapefruit can be spaced closer because they are inside-bearing. The tree height shouldn't exceed 60% of the inter-row width or should approximate 2X the alley width. For example, if a 2 m wide alley is required for vehicles, the maximum height should be 4 m. The citrus leaf operates best at 30% of full sunlight. He prefers pruning to a pyramid shape rather than open-centred as the latter makes the trees wider. (Talk 294)
- G. Albrigo talked on climatic effects on flowering and set. In the humid subtropics, flowering can be the most important factor influencing yield. In Florida they do not have long enough dry spells

to induce flowering so are dependent on temperatures of below 20°C in winter. The optimal range is 10-15°C and an average of 900-1000 hours at these temperatures is ideal. Warmer, wet winters can result in better TSS while colder, dry winters cause lower TSS. If global warming continues, some Mediterranean climates will become intermediate. (Talk 295)

- J. Grosser talked on the role of biotechnology, primarily in breeding. The University of Florida has produced more than 7000 triploid citrus plants using a combination of old and new technology. Somatic cybridisation is a technique used to remove seeds. An example requiring this process is "LB8-9", a seedy Clementine-Minneola hybrid with excellent flavour. Transformation research using Agrobacterium is investigating genes for disease resistance, fruit quality and seedlessness. Alternative protoplast/GFP transformation uses a jelly-fish gene that makes cells glow and facilitates transfer. (Talk 296)
- T. McCollum talked on post-harvest disease control. There are problems with resistance, environmental issues and health perceptions. MRL requirements can also be confusing (see database at: <http://mrl-database.com>). There are some new synthetic fungicides in the registration process e.g., pyrimethanil and azoxystrobin. Thiophanate methyl (Topsin) converts to the same AI as benomyl and should be available for pre-harvest treatments. There is more interest in biocontrol type products but these do not have a kick-back or corrective action. Curing of fruit at 30°C in a water-saturated atmosphere for 48 hours allows wounds to heal without becoming infected. Degreening at high temperatures may therefore reduce waste. The hot-water brush system uses a spray of water at 62°C followed by brushing for 20 seconds. Combinations of hexapeptide and yeast have given good post-harvest control of waste. (Talk 297)
- M. Ismail spoke on better post-harvest handling. In 1980 we had half the number of varieties that we have now. Consumers want a convenient fruit. Fresh cut citrus should be developed and marketed. We need a means of monitoring peel maturity and fruit surface quality so that washing and brushing process can be adjusted accordingly. A worldwide database of the medical and nutritional benefits of citrus would assist in marketing. (Talk 298)

Conclusions

Although we may not be involved in genetic engineering or robotic harvesting it is clear that our research portfolio is serving our growers well and is comparable with any in the world.

International cooperation should be encouraged as worldwide citrus research manpower and funding declines. Ted Batkin (California) and Gerry McEvilly (Australia) are keen to pursue further cooperation. The most obvious area for this is where we share the same research requirements, provided our marketing and phytosanitary positions are not compromised. Entomological examples for this include research on citrus thrips in Australia that is already being funded and may benefit our growers, and research on SIT of Medfly in the Mediterranean that may help us with decisions on SIT of Natal fruit fly. Other international collaborative research with world experts, such as Hendrik Hofmeyr's work with SIT of FCM, is already showing how much time we can save when we collaborate with experts in the field. At the same time, the standard of our research is raised.

Abstracts of talks presented

MULTI-INSTITUTIONAL TASK TEAMS: THE CRI GROUP APPROACH TO CITRUS RESEARCH

T.G. Grout and V. Hattingh

Citrus Research International, P.O. Box 28, Nelspruit 1200, South Africa.

Abstract. Before 1992, levies for research on citrus in southern Africa were collected by the South African Cooperative Citrus Exchange (SACCE) who was the sole exporter of citrus under the brand name "Outspan". Researchers employed by SACCE conducted mostly applied and near-market research and received the majority of funding. Outside research institutions perceived that they only had access to surplus research funds and without any coordination of research effort, duplication and competition occurred. During the period 1992 to 2000, SACCE changed to a private company, other agents began to export citrus without collecting research levies and a Citrus Growers Association (CGA) was formed. The CGA was unsuccessful in collecting sufficient voluntary contributions to meet research requirements but growers voted for the introduction of a statutory levy on export citrus and with that assurance, Citrus Research International (CRI) was formed. In CRI, all major stakeholders now have representation on the board of directors and partnerships have been formed between CRI and other institutions conducting research on citrus: this is referred to as the CRI Group. Recognised experts coordinate research on particular projects or tasks and can create teams of researchers from various institutions to address the research priorities identified by

growers. Allocation of funding is determined by a grower-appointed and grower-chaired committee of experts in each research discipline. In this way, funding is provided to task teams considered most likely to achieve results and there is no duplication.

INDIGENOUS VECTORS OF CITRUS PATHOGENS IN SOUTHERN AFRICA NOW MORE CHALLENGING

T.G. Grout

Citrus Research International, P.O. Box 28, Nelspruit 1200, South Africa.

Abstract. Two citrus pathogens native to Africa are vectored by arthropods in southern Africa. The more infamous of these is African citrus greening or huanglongbing, transmitted by the African citrus psylla *Trioza erytreae* (Del Guercio). The other pathogen has been described as a spiroplasma-like organism, causes concentric ring blotch and the more severe necrotic spot, and is vectored by the citrus grey mite *Calacarus citrifolii* Keifer. Established control measures for these vectors that have been used in IPM orchards for many years, are now being compromised by the withdrawal of plant protection products and the lowering of Maximum Residue Levels in the European Union. Organophosphate stem treatments that have largely been responsible for the control of citrus psylla in the past, are now of limited availability. Neonicotinoids can be applied to soil or stem once a year but are expensive. Other products can be used as foliar sprays but must be IPM-compatible. Any new product must also be incorporated in an Insecticide Resistance Management strategy, as frequent applications are required on young trees. All parasitoids of citrus psylla are compromised by hyperparasitoids, which are likely to attack any introduced parasitoid. Generalist predators are of limited value in preventing pathogen transmission by citrus psylla. Alternative acaricides are available for the control of citrus grey mite but are not all IPM-compatible. Possible predators of citrus grey mite that are closely associated with this pest include the phytoseiid mite *Typhlodromips enab* (El-Badry) and the tydeid mite *Pronematus ubiquitous* (McGregor). New, multi-faceted approaches to the management of both these vectors are required in order to maintain productivity in affected citrus production regions.

8.2 H.F. LE ROUX

10th ISC CONGRESS, Agadir, Morocco. 15-20 February 2004

The 10th ISC Congress was attended by Tim Grout, Hennie le Roux and Graham Barry from CRI. Tim has already given feedback on the part of the Congress attended by himself. This included the plenary session and an overview of the different citrus industries, research and funding as well as the entomology/IPM sessions. In this report the pathology sessions, citrus nurseries, resistance, rootstocks and organic citrus is reported on. In the third report Graham will cover the Horticultural aspects discussed at the congress.

Opsomming

- Na-oesbeheer werk wat gedoen is in Kalifornië toon dat azoxystrobin (Ortiva), fludioxonil (Scholar) en pyrimethanil (Penbotec) *Penicillium* groen en blouskimmel beheer. Al drie die produkte behoort aan verskillende chemiese groepe. Die waarskynlikheid op weerstand is hoog vir al drie produkte en daarom moet dit van die begin af reg bestuur word om die kans op weerstand te verminder.
- Giste soos *Aerobasidium*, *Candida* en *Cryptococcus* spesies is gevind deur die Spanjaarde wat geskik is vir insluiting in geïntegreerde na-oes beheerprogramme.
- Die MRL waarde van TBZ gaan verlaag word van 5 na 3 dpm. Marocco het werk gedoen ter ondersteuning van die hoër waarde.
- Messenger (harpin proteiene) het die insidensie van bruinvrot, suurvrot, blouskimmel en *Botrytis* op suurlemoene verminder deur dit 'n week voor oes op die vrugte te spuit. Wag tot CRI hierdie resultate bevestig het.
- Proewe in Bari, Italië toon dat slegs sekere *Trichoderma harzianum* isolate *Phytophthora* beheer. Dring dus aan op 'n L-registrasie nommer, 'n lot nommer, effektiwiteitsdata en bevestiging van geloofwaardigheid, voordat enige *Trichoderma* produkte in Suid Afrika op sitrus aangekoop word.
- Terugvoer oor navorsing ten bedrae van \$ miljoene is gegee ten opsigte van siektes soos Mal Secco, *Pseudocercospora angolensis*, Greesy spot, CVC, sitruskanker, asiatische vergroening, Citrus Sudden Death en Whiches Broom Disease wat tans nie in Suid Afrika voorkom nie. Die goedkoopste manier om hierdie siektes te beheer is om dit uit suider Afrika uit te hou en te sorg dat die CRI navorsers op hoogte is van elk van hierdie siektes.
- Ida Paul van Suid Afrika het deur middel van die CLIMAX model gedemonstreer dat sitrus swartvlek wel die potensiaal het om in Florida te vestig maar nie in Kalifornië of in die Europese Unie nie.

- Nouer bande moet gesmee word ten opsigte van nuwe kultivars, onderstamme weerstandstelingnavorsing wat gedoen word by CREC in Florida.
- Daar is talle lesse te leer in lande waar daar nie 'n suksesvolle Sitrusverbeteringskema bestaan nie. Suid Afrika s'n is in plek tot waar die material aan die kwekerye oorhandig word. Met enkele uitsonderinge na is die Suid-Afrikaanse sitruskwekerybedryf besig om agteruit te gaan. Hierdie kongres het weereens die noodsaaklikheid van statutêre sertifisering bevestig.

POST-HARVEST

Penicillium

Jim Adaskaveg from the University of California presented an excellent talk on the use of new fungicides for the control of *Penicillium* on citrus. These fungicides are azoxystrobin (Ortiva / Abound), fludioxonil (Scholar) and pyrimethanil (Penbotec), each belonging to a different chemical class of fungicides. These fungicides control both *P. digitatum* and *P. italicum*, including isolates resistant to imazilil and thiabendazole, at rates of 1000ppm each or 500ppm in mixtures. All three these fungicides are classified as "Reduced Risk Fungicides" according to the US-EPA classification, which allows them to go through an accelerated registration process.

However, these compounds are all single site inhibitors and therefore integrated resistance management strategies should be followed from the beginning. This will include programs initiated with sanitation practices using broad spectrum, multi-site action materials to reduce the total pathogen population, tank mixtures or rotations of different classes chemicals, optimal application practices and the limitation of the number applications of a particular fungicide, including pre-harvest treatments. This last factor could pose a problem in South Africa with regard to the pre-harvest use of the strobilurines.

UCR has also developed the so-called "Spiral gradient plates" to mass screen air in pack houses. These plates gives an indication of the level of resistance against the different fungicides and should be used as a standard monitoring tool in all South African pack houses.

Keith Lesar's comments on these new chemicals was that he has tested the strobilurines and found them not nearly as effective as imazilil. He shall be testing the other two during the coming season. Hopefully they will be more promising. Taking into account the pre-harvest use of the strobilurines in the CBS infected areas we should not bank on this chemical group for post-harvest disease control.

Creasing

Carol Lovatt also from UCR, presented results showing that aminoe thoxyvinylglycine(AVG) could reduce creasing. The product , ReTain, which is manufactured by Valiant Biosciences were sprayed at 38 mg ai/l and at 300mg ai/l. The higher dosage was phytotoxic and reduced fruit size . The lower dosage, when applied at petal fall increased fruit set and reduced what we would call the November drop. It reduced creasing with 36%. When applied during the November drop it did not improve fruit set but it reduced creasing with 56%. (Graham Barry will report in more detail)

Clive Kaizer from the University of Pretoria, who will be doing research for CRI in the Eastern Cape this year, has been informed of this product and will try to get hold of it to include it during the coming season.

Biocontrol

The CSIC in Valencia, Spain has over the years tested more than 900 yeasts to include in an integrated post harvest control programme. According to Zacarias only 12 are promising. These include *Aerobasidium*, *Candida* and *Cryptococcus* spp. These yeasts in combination with hexapeptides are showing good results. Ait Ben Aoumar from Morocco also reports a reduction in *P. digitatum* using antagonistic yeasts. CRI should put a greater effort in trying to isolate microbial organisms that could be incorporated into post-harvest biocontrol programmes.

World Trade Regulations

Wally Ewart, president of the California Citrus Quality Council has stressed the point that though trade barriers were reduced through the World Trade Organisation, technical barriers including regulations have become increasingly important. Food safety and the use of pesticides have become issues citrus growers are viewing with increasing concern. Most countries do not render a service to their producers informing them of the different standards in the different countries. The Codex standards which cover the US and

certain other countries do exist. The updated list that exporters in South Africa receive from the CGA each year is unique. It was also mentioned that the MRL of TBZ will be reduced from 5ppm to 3. Morocco has submitted information to support the higher level and the South African industry should do so as well to support them. The maximum residue limits of the USDA can be found on <http://mridatabase.com>

Alternative control methods

In Acireale, Italy, Lanza is looking at novel approaches as possible alternatives to synthetic fungicides. Methods such as storing oranges at 36 C for 72 hours (lemons 24 hours at 38 C), sodium carbonate, sodium bicarbonate peroxyacetic acid and chlorine all in combination with hot water and/or short hot water brushing reduced the incidence of *Penicillium* sp. It increase the phytoalexin scoparone which increase the fruit's resistance. However in many cases this control is not good enough for previously established infections. Growers must see that fruit is treated more careful from picking until it enters the pack house.

Keith Lesar and Tian Schutte must get more support for this type of work especially those combined with the short hot water brushing.

Harpin Protein (Messenger)

From the University of Arizona it is reported that harpin proteins trigger the plant's hypersensitive response (SAR) which impacts on the salicylic pathway. 650ml/ha of harpin protein sprayed 5-7 days before harvesting significantly reduced the incidence of sour rot, brown rot, blue mould and *Botrytis* on lemons. It also reduced the incidence of mechanical injury. The effectiveness of the harpin protein however appeared to vary depending upon the maturity of the fruit prior to harvest and the storage duration.

CRI should definitely investigate the use of harpin protein for the control of post harvest diseases. It was tested by MC Pretorius against citrus greening and failed to give results after several treatments. Producers should restrain from using the product prior to registration, especially on grapefruit, as the effect thereof on the cross protection viruses is unknown.

Formesa Fruitfog

Formesa has formulated smoke generators using ether imazalil, thiofanate-methyl or a combination there-off to replace pre-degreening drenches, to be used in storage areas and to be used on trucks. They claim better degreening results were this product was used compared to the fungicide drenches. CRI is not in favour of this method of application as it increase the risk for resistance to develop. Post harvest chemicals are too few to take any chances and therefore these foggers are not recommended.

PRE-HARVEST

Fungal diseases

Phytophthora

The search for new chemicals and other control methods to control *Phytophthora* is continuing and papers were presented from Morocco, France, Italy and Spain. There are however no other registered options to replace Ridomil (metalaxyl) as fungicide and the phosphonates as fungistat at this stage.

At the University of Bari in Italy, several antagonistic fungi is screened against *Phytophthora* including several isolates of *Trichoderma harzianum*. The most effective organism to reduce the inoculum density of *Phytophthora* in the soil was *T. harzianum* FV178. It is important to note that most of the other *Trichoderma harzianum* isolates did not reduce the inoculum density. So far CRI was not impressed with the strains isolated from South African and Zimbabwean soils and tested against *Phytophthora* in citrus. A more in depth study should be done in South Africa to see what is available and to test all available *T. harzianum* isolates against citrus *Phytophthora*.

In vitro trials with chitosan originating from crab shells at 1mg/ml reduced *Phytophthora* propagules to less than 90%. This provide a promising alternative to synthetic fungicides for organic farming. However, if a product such as copperoxychloride is acceptable for organic farming, phosphonate producers in South Africa should invest money to get the phosphonates approved for organic farming as well. This will be an easier route to follow than to make chitosan economically acceptable for organic citrus producers.

Mal Secco

Mal Secco, caused by the fungus *Phoma tracheiphila*, was once again highlighted as being one of the more important citrus diseases in the Mediterranean countries, especially on lemons. In Morocco new lemon cultivars assessed for resistance showed that all were susceptible. In Tunisia where no chemicals give satisfactory results against the disease screening with antagonistic bacteria also failed to control the disease. Once again the warning should go to the South African growers never to bring any budwood illegally into this country. If Mal Secco is introduced into the Western Cape it has the potential to wipe out the lemon industry in the Cape in a very short time.

Pseudocercospora angolensis

Verniere, whom MC met at CIRAD-FHLOR in Montpellier, France, emphasised that this organism is classified as a quarantine organism in the EU. It was again mentioned that the disease could be found in Mozambique, something that we could not confirm during our Mozambique survey of 2003. Zimbabwe was not mentioned. In the tropics the disease can induce 20 –100% of fruit loss contrary to Zimbabwe where no yield losses occur in commercial orchards. It only causes fruit lesions in neglected orchards with out of season fruit. The route the citrus industry in Cameroon is taking is to evaluate the different citrus cultivars for resistance and to replace existing trees with more tolerant varieties. This implies a move away from navel varieties towards the Valencia types. Verniere will be moved to Reunion and will no more be involved with *P. angolensis*. The researchers who remain involved with the disease are all French speaking which make communication difficult.

Scab

Citrus scab (Elsinoë Fawcetti) is a serious disease of susceptible citrus cultivars eg lemons growing in New Zealand's humid climate. In South Africa this is not common but it can occur in certain cases in lemons. One such case occurred recently in the Eastern Cape. According to Andrew Harty from Kerikeri in New Zealand benomyl, mancozeb and copper were traditionally used to control the disease. Because of resistance developing against the benzimidazoles, certain buyers refusing to buy fruit sprayed with mancozeb and a copper build-up in the orchards because of excessive use of copper he tested some alternative chemicals to control the disease. Trifloxystrobin, azoxystrobin, copper hydroxide, folpet and dithianon all appear to give good control of the disease.

Alternaria

According to Pete Timmer brown spot which was first reported in Brazil in 2001 is now widespread in the States of Sao Paulo, Minas Gerais and Rio Grande do Sul. It is found on Murcott, Dancy, Nova and Ponkan tangerines. Timing of sprays is done according to the Alter-rater model. Using up to 11 sprays the marketable fruit was increased to 28% in the one trial site and 62% in another compared to no marketable fruit in the untreated control plots. My advice to Brazil once *Alternaria* get into a climatological suitable area is to remove the susceptible cultivars and to replace them with *Alternaria* tolerant varieties. It should however be remembered that the easy peelers are becoming more and more in demand with the consumers and CRI should therefore put more money in developing more effective spray programmes.

Greasy spot

This disease caused by *Mycosphaerella citri* does not occur in South Africa. As is the case with Mal secco in the Mediterranean countries South Africa should ensure that budwood is not imported illegally into South Africa from Florida where the disease occurs. There is a definite danger in this regard as the University of Florida has developed several very promising cultivars, which some Cape growers may like to smuggle into South Africa if they got the opportunity. Work conducted by Pete Timmer and Mondal on the ascospore development, maturation, discharge and infection of this organism is very similar to that of CBS. Researchers working on CBS in South Africa should keep a close look on research conducted by Pete Timmer's group on greasy spot in Florida.

Citrus Black Spot (CBS)

Ida Paul from South Africa gave a review of global citrus production with reference to potential areas for citrus production and potential spread of pathogens and pests. The CLIMEX model was used to infer the climatic requirements of citrus species from known production regions. CBS and the Queensland fruit fly was used as examples in these models. The results clearly indicated that CBS had the potential to establish in

Florida but not in California or the EU. This data is extremely important in our argument that CBS should not be classified as a phytosanitary organism for Europe.

BACTERIAL DISEASES

Citrus Variegated Chlorosis (CVC)

CVC is caused by the gram-negative bacteria, *Xylella fastidiosa*. It infects the xylem vessels affecting the transport of water and nutrients. According to Oliveira from Piracicaba in Brazil there is also the possibility that photosynthesis be affected because of toxins produced by this bacteria. This disease should be rated a bigger danger to the southern African citrus industry than citrus canker. The most probable introduction of the disease into Africa will be through missionaries working in Mozambique and Angola. During our survey of Mozambique we emphasised the importance of obtaining citrus bud wood for Mozambique from the CFB . CVC is transmitted by sharpshooters/ leafhoppers and it is much more difficult to control this vector than it is to control psylla (spreading greening) because of its wide host range. CVC is currently the citrus disease that receives most attention in Brazil.

Asian greening

Huanglongbing is caused in India by the phloem restricted fastidious bacterium, *Liberibacter asiaticus*. Because of a lack of an effective citrus improvement programme the disease is prevalent widely in citrus nurseries and orchards throughout Central and Southern India. Trials using foliar applications of the antibiotic ledermycin (600 ppm) on its own and in combination with micro-elements reduced the incidence of visual symptoms substantially. It is recommended that Gerhard Pietersen should investigate the efficacy of ledermycin against African greening further.

Citrus canker

The organism causing citrus canker had a name change since the previous ISC. It is now known as *Xanthomonas citri* pv *aurantifolii*. According to Canteros from INTA in Bella Vista, Argentina an integrated plan to reduce the risk of canker existed since 1994. It involves the following: Certification of selected, registered, symptom-free plots with permanent surveys, windbreaks around every 2-4 ha, sanitation with disinfectants, application of copper containing products to young leaf flushes and to developing fruit to prevent infection, and treatment of fruit in certified packing houses with SOPP at 2% for 2 min. and sodium hypochlorite at 0,02% for 45sec to ensure elimination of any bacterial cell. It was also mentioned that the ban on fruit from Argentina into the EU has been lifted.

VIRUS AND VIRUS –LIKE DISEASES

Citrus Sudden Death (CSD)

Work was presented by Bové, the most knowledgeable citrus virologist in the world, on CSD. This disease was first seen in 1999 in Brazil on sweet orange and mandarin trees grafted on Rangpur lime. As 85% of Sao Paulo's 200 million sweet orange trees are grafted on this rootstock it is a serious threat to their industry. After showing general decline the trees suddenly collapse and die. This is similar to sour orange trees affected by CTV. Below the bud union of CSD –affected trees there is size reduction of the phloem cells, collapse and necrosis of the sieve tubes, overproduction and degradation of phloem, accumulation of non functioning phloem and invasion of the cortex by old phloem. Cleopatra and Swingle showed no symptoms. The association of a Tymovirus has been reported.

Though Rangpur lime is not used as a rootstock in South Africa, Gerhard Pietersen, CRI's newly appointed virologist should be aware of CSD and should perhaps fly via Sao Paulo to Mexico when attending the IOCV.

Tristeza (CTV)

It is reported by IVIA in Spain that there is a direct correlation between the high incidence of the *Aphis* species, *A. gossypii* and *A. spiraecola* and rapid spread of CTV on Clementines in Spain recently. Clementines is the citrus species most visited by aphids.

Davino also reported that CTV is spreading fast in Sicily in the Catania, Syracuse and Taranto provinces. This in spite of the Italian Ministries decree for mandatory control of CTV in 1996. Sixty % of the 200 000ha of citrus in Italy is sweet orange. Ninety eight % of these trees are on sour orange. The Italians may have a

problem which South Africa should keep monitoring as this could create marketing opportunities in the long run.

Witches Broom Disease of Lime (WBDL)

There may be only a few lime orchards in southern Africa but in Oman and its neighbouring countries WBDL has destroyed the lime industry which was the second most important agricultural industry after dates. The fact that Oman could not protect its citrus industry once it got invaded by a new disease sends out a clear message to other citrus industries to ensure that they have properly qualified and knowledgeable scientists in place to deal with these type of crises. Jude Grosser and his team at CREC are once again using somatic hybridisation to develop WBDL tolerant lime trees.

DISEASE RESISTANCE

There are two groups working on molecular characterisation of disease resistance in citrus at CREC. The first group is working under Ken Derrick. An important component of the plants defence system is systemic acquired resistance (SAR). After recognition and hypersensitive response to invading plants, plants show elevated accumulation of salicylic acid (SA). Two distinct signal transduction pathways that conduct to SAR after pathogen recognition by resistance proteins have been identified. Arabidopsis genes are involved with SAR. These and other genes are being characterised or identified from subtracted cDNA libraries constructed from healthy and diseased plants. CRI pathologists should focus more on SAR in order to reduce citrus diseases.

The second group, those of Fred Gmitter is looking at gene introgression into commercial cultivars through sexual hybridisation as well as genetic transformation. They have developed highly specific markers for CTV and citrus nematode resistance genes and they are exploring methods to enable the rapid identification and association of specific gene sequences with resistance to specific diseases. After visiting Mexico (IOCV) Gerhard Pietersen should visit Fred Gmitter in Florida.

NURSERY & PLANT PROPAGATION

In contrast with the citrus nursery industry in South Africa the citrus nursery industry in Brazil has improved the health and vigour of its trees since 2001. The reason being the introduction of a mandatory program for tree certification. In South Africa the citrus nurseries, with the exception of a few, has shown a steady decline since deregulation of the industry. In the past most of the growers were not concerned about tree certification. Hopefully this will change because of EUREPGAP demands. If not, mandatory certification is necessary.

Turkey is still having problems with diseases such as psorosis, stubborn, cachecia and exocortis. It does try to eliminate these diseases through the Turkish Citrus Variety Improvement Programme supported by the FAO. The Citrus and Greenhouse Research Institute has produced 810 000 citrus buds free of virus and virus like diseases since 1992. The CFB has produced at least 2 million/year each year for more than a decade.

ROOTSTOCKS & SCIONS

The group to stay in touch with is Jude Grosser and his team from the Citrus Research and Education Center at Lake Alfred. They have several allotetraploid somatic hybrid combinations under evaluation as well as so called "tetrazyg" hybrid rootstocks which is being selected using high pH, calcareous soil and *Phytophthora* tolerance.

Work done in Corsica with Clementines on six different rootstocks indicates that C35 and C32 citrange should replace Carrizo citrange as rootstock under those conditions. C32 induced earlier colouring and C35 later colouring than Carrizo. The fruit on C35 also had higher juice percentage and total soluble solids than on Carrizo.

As with the rootstocks the CREC in Florida should be noted when looking at new breeding programmes. Fred Gmitter and his team are producing nearly 2000 triploid hybrids annually and several promising new cultivars will be released from that programme in the near future.

Eduardo Stuchi in Bebedouro (Brazil) is working on cultivars which can improve on Hamlin for processing. Two cultivars that showed higher soluble solids were Finike and Torregrosso whereas Westin, Finike, Kawatta, Olivlands and Torregrosso had higher juice colour values.

ORGANIC CITRUS PRODUCTION

Most of the work conducted on organic citrus production was done by the Italians. Their production of organic citrus starts with the seed which may only be treated with thermotherapy or in combination with different fungicides as is described in EU Regulation 2092/91. As growing media peat moss, soil and sand with the addition of poultry manure-based fertilizers and micronutrients gave the best growth. According to the results presented, these researchers were able to obtain yields similar to those obtained with mineral fertilizers, showed better fruit quality characteristics, increased the long term soil potential fertility and reduced the soil mineral N. They also claim that fruit produced organically were more rich in Vitamin C.

In an attempt to get rid of the pulp and peel produced by the citrus industry this citrus waste was composted with olive husks. This combination proved to be phytotoxic. Work done in this regard by Prof Wehner in the late 1990s was highly successful and more advanced than that conducted in Italy.

Organic fruit production like many other new gimmicks does not sell itself and has to be marketed. A small premium is paid for organically produced citrus but not much. In most cases yields in organically produced orchards is lower than in conventional orchards and it does not justify organic production. With increase in consumer demand this may change in future.

CLAM (Comite de Liaison de L'Agrumiculture Mediterraneenne)

South Africa is a member of CLAM. A meeting was held during the congress. Mena Davidson, president of CLAM did invite both myself and Tim Grout to the meeting. Unfortunately the meeting was held in Marrakesh which made it impossible for us to attend.

BIAGRO - Spain

After the ISC, Biagro was visited in Spain. The semi commercial trials conducted with the M3 for fruit fly control has been completed successfully and commercial sales should commence during the coming season. The product cannot be registered because of BASF, the manufacturer of the toxicant. They would like to be the distributor of the M3. If not they will not release the toxicological data needed for registration. However, as the product does not get in touch with the fruit, it can be sold without registration.

A manufacturing plant was also visited to establish whether the M3 moulds would fit onto their machines. It is possible and a costing is being done to ensure that manufacturing of the plastic components of the M3 will take place in future where it can be done most cost effectively. Quest will still formulate the chemical product in South Africa, charge the sponges and send them to Spain for the assembling of the M3.

8.3 GRAHAM H. BARRY

8.3.1 International Society of Citriculture (ISC) 10th Congress, Agadir, Morocco, 15-20 February 2004

Purpose and participation

Approximately 850 people attended the 10th congress of the ISC. Overall, the congress provided a useful vehicle for meaningful interaction among international citriculturists and an opportunity to attend presentations on recent developments in citriculture. I presented three papers at the conference and was co-author on one other paper (see abstracts at the end of this report).

A detailed report on the various sessions has been summarized in Dr. Tim Grout's report, and will not be repeated here.

Production trends

An overview of citrus production in Morocco, the Mediterranean region, China, Japan, Argentina and the USA indicate that citrus production will continue to increase at >3% p.a. and that total citrus production worldwide is now 100 million tons. Combined with increased production is increased per capita consumption and the development of non-traditional markets.

Production practices

Pre- and postharvest crop manipulation with irrigation, nutrition, hormonal and environmental treatments continue to gain attention of citrus researchers worldwide, but only incremental improvements in crop production are being achieved. In general, South African citrus producers are using some of the most advanced production practices currently available. The main research focus appears to be on improving postharvest practices to optimize shelf-life and biotechnology. The latter is certainly receiving the most attention, and funding, and promises to provide breakthroughs in pest and disease control, as well as improved product quality.

Abstracts of presentations

NOVEL APPROACHES TO RIND COLOUR ENHANCEMENT IN CITRUS

Barry, Graham H.

Citrus Research International, Department of Horticultural Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

Rind colour of early-maturing sweet orange and mandarin cultivars is often unacceptable when environmental conditions at the stage of colour development are less than ideal. Therefore, various pre- and post-harvest cultural practices are applied to accelerate chlorophyll degradation and carotenoid biosynthesis. However, these cultural practices are not without some limitation, which requires novel approaches to rind colour enhancement. Pre-harvest treatments with promising effects on rind colour enhancement include elemental sulphur (Thiovit® at 3 g·L⁻¹ applied 2 weeks before anticipated harvest) and a gibberellin biosynthesis inhibitor (prohexadione-Ca at 1 g·L⁻¹ applied 2 to 3 weeks before anticipated harvest). These treatments increased total carotenoid content and improved rind colour rating. Moderating vegetative vigour, i.e. reduced “gibberellin load” in the aerial portion of citrus trees, appears to be integral to optimizing chloroplast-chromoplast transformation as gibberellins have an antagonistic affect on carotenoid biosynthesis. The simulation of sudden cold weather conditions shortly before fruit maturation on colour break, i.e. a cold front, provides a novel post-harvest approach to enhancing rind colour. A “cold shock” was applied to fruit by suddenly dropping fruit temperature from ambient to 4°C within 30 minutes by means of a hydrocooler, and then maintaining fruit at 4°C for 6 hours by cold storage. Thereafter, fruit were “incubated” at 20°C for 72 hours. ‘Satsuma’ mandarin and ‘Navel’ orange did not respond to cold shock. However, in one experiment ‘Nules Clementine’ mandarin fruit showed similar rind colour development to degreened fruit. Unfortunately, this response has not been repeated. Further research is required to ascertain whether a consistent response is achievable, and whether cold shock treatment could partially replace ethylene degreening thereby reducing exposure of fruit to ethylene.

THE QUEST FOR SEEDLESS CITRUS FRUIT

Barry, Graham H.

Citrus Research International, Department of Horticultural Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

In recent years, consumers of fresh citrus fruit have placed greater emphasis on seedlessness as a fruit quality requirement. To complicate matters, the definition of “seedless” has changed with time – in the 1980s, “commercially seedless” fruit were considered to have less than five seeds per fruit. Such a definition would include important cultivars such as ‘Marsh’ grapefruit, ‘Valencia’ orange and ‘Eureka’ lemon, among other less-seeded cultivars. Therefore, an unambiguous definition of “seedless” that fulfils current consumer demand will be presented. To fulfil the requirement of the production of seedless fruit a knowledge of pollen biology and sexual fertilisation is essential to ensure appropriate orchard layout that avoids unwanted pollination. *In vitro* pollen viability of 66 citrus cultivars was determined and pollen of cultivars with strong and weak pollination potential were used for *in vivo* pollen tube growth and seed content studies of ‘Nules Clementine’ mandarin and ‘Delta Valencia’ orange. Data from the *in vitro* assays were rated and categorized into “pollen germination potential categories”, and the relationship between these categories and seed content of ‘Nules Clementine’ was determined by regression analysis. Citrus cultivars with a pollen germination percentage exceeding 2% are likely to set too many seeds in ‘Nules Clementine’ fruit according to consumer requirements. ‘Star Ruby’ grapefruit and ‘Delta Valencia’ were identified as pollen sterile cultivars, in addition to the two previously known pollen sterile cultivars ‘Navel’ orange and ‘Satsuma’ mandarin, that can be used as buffers to avoid cross-pollination between two pollen fertile cultivars. Furthermore, ‘Delta Valencia’ is also ovule sterile. “Pollen germination potential category” accounted for more than 90% ($r^2=0.9192$) of variation in seed content of cross-pollinated ‘Nules Clementine’ fruit.

CLEMENTINE MANDARIN PRODUCTION IN SOUTH AFRICA

¹Barry, Graham H. and ²Rabe, Etienne

¹ *Citrus Research International, Department of Horticultural Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa*

² *Department of Horticultural Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa*

From its humble beginnings in Algeria, North Africa, in the late 1890s, Clementine mandarin (*Citrus reticulata* Blanco) eventually made its way to the southern tip of Africa in 1969. The Clementine mandarin industry in South Africa was initially based on a single Clementine selection, SRA 63, which was imported from Corsica. In the late 1970s, the early pioneers associated with developing the Clementine mandarin industry in South Africa (de Lange, Bredell, von Broembsen and Lee) visited Spain and identified other promising Clementine mandarin selections worth commercialising in South Africa. Approximately 40 000 Clementine trees had been planted by 1980, and this increased to 500 000 trees by 1990. Plantings of Clementines peaked by 2000 when approximately 3.2 million trees had been planted, or 3 000 hectares. Subsequently, plantings appear to have stabilized. South Africa currently produces 80 000 tons of Clementines, of which 50 000 tons are exported mainly to the UK, Europe and USA. More than 95% of Clementine production is confined to the citrus production regions of the Western and Eastern Cape Provinces of South Africa. The main commercial selections are Nules, Oroval, SRA 63, SRA 92 and Marisol. The latter three selections are losing favour among Clementine producers and marketers, and Clemenpons®, an early-maturing Nules type, has recently been planted. Preliminary climatological studies were conducted to establish the potential of Clementine production in South Africa. Thereafter, horticultural practices were required to ensure optimal production in terms of fruit set, fruit size enhancement, rind colour development, and fruit quality. The South African Clementine industry celebrates the first century of Clementine mandarin production.

BIO-CLIMATIC MODELLING – APPLICATIONS WITHIN CITRICULTURE

I. Paul

Centre for Environmental Studies, University of Pretoria, Pretoria, 0002, South Africa

G.H. Barry

Department of Horticultural Science, Faculty of Agricultural and Forestry Sciences, Private Bag X1, University of Stellenbosch, 7602, Stellenbosch, South Africa

Y.C. Collingham and B. Huntley

School of Biological and Biomedical Sciences, University of Durham, Science laboratories, South Road, DH1 3LE, United Kingdom

A.S. van Jaarsveld

Department of Zoology, Faculty of Science, Private Bag X1, University of Stellenbosch, 7602, Stellenbosch, South Africa

Pests and diseases significantly limit citrus production. Trade, in particular the deliberate relocation of citrus plants, provides opportunities for pathogens and pests to establish in new habitats. Such introductions have had devastating impacts on citrus cultivation and, to prevent future introductions, phytosanitary barriers to trade have been imposed. These barriers should be based on scientific principles. The aims of this paper are to highlight the potential of using bio-climatic modelling within pest risk assessments and to determine the potential geographical distribution of Citrus Black Spot and Queensland Fruit Fly using a CLIMEX model. Results indicate that, although climate partially restricts the potential occurrence of these species, that the climate is conducive to the persistence of these organisms in some new areas. Knowledge on the climatic suitability of an area could assist pest risk assessments and in the implementation of phytosanitary barriers to trade.

8.4 W. KIRKMAN

IAEA sponsored scientific visit to Canada (C6/SAF/04004V)

A group of four South Africans visited the Sterile Insect Release (SIR) programme in British Columbia, Canada, from 12-17 July 2004. These were Prof Vaughan Hattingh and myself from Citrus Research International, Hugh Campbell from DFPT Trust, and Gerrit Smit, a grower from Citrusdal.

(a) Programme

Sunday 11/07/04

Arrived in Kelowna at 15H20. Transport to Motel.

Monday 12/07/04

We were picked up by SIR General Manager, Bob Fugger at 08H30. He gave us a general overview of the programme. He explained to us that some of the growers in Zone 2, surrounding Kelowna, were not in favour of the project, as they had not seen the full benefit thereof, where growers in Zone 1, where releases have been done for ten years, are fully in support of the programme, and hardly use any chemical sprays anymore. We were shown a few orchards. We observed monitors on ATV's, and also observed a release driver releasing sterile moths in an orchard. The moths are released at a rate of 2000 per hectare, twice a week, and the release paths taken are 60m apart. We agreed that ants could be a problem in South Africa, as they could attack the moths before they became fully mobile.

We then had a meeting at the SIR offices in Kelowna, where Gavin Young, Manager of Field Operations, joined us. We were given a deeper insight into the programme, and discussed various possibilities relating to the South African situation. We were given a map of the zones and a copy of the 2003 Annual Report.

It became evident that the sanitation or 'clean-up' phase, whereby they removed 116 000 trees in the urban areas, was critical to the success of the program, but also very expensive.

After lunch we visited a grower, Russell Husch, who was very enthusiastic about the programme, and he explained the benefits of less sprays on the environment. We then visited Gerry Shaw, another grower who agreed in principal with the programme, but could not see the real benefits of it, and felt that the \$95 per acre he was forced to pay was making him less competitive than his rival growers in the USA. He questioned why Canada, with a small industry, should be spending the money, and felt that they should just follow what the USA did.

Tuesday 13/07/04

We attended an informal breakfast meeting with Bob Fugger. There were cultivar, irrigation, and crop insurance consultants in attendance, as well as a student. These meetings are held bi-weekly, and any pertinent issues are discussed.

We then went to Safeway shopping mall and viewed some South African fruit.

We viewed a neglected apple orchard where the grower had been given a deadline to remove all the fruit off some of his trees due to high trap catches of codling moth. We observed a high level of codling moth damage in close proximity to a pile of dead trees. We also saw how trees were banded with corrugated cardboard, which form an ideal pupation medium for the larvae. These bands are cleared and replaced weekly, so removing a significant percentage of the population.

Bob then drove us to Penticton, where we were met by Lorne Tomlin, who drove us to Osoyoos. We also met Adrian McClusky, the information officer. We viewed their workshop which is kept busy maintaining their vehicles and 31 ATV's.

On arrival in Osoyoos we booked in to our hotel, and then Lorne showed us around the area and took us to a few fruit stalls where the growers market their produce.

Wednesday 14/07/04

Lorne spent the day informing us of the history of the production facility, and gave us background to its problems and victories.

He explained that normally releases of sterile moths would take place for 20 weeks per year, but this year the feeling was that the sterile moths are not very competitive in spring, so the emphasis was put on mating disruption during this period, followed by releases over the following eleven weeks.

We then went on a thorough guided tour of the whole facility, from diet mixing right through to moth emergence and irradiation. What was particularly apparent throughout was the attention paid to sanitation

and prevention of contamination. Staff have to shower before entering the facility, and there is no flow of personnel from the 'dirty' to the 'clean' side.

The larval and emergence rooms are run at 27°C, but the humidity lowered in the emergence rooms so that the diet can dry out and form an ideal pupation medium for larvae.

In the evening we were picked up by Wilfred Mennell, an organic apple grower, and taken to the Similkameen Valley, the site of the first SIT field release trials done by Dr J Proverbs.

Thursday 15/07/04

Lorne took us to the Okanagan/Similkameen Co-Op Growers Association, where we saw cherries being packed. The Apple line was not running at the time.

We then visited a private packing facility belonging to Greg Norton, where it became apparent that even in such a small facility, safety standards were adhered to very strictly.

On returning to the production facility, we met with Susan Wood, who explained all the quality control procedures to us.

We discussed the export process of moths to South Africa and the results thereof.

After lunch, we spent the afternoon with Scott Arthur, who showed us the computerised control system, and gave us a detailed tour of the impressive air conveyance and temperature control system, and explained various technical matters to us. Each larval and emergence room has independent air flow, humidity and temperature control systems. Air is filtered through a series of three filters, the final filter being a hepafilter, which is so fine (0.3 micron) that it can even catch virus particles.

Friday 16/07/04

We returned to the facility, and viewed various other processes, such as egg sheet harvesting and preparation, which were not seen previously.

Lorne explained that 2 possible improvements that could be made to their facility would be to put the cold room and warm oviposition room further apart, and to have the egg-sheet preparation room in the 'clean' section of the facility.

A further meeting was held with Lorne, where purchasing of diet ingredients and various budget matters were discussed. We were given copies of their procedures and quality control manuals.

After lunch we visited the Pacific Agri-Food Research Centre in Summerland, and were given a tour of the impressive facility by a technician, Mark Gardiner. We then met with Dr Howard Thistlewood, who had previously managed the SIR programme, and he gave us his views on the programme.

Lorne then drove us back to Kelowna.

(b) Itinerary

Date	Place	Institution	Activity	Mode of Transport
11 July 2004	Calgary	Calgary Airport	Entering Canada	Air
12 July 2004	Kelowna	Airport	Scientific Visit	Air
13 July 2004	Osoyoos	Okanagan-Kootenay Sterile Insect Release Program	Scientific Visit	Road
16 July 2004	Kelowna		Scientific visit	Road
18 July 2004	Kelowna	Airport		Air
18 July 2004	Toronto	Pearson International Airport	Exiting Canada to return to South Africa via Amsterdam	Air

(c) Value of visit

I am involved in the development of a technique for mass rearing of sterile false codling moths for SIT purposes. The methods used by SIR to rear Codling moth can be of great value to us. We have done several trials experimenting with the SIR diet, but have found some of the ingredients to be very expensive, and not easily available in South Africa. I now have a deeper understanding of the role each ingredient plays in the diet, and can now look for substitutes for those ingredients which are very expensive or unavailable in South Africa.

We have seen a highly sophisticated rearing facility running very smoothly and efficiently. We will struggle to set up as lavish a facility in South Africa, but I feel that by keeping the few basic principles in mind we can achieve good results on a slightly simpler scale. These basic principles include air flow and filtration, pressurisation, and an absolute emphasis on sanitation and human traffic flow.

We were given a very balanced look at the whole program, with visits to growers and researchers who were very pro the system, and also some who had reservations about its value to them. It was good to see the benefits of the program, and also hear the reservations of a certain few who would probably not be involved if they were not forced to.

The visit emphasised the importance of an area-wide approach to pest management, as well as the necessity of cooperation by all parties involved if we are to succeed with SIT in South Africa. It was an eye-opener to see what can be achieved when people have a common goal, albeit with some statutory help.

My colleague, Dr Sean Moore, was privileged to visit the facility last year, and he had discussed the program with me at length. However, one cannot envisage and understand the scope and detail of the program unless you have seen it for yourself. The value of a visit can not be underestimated.

The visit to the Pacific-Agri Research Centre in Summerland was also highly valuable, and hopefully we can follow up with some of the contacts we made there and add value to our programme, as well as other areas of research, such as virus production and the use of entomopathogenic nematodes.

I am extremely grateful to the IAEA for affording me the trip and am looking forward to judiciously implementing what I have learned.

8.5 V.H. HATTINGH

8.5.1 IAEA sponsored study tour of the Sterile Insect Release programme for the control of codling moth in the Okanagan Valley, British Columbia, Canada, 10 – 19 July 2004

Summary

The Okanagan Valley Sterile Insect Release (SIR) programme was visited by four South Africans from the citrus and deciduous fruit industries. The objective of the authors' participation was to gain exposure to the various facets comprising the successful development, implementation and operation of a SIR programme, aimed at the control of a lepidopteran pest of fruit. Whereas the SIR technique has been applied to the control of codling moth in Canada, there are lessons to be learned from this programme that would be of value in the potential application of the technique for the control of False Codling Moth (FCM) *Cryptophlebia leucotretata* in the southern African citrus industry. An overview of the major components of the programme was obtained. Discussions were held with a range of parties reflecting diverse perspectives on the technical details of the programme implementation, including the production of sterile moths, preparation of orchards for SIR releases, pest management on an area-wide basis, administration of the programme, funding of the programme and stakeholder relationship management. The insight gained is considered to be valuable in managing the process of further developing the SIR technique for the control of FCM in the southern African citrus industry.

Itinerary

Sat & Sun 10 & 11 July	Travel: Cape Town – Kelowna
Monday 12 July	Observe field operations, hold discussion on administration, management and funding of programme, hold discussions with growers.
Tuesday 13 July	Participate in fieldsman, breakfast discussion, inspect orchards, discussion on public relations, transit Kelowna – Osoyoos.
Wednesday 14 July	Tour of sterile moth production facility

Thursday 15 July	Visit local packhouse, study of sterile moth production facility
Friday 16 July	Conclude study of production facility, transfer to Kelowna
Saturday 17 July	At leisure – Kelowna
Sun & Mon 18 & 19 July	Travel: Kelowna – Cape Town

The study tour group consisted of the following:

V Hattingh	CEO Citrus Research International, Stellenbosch
W Kirkman	Research - Citrus Research International, Port Elizabeth
G Smit	Citrus grower, Citrusdal
H Campbell	GM Deciduous Fruit Producer's Trust Research, Stellenbosch

Background

FCM is a pest of considerable concern to the southern African citrus industry. The potential for applying the SIR technique to control this pest has been under investigation in South Africa for the past three years. The radiation biology of FCM has been studied, release and re-capture field trials and field cage trials have been successfully undertaken in South Africa. During the 2004/2005 citrus production season, small scale field releases of sterile FCM are planned as part of a pilot project. In the near future industry decisions will need to be made regarding the potential scale up of the pilot project to a full-scale programme.

South African scientists involved in this research, have previously visited the SIR programme in Canada, with the support of IAEA. However, this follow-up visit included representation from industry research management, citrus growers and scientists. Inclusion of industry management and grower representatives will be valuable in facilitating forthcoming industry decision-making and the potential further development of the technique in the industry.

Kelowna

This part of the trip was hosted by Mr Bob Fugger, as General Manager of the SIR programme. Orchards in the area were visited and the activities of programme monitors and release drivers were observed. Discussions were held with Mr Fugger and the programme's Field Manager Mr Gavin Young. An overview of the historic development of the programme was obtained, including the funding, administration and management of the programme. Discussions were held with two prominent growers who held divergent opinions about the programme. The study group participated in a two-weekly breakfast meeting of local fruit industry representatives. A discussion was held with Mr Adrian McClustkey, who is responsible for the programme's public relations.

Osoyoos

This portion of the trip was hosted by Mr Lorne Tomlin, Plant Manager of the SIR moth production facility in Osoyoos. The operation of the production facility was thoroughly inspected and extensive discussions held with Mr Tomlin and Mr Scott Arthur, the facility's Maintenance Supervisor. The technical, operational, managerial, personnel and financial aspects of the production component of the programme were discussed. Quality Control details were discussed with Ms Susan Wood, the Quality Control Supervisor.

Pacific Agri-food Research Centre (PARC)

A visit to PARC in Summerland was organised by the SIR hosts. A tour of the PARC facilities was followed by a discussion with Dr Howard Thistlewood. Additional perspectives were provided by Dr Thistlewood, who also provided valuable advice on the potential development of the technique for application to FCM control in the southern African citrus industry.

Conclusions and recommendations

The trip is considered to have been successful, in that valuable insights were gained into a range of relevant aspects affecting the success of such a programme. The following points reflect a number of key conclusions and recommendations:

- The importance of industry stakeholder buy-in is particularly high for such a programme to be successful.
- The area-wide nature of such a programme, is of considerable value in effectively reducing pest populations to very low levels.

- It is important to ensure that beneficiaries of the programme are provided with appropriate information that will support the establishment of realistic expectations for such a programme. The following are some pertinent issues that should be well understood by all stakeholders from the outset: suppression versus eradication, the ongoing need for additional control practices, costs of the strategy and the area-wide intensive commitment required from growers.
- Unlike the Canadian programme, a SA FCM SIR programme will be more reliant on the citrus industry taking responsibility for funding. Government or international donor funding may be obtainable for the start-up capital requirements (particularly for infrastructure establishment), but the growers will most likely have to carry the ongoing cost of operating the programme. Consequently, the cost effectiveness of the SIR approach to FCM control, relative to other control options, will require careful consideration before an industry decision to implement such a programme can be made. It is critically important for the potential success of the programme, that local industry partners, with appropriate insights and credibility conduct this economic feasibility evaluation and that it is not conducted by external experts on behalf of the industry.
- The apparent pre-requisite of first having to reduce FCM population levels to appropriate levels before SIR releases can be successful, needs careful consideration. This will be an important point that growers will need to be aware of, before the industry potentially commits to implementing the technique. Likewise, the need for ongoing supplementary controls, in addition to SIR, needs to be incorporated into the economic feasibility study and growers need to be aware of the need for such ongoing additional control inputs.
- The mechanisms by which the programme is potentially implemented require careful consideration. Enforced participation in such a programme in SA, is unlikely. Nonetheless, there is much to be gained from a co-ordinated, area-wide approach to application of such a technique. This highlights the critical importance of following appropriate industry-driven processes in the further development of this technique within the SA citrus industry. It will be important to involve all parties currently involved in the programme, in an industry-managed discussion forum. Likewise, as the programme develops, the inclusion of additional stakeholders in a co-ordinated, industry-driven process will be critically important for successful implementation.
- The ongoing support of IAEA and the willingness of parties involved in other successful SIR programmes, to provide the SA programme with assistance and advice, will be essential for the success of the SA programme.
- Information on numerous technical details of the Canadian SIR programme have been gleaned from this trip and will be of great value in the further development of the SA programme.
- CRI is mandated by the citrus growers of southern Africa to oversee the provision, co-ordination and funding of research and technical support services to the citrus growers of southern Africa. Hendrik Hofmeyr (employed by CRI), is the SA citrus industry scientist responsible for the coordination of industry FCM research. Hendrik has conducted the preparatory FCM SIR research in collaboration with Stephanie Bloem (IAEA) and Jim Carpenter (USDA ARS), with the support of both IAEA SIT programme in SA (Dr Brian Barnes being the SA IAEA counterpart) and the southern African citrus industry (CRI). CRI should henceforth take a leading role in the further development and potential application of the SIR technique for FCM control in the southern African citrus industry. This will need to be conducted in collaboration with IAEA and all other stakeholders.

Acknowledgements

- IAEA and Dr Brian Barnes for funding and arranging the study tour.
- The Canadian Codling Moth SIR Programme and its staff for hosting the visit, with special thanks to Bob Fugger, Lorne Tomlin and Coralee Harrison for their hospitality and assistance.

8.5.2 Participation in the XXII International Congress of Entomology, Brisbane, Australia and a post-congress study tour in SE Australia: 12 – 26 August 2004

Introduction

The trip consisted of two sections. The first component entailed participation in the four-yearly International Congress of Entomology (ICE) in Brisbane from 15–21 August 2004. The author presented a paper entitled: “*Changes in the driving forces behind pest control strategies in export fruit industries such as the South African citrus industry*” in the symposium section dealing with “*Pest management in tropical and subtropical tree crops*”. The second part of the trip consisted of a self-organised study tour in SE Australia, aimed at gaining a better understanding of Research & Technical services in the Australian citrus industry, in comparison with the SA citrus industry.

A. International Congress of Entomology, 15 – 21 August 2004

Itinerary

Thursday & Friday	12 & 13 August	Transit: CapeTown– Johannesburg – Sydney - Brisbane
Sunday - Saturday	15 – 21 August	Congress: Brisbane
Sunday	22 August	Transit: Brisbane - Sydney

Overview of Congress

The primary objective of the author's participation in the congress, was to gain an overview of the current status of cutting edge developments, across the scope of applied entomological research, on a global scale. Since the International Congress of Entomology is the only global, periodic forum for all aspects of entomological science, this should be an appropriate event for pursuit of this objective. The following were secondary objectives: (1) Contribute towards maintaining a participatory status in an appropriate specialist field, by presenting a paper; and (2) strengthen and broaden an international peer network of contacts.

The Congress attracted approximately 3500 entomologists from across the world. A very wide range of topics was covered in the 7-day programme. Of particular relevance to the author, as the industry co-ordinator of market access technical issues, was a session on regulatory entomology. This essentially relates to phytosanitary and quarantine aspects of applied entomology. A large proportion of existing international contacts participated in the congress, and the scope of relevant contacts was considerably enhanced through the establishment of new contacts. This was particularly the case for contacts in the area of market access issues. There was a strong participation from Australia, Asian countries and USA, whereas European participation was markedly scarce. Although participation from Africa as a whole was low, South Africa was well represented with approximately 35 delegates.

Both the number of presentations and interest expressed by the audience, indicated a tremendous current focus of attention on the following fields of entomology: (1) conservation ecology; and (2) biodiversity preservation. Conversely, there was an alarming dearth of contributions, or interest in, applied agricultural entomology. In addition to neglect of the broader range of applied entomological topics, there was a near complete absence of contributions from the agricultural chemical industry. The most interest shown in any aspect of applied agricultural entomology, was in the area of organic production practices, but this amounted to little more than a catalogue of what is not achievable in the field of organics.

The lack of interest in applied agricultural entomology at such a congress, must signal alarm for any agricultural industry that is heavily dependant on applied entomology, such as the citrus industry. In more general terms, this experience is reflective of a broader development. Applied agricultural entomology has traditionally been viewed as a lower level of academic pursuit. However, the considerably higher income opportunities presented by the applied sector, had ensured that there were sufficient entomologist of high calibre that were prepared to make this compromise. Although not as yet equally evident in SA, this differential in economic opportunity has largely disappeared in the international entomological community. This has been driven by a multitude of factors, but the net result is a retreat of entomologists from the applied research field.

Notes on selected topics of special interest

The abstracts of presentations at the congress are available from the author in digital format. A copy of this has been deposited in the CRI library in Nelspruit.

- The section dealing with **regulatory entomology** had a strong focus on Australia and USA, but was also of international relevance. The primary value of this session, was an opportunity to strengthen existing contacts and make new contacts in this field. The value of having a strong specialist statistical expertise in this subject area, was highlighted. Likewise, the great potential value of increased international research cooperation in this field, was apparent. This is particularly relevant in the area of "physical quarantine treatments". It was established that an International Standard for Phytosanitary Measures (ISPM), is on the agenda for drafting by the International Plant Protection Convention (IPPC), the body mandated to develop quarantine guidelines, in accordance with the World Trade Organisation (WTO) agreement on Sanitary and Phytosanitary (SPS) controls. The framework for optimising this opportunity was discussed with Robert Griffin, the former IPPC coordinator for the drafting of ISPMs.

Firstly, it is necessary to ensure that the drafting of this ISPM is prioritised on the IPPC agenda. This ISPM should cater for Addenda to be compiled, that will constitute internationally standardised physical quarantine treatments; for example, cold treatment of citrus for the disinfestation of various fruit flies. Once such an ISPM is agreed upon, it provides an opportunity for the establishment of an international network of research specialists, who are thereby provided with a mechanism, whereby scientific findings can be transposed into legislative regulations. This provides an opportunity to minimise political and bureaucratic interference. The potential significance of this opportunity is considerable for the southern African industry and should be pursued as a priority.

- There were many contributions on *Xylella fastidiosa*, the bacterium responsible for a number of serious plant diseases, including CVC (Citrus Variegated Chlorosis), that has reached highly damaging proportions in Brazil. A lot of work is being done in attempting to control the disease and gaining a better understanding of how it is transmitted. In Brazil, it is known to be transmitted by sharpshooters. It is apparent that transmission of the pathogen is far less vector specific than previously reported. It has now also been established that certain leafhoppers can transmit the disease. Since leafhoppers do occur in SA, and are sporadically a pest on citrus in SA, this information elevates the status of CVC as a potential phytosanitary threat for the SA citrus industry, if the pathogen should be introduced into the region.
- **Organic production.** There was a session dedicated to presentations on organic production. However, these presentations provided no new direction on the topic, and reflected very little real progress with practical implementation of the practice, particularly with tree fruit crops.
- **Genetically modified organisms (GMO).** There were many presentations on the topic of GMOs. However, the usual debate does not seem to have changed or progressed. There remains very little concrete evidence of any significant deleterious environmental impact. Concerns continue to be based on the prospect of something going wrong. The inability to conclusively demonstrate that such concerns will never be realised, ensures that the debate fails to be concluded.
- **Sterile insect releases (SIR/SIT), agrochemical pest control and biological pest control.** Although all of these topics are fundamental to pest management and agricultural entomology as a whole, there were no presentations on irradiation techniques, and very few contributions on agrochemical or biological pest control.

B. Post-Congress study tour

Itinerary

Sunday	22/08	Transfer: Brisbane – Sydney
Monday	23/08	Sydney meetings: Horticulture Australia & AusCitrus
Tuesday	24/08	Mildura: Australian Citrus Growers Association & Dareton Research Stations
Wednesday	25/08	Renmark: Yardilla Park (growers, exporters & industry representatives), transfer: Mildura – Sydney
Thursday	26/08	Transfer: Sydney – Johannesburg

Objectives

Whereas the author has maintained a long-standing relationship with Research and Technical Advisors operating in Queensland, the majority of Australian Citrus is produced in the SE regions, that have not previously been visited. Personal contact has been maintained with the Horticulture Australia Limited (HAL) co-ordinator of the Australian Citrus Research portfolio. However, HAL has recently undergone restructuring and this function has been assigned to another individual within HAL. The objectives were to establish contact with the new HAL citrus co-ordinator, some key citrus industry individuals and gain an insight into the Australian Citrus Industry approach to Research, Extension, Citrus Improvement and Market Access issues.

Industry structures

The Australian citrus growers have formed a national citrus growers association, represented by a Board of Directors with approximately 2500 grower members. A large portion of the membership consists of growers that produce small quantities of citrus (< 11 ha), in combination with other crops. The national citrus growers association makes decisions on the implementation of national research and marketing levies. The National Growers Association elects grower representatives, to serve on a national Market Access Committee, an

Industry Advisory Committee for Research, and employs a communications manager. In addition to the national level, there are also a number of State (Provincial) grower associations and State Levies. The National Growers Association strives to achieve interaction and co-ordination between the various groupings.

Horticulture Australia Limited (HAL) was formerly a Governmental organisation, but has privatised and provides a range of services to the horticultural industries, including citrus. HAL collects levies and manages the funding, execution and reporting of research and marketing projects. In addition to the levies, HAL also invites voluntary funding contribution from any stakeholder in the Australian horticultural sector, for the funding of specific research projects required by the particular stakeholder. Industry research funds (not marketing) are equally matched by a Government fund, that may not exceed 10% of the Horticulture Sector's turnover (10% of \$600m pa = \$60m pa = approximately R270m pa).

Several posts within HAL are assigned to interface with the various industries. Whereas Mr Gerard McEvilly was responsible for citrus within HAL for several years, Mr Ross Skinner has recently been assigned this responsibility. Mr McEvilly will in future be focussing on projects aimed at realising efficiencies in the supply chain. HAL has a newly formed commercialisation division, that aims to pursue an income stream from IP arising from the research funded by HAL. It is noteworthy that this is a parallel strategy to that being followed by CRI in the formation of the commercialisation subsidiary, River Bioscience.

HAL also hosts a Market Access Committee, which provides a forum for the coordination of the Market Access interests of the various fresh produce exports. Mr Mark Chown, Vice President of the National Australian Citrus Growers Association, represents the Horticultural industries on this committee. HAL in turn provides staff that interact with Government officials on the various Market Access issues.

Funding

The current national citrus research levy raises approximately AUS\$1.5m pa and an additional \$0.5m is derived from voluntary contributions. With the 1:1 matching government funds, there is approximately \$4m pa available for research. At an exchange rate of R4.5/\$ this is equivalent to R18m. In comparison, including the leveraging of grower funds through government subsidising schemes and other sources, the SA citrus industry research receives approximately R13m pa. However, comparison of the South African and Australian research portfolios, indicates that the SA portfolio is approximately 2 to 3 times larger than the Australian portfolio.

The matching Australian funds apply regardless of where, or by whom, the research is conducted. This is in stark contrast to the SA THRIP scheme for government matching of industry research funds, where only tertiary education institutions qualify, and a multitude of other additional qualification criteria are applied within the SA system, making SA funds far less accessible than in the Australian system.

The affect of the Australian model is that it attracts a range of stakeholders beyond the grower community, to also contribute towards research and encourages a wider range of potential research service providers to offer research services. This is the type of research enhancement scheme that needs to be considered by the SA National Agricultural Research Forum (NARF), in striving to improve the SA National Research System.

It seems to be commonly perceived in Australia, that available funds are insufficient to address the industry's research needs. Researchers complaints include the high administrative input required to secure funds, the low funding rate that has resulted in insecurity, the loss of expertise and an inability to attract and retain expertise, all resulting in a failure to provide for succession planning. A common criticism is that there are too many groupings, too much fragmentation and too many parties taking up levy funds for activities other than research. Some parties commented that there is a problematic lack of commitment, responsibility, sense of ownership and common vision for research, among the growers.

Nature of the research portfolio

Comparing the SA and Australian research portfolios, gave rise to the following observations: The SA portfolio is approximately 2 to 3 times larger, covers a wider range of topics, but a great deal more detail is specified in the research objectives. The Australian portfolio addresses a far narrower range of research topics, is considerably smaller in terms of content and research expertise that it mobilises, and the research projects tend to be more broadly defined rather than specifying detail.

Several topics that would not be considered research in SA are included in the Australian portfolio. A topic that is addressed within the Australian portfolio, but not in the SA portfolio, is specific market research. A

second associated topic, that is to receive attention by Australian scientists, and should be considered in SA, is the development of sensory profiles for SA citrus fruit.

Research capacity

There are no scientists dedicated exclusively to citrus research in Australia. There are only a handful of researchers (approximately 6) that have a long-standing and routine citrus portfolio. Approximately half of the "citrus orientated" researchers are close to retirement (within 3 years), and there is little apparent succession planning. Research that supports the citrus industry, is clearly in a current state of challenge in Australia.

CIP

The Australian CIP appears to be in good order, due to the leadership and dedication of individuals such as Pat Barkley and Graeme Sanderson, although there are surely other individuals worthy of recognition that were not encountered during the brief visit. The Australian industry appears to be strategically well positioned with regard to its CIP and cultivar development.

Of particular interest is the close collaboration of Australia with China. The Australian citrus industry seems to have achieved something that the rest of the citrus world must be envious of, being access to citrus genetic material from China. An example of this is evident in two promising new rootstocks acquired from Chinese material, with one performing exceptionally well in initial trials.

There is strong evidence of a long term strategic approach to cultivar development within the Australian industry. There has been a concerted effort made over the past 10 years to bring in new cultivars. However, at this point, the Australian industry has also fallen into some of the same pitfalls as the SA industry. Australian industry opinion leaders, are now thinking in the same direction as the SA citrus industry has recently gone, with regard to the creation of an industry cultivar manager post.

There is now a strong push within Australia, to ensure that all cultivars, regardless of ownership or origin, are included in impartial, comprehensive, industry operated, comparative evaluations. It is interesting to note that the emphasis is on ensuring representative geographic spread of evaluation plantings, as a priority above statistically replicated trials. This is also in line with the approach that has been identified for future evaluation in SA.

There is also a recognition within the Australian industry, just like in the SA industry, that a serious strategic error was made in the industry, by not engaging directly in the commercialisation of protected cultivars. Both industries are now attempting to effect damage control on this front.

Cultivar commercialisation

Discussions on this subject were held with various prominent Australian role players. The two industries have apparently had similar recent experiences. Like the SA citrus industry, the Australian industry also initially withheld from direct involvement in the area of commercialising protected cultivars. The Australian industry has also concluded that this was a mistake, and most prominent Australian role players were optimistic about the route that the SA industry is now taking by appointing a cultivar manager. There was strong support for having inter-industry collaboration in this regard rather than encouraging individual parties to make deals in isolation. There is considerable discomfort within the Australian industry around the current ARC-ANFIC interaction on cultivars. This has been heightened by awareness of the failure in SA to get an agreement between the ARC and SA citrus growers on cultivar matters. CRI was requested to make the new cultivar manager available to clarify these matters to the Australian citrus industry as a priority.

Research cooperation

Given the consideration that research in both Australia and SA is funded by growers, who are concerned about protecting their respective competitive advantages, research collaboration is a sensitive issue. Keeping this in mind, the prospect of research collaboration was discussed with various industry groupings, including HAL and the Australian Citrus Growers. The absence of a CRI counterpart in Australia was generally recognised as a hindrance to the potential implementation of collaboration, that could be mutually beneficial, and not comprise local grower interests. Given this finding, it was concluded that, instead of striving for an over-arching general agreement, the focus should be on individual researchers establishing inter-industry collaborative ties on suitable topics.

Considering that research results themselves are of little value relative to the availability of appropriate expertise, it would indeed be detrimental, for one industry to assist in the establishment of key expertise in a competing industry. However, should both industries already have the necessary expertise, collaboration in certain research fields, should be mutually beneficial. It is especially attractive in areas of research, where neither party is making progress in isolation, and where an injection of new thinking is required, or where a pooling of resources would make it possible to do work that neither group could attain on its own.

The following research fields were identified as being prospective areas for collaboration: Cultivar and rootstock evaluations, rind disorders, the control of various pests and diseases of mutual concern. Each specific collaboration proposal should get prior endorsement by suitably mandated bodies in both industries. The primary interaction channel was identified as being CRI – HAL. The CEO CRI met with both the outgoing, and newly appointed, HAL citrus research co-ordinators and it was agreed that this would be a suitable route to follow.

Market Access

The Australian citrus industry's organisational structures involved in market access, are in principle similar to the southern African industry's approach. The frustrations and challenges experienced by the SA citrus industry, in terms of interaction with local and foreign plant health authorities, are largely shared by the Australian industry.

The Australian citrus industry is fortunate to have strong support from a Government that allocates huge resources towards protecting Australian producers from the introduction of exotic pests and diseases. This has enabled the Australians to effectively eradicate fruit fly invasions and the recent outbreak of citrus canker, appears to have been well contained, with good prospects for eradication. The strong support from government is also evident in their ability to maintain fruit-fly-free zones, through strictly enforced regulations prohibiting the movement of fresh fruit between regions within Australia. This includes both permanent and periodic roadblocks. The agriculture-friendly nature of the Australian government is abundantly apparent.

Summary of conclusions and recommendations

- Industry, Research and Market Access structures within SA, are comparable with the Australian operations, although the SA system are apparently more cohesive and have more common direction.
- The cost-effectiveness of the SA citrus research enterprises, compared favourably with the Australian portfolio.
- The role of government in supporting research, is apparently more effective and better aligned with the citrus industry in Australia than in SA. The SA industry stands to gain considerably by locally addressing this issue.
- Both industries are proceeding similarly with regard to deriving additional research income from the commercialisation of Intellectual Property, arising from research funded with grower contributions.
- The new SA citrus industry approach to grower cultivar interests, through the appointment of a cultivar manager, received a favourable response from the parties consulted in Australia. There is an urgent need for the SA cultivar manager to engage with the Australian industry to ensure protection of SA citrus growers' cultivar interests in Australia.
- The prospects for mutually beneficial research collaboration, that will not jeopardise the competitiveness of the respective industries, should be centred around individual researchers and be vetted by both CRI and HAL.
- The following topics should be considered for inclusion in the SA research portfolio, as they are in Australia: (1) specific market research and (2) development of market-related sensory profiles for southern African citrus fruit.

8.5.3 Participation in the 2004 CLAM General Assembly in Antakya, Turkey, 07 – 15 October 2004

Itinerary

Thursday	07/10	Transit: Cape Town-Johannesburg Zurich-Istanbul, Kayseri
Saturday	09/10	Pre-meeting tour of Cappadocia region with CLAM delegates
Mon-Wed	11-13/10	General assembly in Antakya Post-meeting tour of Turkish citrus and agricultural development projects with CLAM delegates
Thu-Fri	14-15/10	Transit: Adana – Istanbul – Zurich – Johannesburg – Cape Town

Background and objectives: the strategic significance of CLAM participation

CLAM (Liaison Committee for Mediterranean Citriculture) is an organisation that represents citrus producers, importers and exporters in the Mediterranean countries. Membership consists of representatives from Spain, France, Italy, Greece, Cyprus, Turkey, Israel, Egypt and Morocco. The objectives of CLAM are to provide a forum for exchange of information on production, markets and relevant technical issues affecting the Mediterranean citrus countries. In addition to members, there are associate CLAM members that have for many years been USA, Argentina and South Africa. On deregulation of the South African citrus industry, Capespan temporarily continued to participate to retain continuity of SA participation.

In 2001 the Secretary General (CEO) of CLAM (Luis Calabozo) invited the CGA to participate in CLAM's activities. The principal advantages to be derived by CGA's participation in CLAM are as follows: (1) It provides access to information on the production, exports, development trends and market performance of approximately 70% of the world's citrus production, (2) It provides access to information on the performance of citrus in the EU market preceding southern African entry on an annual basis; (3) It provides a forum for the development of inter-personal relationships with key individuals in the European citrus production base; (4) It provides an opportunity to have inputs into the Mediterranean citrus industries' actions in terms of EU legislation, particularly pertaining to SPS issues and this is the most significant aspect of CLAM participation from the southern African citrus industry perspective. The CGA, being responsible for representing industry Market Access interests, is therefore the appropriate party to represent the southern African citrus industry at CLAM.

It is therefore not surprising that the CGA accepted the offer to participate in CLAM activities. The author, being responsible for the co-ordination of industry technical market access issues, being familiar with the EU SPS position and already being acquainted with some of the CLAM member representatives, was nominated as the SA CLAM representative, and has participated in CLAM activities for two years preceding this year's General Assembly in Turkey.

This strategically important decision, paid handsome dividends in 2003 when, in the light of the 2,4D crisis in the EU, the author was able to mobilise the weight of the Mediterranean citrus industries behind SA's engagement with the EC, to establish import residue tolerances for 2,4D on citrus, through unprecedented EC procedures.

Of even greater strategic significance, is that CLAM participation provides a mechanism to counter-act EU Mediterranean citrus producers' endeavours to effect protectionist positions through EU phytosanitary legislation. This can be achieved by virtue of CLAM also being representative of Mediterranean importers, exporters and non-EU Mediterranean member countries.

Points of particular interests arising from the meeting

1. The network of relationships within the CLAM grouping was greatly advanced by participation in these meetings.
2. Comprehensive statistics were obtained for CLAM members' 2003 industry performance. Copies of these statistics have been deposited at both the CGA office in Durban and the CRI library in Nelspruit.
3. The Spanish citrus industry gave a presentation on the trends, current status and future prospects for cultivars in the Spanish citrus industry. Discussions were held with the individual responsible for the commercial aspects of cultivar development in the Spanish citrus industry. This has established a platform for further discussions by the new CRI cultivar manager.

The Spanish nursery industry presented information on cultivar development and planting statistics in the Spanish industry. The Spanish nurseries supply the industry with approximately 7 million trees pa. There is a strong shift to planting late naves and a wide range of soft citrus. Six of the 10 most-planted cultivars over the past 5 years are patented cultivars. Over the last two years Oronules, Capola, Arufatina and Hernandina have been planted abundantly. The new cultivar Clemenrubi, a natural mutation of Oronules, is expected to dominate tree sales in 2004. It is two week earlier than Oronules and has good quality. Although Clemenules is still the most planted soft citrus cultivar, these are now mostly older plantings.

4. A long-standing relationship has been maintained with leading parties in the Israeli citrus industry. The CLAM meetings provided an opportunity for ensuring continuity in this relationship, given that there has been restructuring within this industry. Mena Davidson has retired and Tal Amit has replaced Mena in an equivalent position. Preparatory discussions were held, to set the scene for the cultivar manager to take up negotiations with the Israeli industry, regarding commercialisation rights to Israeli cultivars.
5. In 2001 the development of relationships with appropriate parties within France, was identified as a strategic EU phytosanitary objective for the SA citrus industry. Although various contacts have been developed towards this goal, strong relationships with suitably influential French parties have remained elusive. The previous three years' endeavours appeared to be coming to fruition during the Turkey meetings, when discussions were held with a French party that has the required profile. This encouraging progress will now be actively pursued.
6. The author has endeavoured to gain participation in relevant CLAM activities over the past. This has proven to be a slow process. However, CLAM has a technical co-ordinating committee, that will in future become more involved in EU SPS legislative issues. The Chairman of this committee and General Secretary of CLAM have agreed to actively engage the author in the activities of this committee in the future.
7. The membership status of SA in CLAM has been agreed upon by the CLAM Board, as "associate CLAM member" status. There is a membership fee of approximately Euro2500 pa. This is equivalent to the cost of a one week trip to Europe and the strategic significance of the opportunity will surely provide for a very attractive return on investment.
8. The prospect of hosting a future CLAM General Assembly in SA has been raised by the CLAM Secretary General and bears further consideration by the SA industry.
9. Information was obtained on a previously unknown EU SPS legislative development, that warrants noting by SA. The Directive (2002/89) relates to phytosanitary inspection of fresh produce imported into the EU. It caters for inspection at points other than ports of entry and allows for reduced inspection intensity of low-risk commodities. To be recognised as a low-risk commodity, an EU member state must put the proposal to the Commission. This legislation should be carefully studied and a course of action established. However, in the light of the balance required between a multitude of issues currently relevant within the EU SPS environment, it may be prudent for SA not to currently raise its profile on this particular issue.
10. The CLAM technical committee is taking up the issue of declaring the use of post-harvest preservatives on cartons in the EU market. The precise legislative requirements are unclear and German retailers are objecting to the declaration of Imazalil on cartons.
11. CLAM has resolved the issue of German clients objecting to the use of TBZ on imported citrus for juicing. The German national legislation that prohibited TBZ on processing fruit, which is contrary to EU legislation, has consequently been rescinded.
12. CLAM countries anticipate overall production and export volumes for 2004/5 that are much the same as 2003/4, and to date quality appears to be better than last year. More specifically, production of oranges and grapefruit is expected to decline slightly, lemons to remain unchanged and soft citrus to increase by 15%. Colour development in Spanish soft citrus has been delayed and their entry into the market will be delayed by 2 weeks.
13. The previous rapid growth in the Egyptian industry has levelled off. This is primarily due to dismantling of various Government subsidies to growers and exporters.
14. The Turkish industry continues to grow. Grapefruit and lemon plantings have tailed off, but soft citrus and orange plantings are continuing strongly.
15. Morocco has recovered from the previous season's drought.
16. Israel has commenced with a replanting programme and for the first time in many years, there is a good level of confidence in the industry. The Israeli citrus industry has undergone restructuring and the Citrus Marketing Board of Israel has now been taken up as a division within the Plant Production and Marketing Board.

17. Greece is expecting a reduced production due to frost during blossom. The Greek industry is currently embarking upon an industry rejuvenation and replanting programme.
18. Italian production continues to decline. Citrus Tristeza Virus is having a major impact on the industry and it is anticipated that this will continue and worsen in the near future.
19. Floridian production is expected to be reduced dramatically by the Hurricanes. It appears that there has been relatively little damage to the trees and the losses have been primarily in terms of crop loss.
20. Argentina has gained access for lemon exports to the Japanese market. The phytosanitary controls applicable to these exports should be ascertained, to determine the competitive implication for South African exports.
21. The Russian import agent JFC, gave a presentation on the Russian market. Whereas southern Africa is the largest supplier of oranges during the summer season, Argentina dominates the lemon supply. Chinese exports of soft citrus, are starting to compete strongly with Spanish exports to Russia. It was reported that there is a strong demand for lemon and red grapefruit in Russia. Lemon consumption in Russia is reported to be particularly high.
22. Spain has gained access for clementine exports to Japan. These exports will begin in the 2004/5 season. Japan's granting of access for Spanish clementines, is in stark contrast with Japan's steadfast refusal over the past 5 years to open the market for South African clementines. It seems that the Spanish-Japanese deal was concluded when a high level EU trade delegation visited Japan. It seems that SA will also have to resort to political intervention before the Japanese market will be opened for SA clementines.
23. The second reading of the revised EU MRL frameworks legislation is envisaged for December 2004, with anticipated implementation in 2005. A number of critical details remain unclear. The progress of this legislation needs to be tracked very closely as a matter of priority.
24. Confirmation was obtained that SA's interpretation of EU legislation pertaining to the use of SOPP is correct. SOPP may continue to be used as a preservative until January 2006, and then the MRL will continue to apply, but SOPP will no longer be classified as a preservation. Depending on the outcome of current investigations into the EU preservatives legislation, this may imply that as of 2006, SOPP should not be declared on cartons as a post-harvest preservative. These developments should be tracked to ensure that the industry is provided with adequate notification of impending changes. The Freshfel websites freshquality.org and freshfel.org, should be monitored continuously for notification of changes to EU legislation.
25. It was announced that complete kits of equipment required for "fresh cut" citrus are now available on the market for approximately US\$150 000.
26. LAM is supporting the development of a Mediterranean-wide SIT programme for the area-wide control (suppression) of Medfly. The EU has funded a three year initial development proposal. These developments have very serious potential implications for the southern African citrus industry. This may lead to Medfly becoming an "officially regulated" pest in Europe, and this in turn, would provide a legal foundation for treating Medfly as a quarantine pest of imported fruit, similar to the current phytosanitary status of CBS. It is critically important that the southern African industry tracks these developments very closely. The CLAM Secretary General and Chairman of the CLAM Technical Committee, have agreed to keep the author informed of the technical committee's activities and discussions.
27. The CLAM Technical Committee has launched a project to co-ordinate efforts, aimed at protecting the Mediterranean citrus industries from the introduction of new pests and diseases. This development adds to the vital importance of the southern African citrus industry, remaining associated with CLAM.

Conclusion

Participation in this CLAM event was most valuable in pursuit of the objectives set by the local industry for participation in CLAM activities. The strength of networking with CLAM members has benefitted greatly. Insights gained into the technical-legal developments within the Mediterranean citrus countries and the EU, are of immeasurable value. It is recommended that CGA cement SA's position within CLAM, by subscribing

to official "Associate CLAM Membership". The annual membership fee is approximately EU2500, equivalent to the cost of one overseas trip.

8.6 A.B. WARE

8.6.1 Visit to Australia - 15-21 August 2004

The primary purpose of the trip was to attend and deliver papers at the XXII International Congress of Entomology held in Brisbane, Australia, between 15 and 21 August 2004. The meeting was attended by some 3100 delegates who presented in excess of 1700 papers and more than 900 posters. The congress was divided into 20 sessions resulting in 17 papers being presented at any one time. Physically one could only attend a maximum of 125 oral presentations which meant one had to plan one's attendance carefully. Subject matter was wide and ranged from "Managing insects on plants", "Pesticides, resistance, transgenics and genomics", "Integrated pest management", "Biological control, entomophagous insects and insect pathology", "Urban and stored products", "Medical and veterinary entomology", "Insect/plant interactions", "Ecology and population dynamics", "Chemical and physical ecology and behaviour", "Reproduction and development", "Genetics and evolution biology", "Neurobiology and behaviour", "Physiology and immunity", Biodiversity and biogeography, systematics and Phylogeny", "Acarology", "Insect invasions", "Social insects", "Entomology and the public". The final sessions involved society and special interest group meetings.

Many of the presentations were reviews of the particular presenters' research or highlighted the state of research in a particular field and provided little scientific substance. An example of this was Andrew Beattie's team on mineral oil research (Beattie *et al.*; (a) Beattie *et al.* (b) Rae *et al.*; Hassain *et al.*; Liong *et al.*). In the "Insect invasions" section many of the presenters gave talks from a regulatory perspective (Hallman; Davis & Grimm, Bansiddhi; Yokoyama and Miller; Griffan, Jamieson, Stynes, Qin). Kopittke *et al.* gave a biometrician's account of disinfestations treatment. However, in discussions with some of her colleagues they were of the opinion that practical considerations and the prior agreement of research protocols took preference over the "science" of numbers. Nancy Cunningham and co-workers indicated 'Tsumanii' as a possible packhouse treatment for quarantine pests of navel oranges. Andrew Jessup gave little away in his presentation of trapping female *Bactrocera* fruit flies. Francis de Lima gave a review of fruit fly eradication in Western Australia – basically old hat.

In the pest mitigation section the identification of potential threats to non-target species (van Lenteren *et al.*), mapping of the potential ranges (Sutheist), prioritising threats (McKirdy) and analysing the threat of a potential invasion (Qin) were addressed. A few papers addressed the issue of habitat manipulation by providing natural enemies with floral nectar (carbohydrate source) and pollen (protein source) (van Eenden; Andow *et al.*; Wrattton *et al.*) thereby ensuring their survival and reducing the necessity to intervene chemically. Techniques for marking insects for mark-release-recapture studies were described by Hagler and Jones (TgG) and Hagler and Williams (animal protein) and ELISA. Rubidium techniques were described by Scarratt and Wrattton and by Bancroft. The former technique may be useful in augmentation studies. Video techniques were described by Wrattton *et al.* for assessing predation of thrips eggs.

Fumigation using essential oils was the subject of three presentations (Perera *et al.*; Bailey, Korgetts, Hassan and Uddin; Kalinovic and Rozman). The oils, derived from a number of sources including eucalyptus, lavender, thyme and *Cinnamomum*, were successfully used in stored products. The active ingredient is apparently 1,8 cineole and is considered a friendly alternative to phosphine and methyl bromide. There was a notable absence of "new chemicals". Daniella Le Lagadec reported that thiamethoxam (neonicotinamide) was good in controlling scale and mealybug when used as a soil drench. It was better when applied in the dripper zone rather than around the trees. This observation raises the question whether Confidor and Mospilan would be better if applied in a similar fashion. Salt was reported to be an option to manage sucking insects (Khan and Murray; Brier *et al.*).

Two hours of the conference were set aside for fruit fly biology, ecology and management. Don McInnis *et al.* gave a presentation on the development and field evaluation of a pupal colour sexing strain for the melon fruit fly. Ultimately an SIT programme will be dependent on the development of a sexing strain for Natal fruit fly. Welden presented a poster entitled "Mass rearing and sterilization after the mating behaviour of male Queensland fruit fly." Two papers on the effect of diets have on behaviour and development of fruit flies. Ginger oil was found to increase male Medfly calling (Bosco *et al.*) and this observation may have some application in SIT and fruit fly monitoring. Niaan was found to improve Medfly larval weight (Chang & Li). Juanita Heunis and Glenn Bowman gave their results of fruit fly behaviour in various temperatures (Medfly) and wind, rain and ambient temperatures (Queensland fruit fly) respectively. The use of orange to attract and trap Mexican fruit flies was reported by López-Martínez & Acosta-Durán. A poster by Baliraine *et al.* demonstrated the displacement of Medfly by Natal fly on the island of Reunion.

Pest exclusion using netting (Lloyd pers. Comm.; Waite *et al.*) needs to be examined under South African conditions as it is a single treatment that can potentially create FCM and fruit fly-free areas. Other aspects that may make the technique economical is the fact that no hail insurance would be necessary and that wind damage should be minimal. In the case of mandarins in the Patensie growing area bird damage would be avoided.

Two talks were presented. The first was the "South African experience in cold disinfestations research" (Ware [a]), and the second reported on field results using the M₃ bait station (Ware [b]). However, there were many talks of interest that I was unable to attend. Some of these were integrated pest management with ants (Peng and Christian; Peng), thrips semiochemicals (Teulon, *et al.*), molecular response to cold and desiccation (Denlinger *et al.*), olive fruit fly research (Yokoyama and Miller; Fathi & Reuvin; Pickett *et al.*, Economopoulos), pest control changes in research strategy caused by import requirements (Hattingh), to mention just a few.

The South African Entomological Society successfully bid for the next International Congress of Entomology. This will be held in Durban in 2006.

Perhaps the greatest benefit of these regular get-togethers is the opportunity to network, renew old acquaintances and meeting "new" people. I was able to establish contact with Queensland Department of Primary Industries and made a post-congress tour of their facilities in and around Cairns. The first port of call was a facility at Cairns. This was built in response to the papaya fruit fly invasion. Currently it is being used to rear *Cactrocero neohumilis* and *B. trivialis*. The latter considered to be potentially as important as Queensland fruit fly *B. tryoni*. The unit has been doing a lot of post-harvest disinfestations. This includes vapour heat treatment. Peter Leach is of the opinion that citrus will tolerate the treatment. He believes that previous research results were compromised as the fruit had undergone cold storage and then heat vapour treated. To be effective the fruit must be vapour heated before cold storage.

Pedro O'Conner, facility manager of The Centre for Wet Tropics Agriculture, South Johnson, took me on a tour of the research station. Bananas, pawpaws, cocoa, durian and mangosteens are the main thrusts of their research. The final visit was the DPI & F and Aquis research facilities at Mareeba. Here I spent some time with Kerrie Huxford, the entomologist responsible for surveying fruit flies in the islands of the Torres Strait (situated between Papua New Guinea and Australia). It was interesting to find out that the Australians know more (than we do in South Africa) about the dispersal and distribution of the *Bactrocera* sp. recently recorded in Sudan, Tanzania, Uganda and Kenya. This species originated from Sri Lanka and is apparently more aggressive than *B. zenatia* and should be considered a serious threat (Luckman, 2004).

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8.7 P. CRONJÉ

8.7.1 Visit to Sheerness UK, 11-15 September 2004 for research purposes

The main reason for the visit to the UK was to retrieve the CO₂ measuring equipment on board the "Snowflower" that sailed from PE on 26 August 2004.

Installation of equipment

The CO₂ measuring equipment was installed on the "Snowflower" (hold 4B) that was carrying Valencias from the Eastern Cape. Most of the pallets were made up of telescopic cartons but several pallets, with two bulk bins per pallet, were also in the shipment. The equipment was installed onboard with the help of Dewald Millard from Capespan in PE harbour. The ship sailed on 26 August to Vigo (unloaded fruit on 10-11 September) and arrived in Sheerness on Sunday 12 September.

Retrieval of equipment

I arrived in the UK on Saturday 10 September and drove to Sheerness on Sunday 11 September. Although the ship was expected during the day it arrived at night resulting in unloading only commencing on the morning of 13 September. The unloading started at 6 a.m. and continued for 2 hours before being stopped by rain (said to be a spin-off from the hurricanes in the Caribbean!). Luckily after a 2-hour delay the rain stopped for the rest of the day and the whole ship was unloaded.

The CO₂ equipment was retrieved from the hold and the data were downloaded from the logger. I stayed on during the unloading and saw a lot of damage to the pallets that was caused during the unloading process. Most of this damage was the result of forklift operators that rammed a pallet into too small a space. Damage caused by forklifts "teeth" on the wooden pallet base was also seen regularly (the blue wooden bases from CHEP were in better condition than the no-name brand ones). The other main source of fruit damage was bulk carton bins that bulged out at their base and during loading got torn by the placement of the adjacent pallet. When the pallet was unloaded the fruit spilled out in the hold and caused a lot of delays.

After the unloading, I got the CO₂ values that the ship's datalogger recorded from the Captain of the ship. The values were at an average of 2000 ppm and corresponded with those from my logger. The values that I recorded reached a maximum before the ship sailed of 2500 ppm and decreased to a level of 1600-1800 ppm during the voyage. These values are not reported in literature to be detrimental to fruit quality.

Capespan facility at Sheerness

All the staff at the facility were very helpful in the retrieval of the equipment (Tony Costello from Cape Reefers and Paul Jeffrey from Medway Ports), and Jaque Vriese from Capespan who showed me around the cold rooms and repacking and inspection facilities.

Meeting with Jason Perrott

A meeting was arranged with Jason to discuss doing a project with him that would be part of his MSc studies at Writtle College in the UK. We met on 15 September and he proposed to do an experiment on the effect of Modified Atmosphere Packaging on the development of rind disorders of Satsumas exported to the UK. We agreed that it would be mutually beneficial to do this work and I would include it in my project proposals for 2005.

Conclusion

The visit to Sheerness was very valuable for me to see what happens with the fruit once it arrives at the import harbour and under what constraints the whole process is exposed to. The equipment that I retrieved arrived in Stellenbosch in good working order and after a calibration check was re-installed on the "Sun Claudia" that sailed to the USA on the 28 October 2004. The data from this project will be reported on in the research report of 2005.

8.8 H.F. LE ROUX, S.P. VAN VUUREN & G. PIETERSEN

8.8.1 Visit to São Paulo State, Brazil, to investigate Citrus Greening Disease problem. November, 2004

Background

Fundecitrus, Brazil, requested a visit by H.F. le Roux, S.P. van Vuuren and G. Pietersen, three South African plant pathologists, to assess the situation regarding an outbreak of citrus greening disease, occurring in 2004. This disease had not previously been reported from Brazil, but has been known in South Africa since the 1920s. The Brazilian Institute required comparisons with South African greening and inputs on control.

Fundecitrus agreed to cover all costs, and in exchange they would demonstrate diseases not occurring in South Africa to aid in the initiation of a capacity in South Africa to be able to detect these diseases pre-emptively. The Brazilian industry furthermore made use of the visit as part of an awareness programme to illustrate the importance of greening and its control.

Vergroening is vir die eerste keer gedurende 2004 in Brazilië positief geïdentifiseer. Die teenwoordigheid van twee candidatus spp nl. *Liberibacter americanus* en *L. asiaticus* is bevestig. Drs. Hennie le Roux, Fanie van Vuuren en Prof Gerhard Pietersen het op versoek van Fundecitrus, CRI se eweknie in Brasilië Sao Paulo en Mina Gerais provinsies besoek. In die proses is nie alleen kennis ingewin omtrent Brazilië se vergroening nie maar ook t.o.v. sitruskanker, "citrus variegated chlorosis, sudden death, pink disease, post bloom fruit drop en Leprosis virus". Brasilië is 'n broeiplek van sitrussiektes en Suid Afrikaanse sitrusprodusente word aangeraai om hierdie dele van die wereld te vermy.

The Brazilian citrus industry

The Brazilian Citrus industry expects to produce about 360 million boxes (40kg/box) citrus in 2004, about the same as in 2003, with current projections suggesting that 2005 production would be down to 300million boxes.

The number of producers in the industry declined from 20000 to 11000 over the last 4 years since the advent of the disease Citrus Variegated Chlorosis (CVC) caused by a xylem-limited bacteria *Xylella fastidiosa*. A tendency exists in Brazil that larger Citrus producers continue to expand their production through increased acquisition of land while smaller producers stop the production of Citrus and tend to change to sugarcane production. Currently, there are 15 million replacements of trees per annum in Brazil without an increase in acreage. There are some changes from dry-land to irrigation, resulting in yield increases from 45 t/ha to 65 t/ha. This could imply an increase in juice production in Brazil.

The industry (processors) pay a statutory levy per box resulting in an income of about 12-14 million US\$ for Fundecitrus. Of this, about 10-11 million US\$ is used to fund the eradication of citrus canker, while the remaining funds are employed for research at Fundecitrus. Fundecitrus have about 2000 people working on the canker eradication programme. For research, 9 researchers are employed fulltime to investigate a number of pathogens and diseases (CVC, CBS, *Alternaria*, Leprosis, Sudden death and Greening). These researchers are all stationed at Araraquara where excellent laboratories and growth facilities exist. The personnel are well-qualified, enthusiastic and motivated about their work. They have good working conditions and have 30 working days leave per year.

The citrus industry in Sao Paulo State used to be mainly situated around Araraquara but moved north over a number of years, however with the advent of CVC and Sudden Death it is currently moving further south of Araraquara to places like Itipitanga and Conchal.

A boom period is occurring in the sugarcane industry, and if great strides had not been made with higher density, irrigation and improved nursery practices, citrus production would have declined. Some processing plants that closed down a few years ago have subsequently been re-commissioned over the past two years.

Two organizations exist within the Brazilian industry, the grower association Associtrus (looking after the grower interests) and a processors organization (compare CGA and citrus exporters forum). Income per box in Brazil to the grower is US\$ 2/box.

Export of fruit remains unimportant and the chance of Brazil entering this market in a more prominent way is becoming increasingly remote due to CBS, *Alternaria*, Rust mite and other diseases.

Greening

The organism causing greening in Brazil differs from that found in South Africa. In Brazil, two bacteria *Candidatus Liberibacter asiaticus* and *L. americanus* are the causal organisms of the disease with *L. americanus* predominating. In South Africa the disease is caused by *L. africanus*.

Similarly, the vector in South Africa is the citrus psylla, *Trioza erytreae*, whereas in Brazil a different psyllid, *Diaphorina citri*, is known to transmit *L. asiaticus*, and also suspected to be the main vector of *L. americanus*. It appears as if the virulence of the disease-producing organism in Brazil is considerably higher than in South Africa, or the vector may be more efficient at transmitting the disease. About 90% of infected trees are within a 30 km radius area around Araraquara, while the remaining 10% is intermittently spread over a diameter of 300 km.

While the disease occurred in March 2004, some indications by growers suggest that the disease was observed from about 4 years ago. Since March, Prof. Bove (France) and Dr. Marco Machado (Silvio Moreira Institute) have determined the causal organisms. A delineating survey was made and an immediate voluntary eradication programme was embarked on. Furthermore, excellent training of scouts to identify the disease was done.

Symptoms of greening in Brazil appear closer to those of Asiatic greening, where more marked yellowing and branch die-back occur. It is also easier to detect these symptoms in hotter months than with African greening. The disease in Brazil appears to thrive on higher temperatures.

During the visit a greening workshop was held at the University Paulista (UNIP) in Araraquara, during which 450 delegates from the Citrus industry attended, representing 80-90% of citrus production in São Paulo State. It was held at such high level that media coverage of interviews with the South African researchers appeared on television during the same afternoon and evening and appeared on the first page of major newspapers the next morning.

The workshop were also attended by senior personnel of the Ministry of Agriculture of São Paulo State. Moves to initiate legislation for the eradication of greening infected-plants were immediately initiated after the meeting. The urgency and pressing need for legislation is something the South African industry has scandalously neglected over the past 40 years.

Sudden death

This devastating disease affects sweet orange on Rangpur lime rootstocks and also to a lesser extent on Volkameriana rootstocks. Whole groves with the disease were observed during the visit where trees were dead or dying. The industry is losing 2 million trees per year in the North of São Paulo State due to this disease and CVC. The Minas Gerais area appears to contain even greater infections. Controversy exists around identification of the causal agents, with citrus tristeza virus occurring in all affected plants but a second virus, Sudden Death associated virus (SDaV), a member of the Tymoviridae, also being identified from infected plants. Different research groups support various theories regarding the cause of the disease. These theories include the possible mutation and spread of CTV to a more severe form, secondly, synergistic responses due to the presence of both viruses, and thirdly, the possibility that SDaV as the cause of the disease.

Control is achieved by converting groves to trees on tolerant rootstocks such as Swingle, Sunki, Cleopatra and to a lesser extent Troyer, and Carizzo. A second control strategy, that of inarching infected trees, or trees at risk, on Rangpur lime rootstocks with Swingle rootstocks, is also used. Unfortunately Swingle is less tolerant than Rangpur lime to drought, and this may require a conversion to irrigation. There are indications that while using this procedure the Rangpur lime does not die and that drought tolerance may remain.

The disease, however, has been devastating, for example the Campo Grande farm of Cutrale on the Sao Paulo border with Minas Gerais, has replaced 640000 trees per year for the past 4 years on alternate rootstocks.

It is the opinion of the South African team that the control strategy employed in Brazil to control the disease is not the final solution and just represents the buying of some time while a number of research avenues are pursued by the Brazilian researchers.

Citrus Variegated chlorosis (CVC)

The disease is caused by *Xylella fastidiosa*, a xylem-limited bacteria. (The greening-inducing bacteria are all phloem-limited).

CVC reached its zenith about 10 years ago in Brazil, where-after a number of representatives from the Brazilian industry visited South African nurseries to improve their own. This resulted in huge improvements in their nursery industry, surpassing those of South Africa, and becoming arguable the best in the world.

As mentioned earlier, because of trees affected by CVC and SD, two million trees are removed in Brazil each year, and this is a direct cause of the industry moving further south into the cooler areas. The affected trees have chlorotic leaf lesions and small fruit that can still be processed in contrast with greening where the fruit is unusable.

The fact that Brazil can co-exist with the presence of CVC can be directly ascribed to measures in improving nursery standards. CVC inspection and eradication are done by producers themselves, and therefore negligent practices can affect neighbouring farms.

Leprosis

Severe symptoms were detected around Araraquara and north (north more severe on fruit and trees). The disease is spread by *Brevipalpus* mites, and the causal organism, is thought to be rhabdovirus-like viruses, two of which have been found associated with infected plants. This disease requires the highest input costs for vector control amongst the diseases of citrus in Brazil due to continued, intensive spray programs needed to control the mite. Although the same mite occurs in South Africa, the virus is not present in South Africa. South Africa is therefore at risk to this disease through the accidental import of this disease on infected material from Brazil. South African producers are urged not to illegally import material and to take maximal precautions when visiting Brazilian citrus farms to sanitize themselves, their clothes and shoes.

Alternaria

Alternaria was observed a year ago for the first time in Brazil. Subsequently an explosion of the disease has taken place, especially on Ponkan and Murcott varieties. Eight fungicide applications per year do not appear to guarantee lesion-free fruit.

A variety trial showed that 15 of the 22 mandarin types tested were sensitive for the organism. Good results for control were, however, obtained with strobilurins with the best being pyrochlorobin.

Pink Disease

Pink disease affects the bark and limbs of mature trees under high rainfall conditions and produces necrosis and gumming. The disease is caused by the fungus, *Erythricium salmonicolor*, formerly known as *Corticium salmonicolor*. It is believed that the Brazilian industry is underestimating the disease which is spread throughout the citrus producing areas. Copper sprays and pruning is used to control the spread of the disease.

Post-bloom fruit drop (PFD)

PFD is caused by *Colletotrichum acutatum*, and can cause up to 90% post-bloom fruit drop in certain years. It occurs mainly in cooler areas of Conchal and Itipitininga. During this visit it was observed on Valencias and Tahiti Lime. The fungus may invade entire petals that turn orange brown, become dry and hard and remain attached to the inflorescence. The fruitlet abscises at the base of the ovary and the floral disk, calyx and peduncle remain attached to the tree, forming structures commonly referred to as buttons.

Citrus Blight

Brazil is still losing thousands of trees per year due to Blight, making it one of the three most economically important diseases in this country. All the areas visited were infested with Blight. The cause remains unknown. Rangpur lime, the rootstock used most frequently in Brazil is highly susceptible to Blight. Where orchards are replaced as a result of Blight they are replanted either with Swingle or Sunki.

Citrus Canker

As mentioned earlier, Fundecitrus spends between U\$10-11 million per year on the eradication of canker. No infected trees were observed during this visit because of the strict eradication program enforced in Sao Paulo. Two thousand people are involved in this program. However, while eradication takes place in Sao Paula state none is done in the adjacent Parana state.

Citrus Black spot (CBS)

The incidence of CBS, which started in the Conchal area approximately five years ago has increased dramatically and now also occurs as far north as Minas Gerais. The Brazilian industry is unlikely to retain any CBS free areas. The disease is more severe in Brazil than South Africa. This may be due to a more suitable climate and multi-cropping. Evidence exists suggesting the fungus may have come from South Africa (DNA markers similar to those in South African and Mozambican strains were found). Though ascospores are the major source of infection, pycnidiospores also play a prominent role to infect some of the latter croppings.

The strobilurins give good control with pyroclostrobin being superior. Benomyl has been removed from Brazil by the manufacturer (Du Pont) but is not banned for use. Carbendazim remains available for control of CBS. This fungicide is not as effective as Benomyl.

Phytophthora

As is the case with the improvements in their nursery industry the Brazilian industry did an excellent job in copying Phytex. The incidence of *Phytophthora* has reduced dramatically in Sao Paulo as a result of the four phosponate sprays they apply per year. This combined with a special selection of sweet orange rootstock allow Setae Lagoas farm to interplant where old *Phytophthora* infected trees have been removed. Setae Lagoas also has a Trifoliata selection which differs genetically from that available in Brazil's genetic sources. This Trifoliata is more vigorous than the sources used in Brazil and gives a bigger tree.

Other institutions visited:

Citroson Nursery

This nursery belongs to Juliano Ayres. It produces 1 million trees per year and is by far the best citrus nursery that any of us has seen. SACNA should send a delegation to Brazil to see what nursery standards should be. Sao Paulo moved from open soil nurseries in the 1990s to the best citrus nurseries in the world. All these nurseries are covered with plastic roofs and psylla netting on the sides. The trees are lifted from the soil and are in containers. Clean budwood is supplied to them by the Instituto Agronomico, Sylvio Moreira at Cordeiropolis. However, each nursery has its own mother blocks. Tree certification is in place.

Allelyx

F. van Vuuren and G. Pietersen visited this facility after being invited to do so at the IOCV meeting in Mexico by one of the co-founders Dr. Ana-Claudia Raseria Silva. The company was founded by members of the consortium that had sequenced the entire genome of *Xyllela* after recognising the financial potential in work of this nature. 150 million US\$ were invested in equipping this laboratory with state of the art sequencing facilities, bioinformatics facilities, tissue culture facilities and plant growth facilities. The company has grown over the two years of its existence from 20 researchers to a hundred, and is expected to increase to 150 by the end of 2005. The company focuses on plant biotechnology and seek projects requiring cloning, sequencing and transgenic plants, which would generate financial returns for the company. They were very interested in pursuing the sequencing of the entire genome of *Liberibacter americanus* for an undisclosed application in Brazil. They are currently sequencing the entire genome of one of the rhabdovirus-like viruses involved with leproses and did some excellent work towards finding the causal organism of sudden death disease of citrus. During the last-mentioned study, 85000 clones were sequenced obtained from randomly-primed cloned libraries of 8 infected trees. Large numbers of CTV sequences were generated as well as some clones of a member of the Tymoviridae. This virus has now been completely sequenced by the company and is called Citrus Sudden death associated virus (CSDaV).

Bebedouro

The research station at Bebedouro was visited as H le Roux was of the opinion that he saw greening like symptoms there back in the 1990s. No symptoms could be found and it is believed that what he saw was

mineral deficiencies. The greening found in Brazil is so aggressive that it would have wiped out the research station if it was present.

Fazenda Setea Lagoas

The farm of Carlos van Parys de Witt was visited near Conchal. He is one of the largest exporters of fresh fruit from Brazil. He exported 1 million cartons to the Netherlands this season and is very pleased with the money returned for his produce. Though greening was found on this farm every tree was removed and mapped to monitor the spread of the disease.

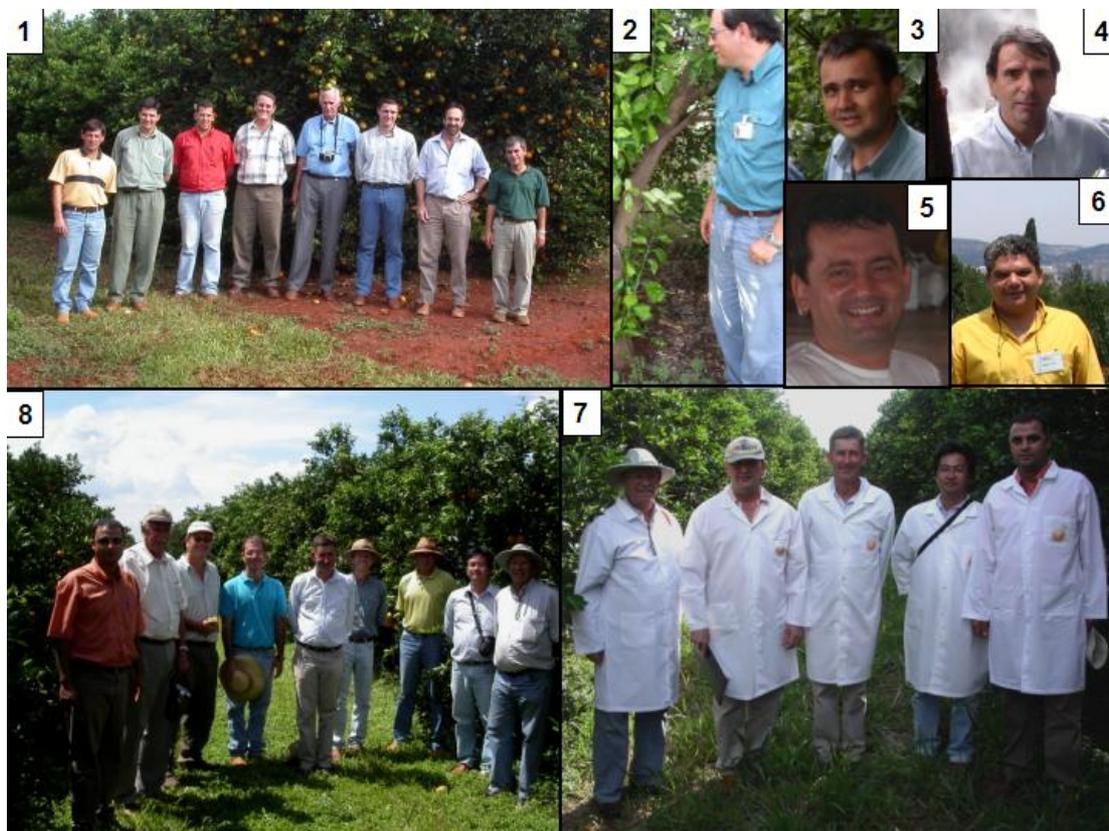


Figure 8.8.1.1. Photographs of Brazilian Researchers and Citrus Industry representatives met during the visit to Brazil. Photographs clockwise from top left corner.

- (1) Group photograph of (from left to right) *, Renato Bassanezi (Fundecitrus), *, Hennie le Roux (CRI), Fanie van Vuuren (CRI), Jorge Mangussi (Cutrale), Gerhard Pietersen (CRI), Silvio Lopes (Fundecitrus).
 - (2) Fernando Tersi (Cambuhy) at a citrus tree, pruned, but re-sprouting.
 - (3) Ento * (Fischer).
 - (4) José Luiz Rodrigues (Cambuhy)
 - (5) Marcell Sposito (Fundecitrus)
 - (6) Rogerro * (Cutrale)
 - (7) Group Photograph (from left to right) Prof. Geminez (Fundecitrus), Eduardo Stuchi (Embrapa), Gerroto * (Cutrale), Pedro Yamamoto (Fundecitrus), Waldir de Jesus Junior (Fundecitrus).
 - (8) Group photograph (left to right) Waldir de Jesus Junior (Fundecitrus), Fanie van Vuuren (CRI), Hennie le Roux (CRI), Andrea * (Fundecitrus), Gerroto * (Cutrale), Edison * (Cutrale), * (Cutrale), Pedro Yamamoto (Fundecitrus), Prof. Geminez (Fundecitrus).
- *Unknown

Contact List

Dr. Juliano Ayres
Fundecitrus (Scientific Manager)
ayres@fundecitrus.com.br

Prof. Nelson Gimenes
Fundecitrus (Executive secretary)

gimenes@fundecitrus.com.br

Dr. Waldir Cintra de Jesus Junior
Fundecitrus (Plant pathologist: Viruses)
wcintra@fundecitrus.com.br

Dr. Renato Beozzo Bassenezi
Fundecitrus (Plant pathologist: Viruses)
rbassanezi@fundecitrus.com.br

Dr. Pedro Takao Yamamoto
Fundecitrus (Entomologist)
ptyamamoto@fundecitrus.com.br

Dr. Silvio Lopes
Fundecitrus (Plant pathologist)
slopes@fundecitrus.com.br

Me. Diva do Carmo Teixeira
Fundecitrus (Plant pathologist)
diva@fundecitrus.com.br

Dr. Marcel Bellato Sposito
Fundecitrus (Plant pathologist: CBS)
marcel@fundecitrus.com.br

Mr. Nelson Arno Wulff
Fundecitrus (Plant pathologist)
nelsonwulff@fundecitrus.com.br

Mr. Jose' Luiz Amaro Rodrigues
Cambuhy (Director General)
joseluiz@cambuhy.com.br

Mr. Fernando E.A. Tersi
Cambuhy (Production Manager: Citrus)
ftersi@cambuhy.com.br

Mr Agnaldo de Tarso Rigolin
Cambuhy (Production Manager)
arigolin@cambuhy.com.br

Mr. Fabio Mitusuru Saito
Citrosuco (Process Engineer)
fsaito@citrosuco.com.br

Helton Carlos de Leao
Fischer (Supervisor: Technical Department)
hleao@citrosuco.com.br

Celso Nogueira
Fisher (Agronomist)
cnogueira@citrosuco.com.br

Mr. Flavio de Carvalho Pinto Viegas
Associtrus (President)
fviegas@ipco.com.br

Douglas Kowarick
Associtrus (Vice President)
associtrus@mdbrasil.com.br

Mr. Rogerio Visconti Vieira

Cutrale (Area Manager)
(016) 236 9187

Mr. Jorge A. Mangussi da Costa
Cutrale (Area Manager)
(016) 236-9187

Dr. Ana Claudia Rasera Silva
Allelyx
Ana@alelyx.com.br

Dr. Diceu de Mattos
Centro de Citriculture; Sylvio Moreiera (Director)
ddm@centrodecitricultura.br

Dr. Alexandre Morais do Amaral
Centro de Citriculture; Sylvio Moreiera (Molecular microbiologist)
ddm@centrodecitricultura.br

Mr Jose Dagoberto de Negro
Centro de Citriculture: Sylvio Moreiro (Agronomist)
dagoberto@centrodecitricultura.br

Dr Eduardo Feichtenberger
Secretario da Agricultura, APTA. (Plant Pathologist)
e.feichtenberger@splicenet.com.br

Mr Eduardo Lopes
Citrovita (Production Manager)
Eduardo.lopes@citrovita.com.br

John Redfern
Defensive (Marketing Manager)
mkt@defensive.com.br

Mr Marcos Pozzan
Montecitrus (Area Manager)
Marcos.pozzan@montecitrus.com.br



Figure 8.8.1.2. Symptoms of greening disease observed in Brazil. Clockwise from top left: Typical sectorial yellowing within older trees. A young tree showing systemic greening disease planted * months earlier, within 20m of a severely Greening infected block . Typical necrosis of phloem vessels at base of fruit. Typical lopsided formation of Greening affected fruit.



Figure 8.8.1.3. Symptoms of sudden death in Brazil. Clockwise from top left: Rapid death of trees leaving fruit still hanging. Death of whole groves. Yellow stained (inactive) phloem of rootstock. Inarching to allow trees to recover. Prof Gemeniz, who suggested the use of inarching to save sudden death affected trees with Fanie van Vuuren and Hennie le Roux.



Figure 8.8.1.4. Various other disease observed in Brazil: Clockwise starting Top left. Leprosis symptoms on fruit. Rubilosis (Pink) disease on trunk of affected disease. Rubilosis on twigs. Citrus exocortis virus on rootstock.



Figure 8.8.1.5. Leprosis symptoms observed in Brazil. Clockwise starting top left. Twig and small branch lesions typical of leprosis. More twig symptoms of Leprosis. Fruit lesions typical of leprosis. Concentric ring lesions on leaves, typical of Leprosis.



Figure 8.8.1.6. Postbloom Fruit Drop (PFD) caused by *Colletotrichum acutatum*. Note the orange brown petals on the left and the buttons in the right hand picture where the fruitlets abscised.

8.9 G. PIETERSEN

8.9.1 Sixteenth Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 07-13

Itinerary

- 1 November: Leave JHB Intl. Airport for Weslaco, Texas, via Amsterdam- Netherlands, Houston-Texas and McAllen- Texas. Arrive 2 November 21:00.
- 4-5 November: Attend pre-conference tour of Texas Citrus Industry. Visit Texas A & M University, Kingsville Citrus Centre, Tour of Valley Citrus groves, packing shed, juice plant and nurseries.
Visit USDA-APHIS-PPQ facility at Moore Base.
- 6 November: Leave by bus for Monterrey, Mexico,
- 7-12 November: Attend the Sixteenth Conference of the International Organization of Citrus Virologists, Monterrey, Mexico.
- 13 November: Leave Monterrey Intl. Airport for Johannesburg via Houston and Amsterdam. Arrive Johannesburg 14 November, 21:40.

Introduction

This conference and pre-conference tour in the Citrus Production region of Texas, USA, proved to be exceptionally valuable in terms of acquaintances made, technical and strategic information acquired and research materials obtained or sources identified, and will play a significant role in the rapid establishment of the CRI@UP Citrus Virology program. The timing of the conference could not have been better for the delegate who had started working on the Citrus Virus program four months earlier and had had just enough time to start finding his feet in the Citrus virus study area and new environment. It was just barely preceded by a planning phase for the CRI@UP program, with proposals for research being submitted just prior to the conference. Priorities and future research strategies could therefore be supported or modified/alterd for the program against a background of some knowledge on the status of research worldwide. Even presentation introductions (oral and poster), often of limited value to persons already entrenched in a given field of study, proved valuable in providing background knowledge for the delegate.

On a technical level the conference yielded a large number of valuable presentations, many of which no doubt being valuable mainly because of the novelty of the field of study to the delegate. These factual technical points are too numerous to include within this report and are also of such a detailed technical nature that they would be of little general use. However in general presentations have confirmed the general future research approach identified at CRI@UP and will be presented using the planned research programme as backdrop.

Information directly relevant to CRI@UP Plant Virology Program short-term goals

1) The IOCV conference clearly illustrated that, amongst the viruses, citrus tristeza virus needs the most attention, with the huge genetic variability of the virus confounding most control strategies. Of thirty-one oral presentations on this virus, twenty-one directly or indirectly addressed aspects of CTV variability. Characterisation of the CTV variability is a priority worldwide and increasingly powerful techniques are being developed to address this. The research proposal submitted to CRI to use micro-array technology to address this is supported by a presentation from the University of Arizona (Xiong *et al.*, "Rapid analysis of Citrus Tristeza Virus genomes using a re-sequencing oligonucleotide microarray"), where a much more sophisticated (but prohibitively expensive), related approach is being developed to address the problem. The proposed CRI@UP approach however remains novel and, in our opinion more practical given the local resources and conditions. Furthermore it was clear from presentations that the need to establish the scale of variability in a given locality was an essential component to establishing successful control strategies.

2) Presentations at the IOCV conference also illustrated the fact that disease spread to new locations and New diseases can have devastating effects (Bove', "Citrus Sudden Death in retrospect"; Bove' *et al.*, A new *Liberibacter* species, *Candidatus Liberibacter americanus*, is associated with Huanglongbing in Sao Paulo State, Brazil), and require rapid identifications in order to develop control strategies. This also supports the second main research priority identified, that of establishing detection methods for the rapid and sensitive identification of citrus viruses and other graft-transmissible pathogens whether they occur in South Africa or not.

3) Presentations also illustrated that with the advances in molecular methods, increased sequence information available, and common PCR, cloning and sequencing protocols between plant viruses work and for other graft-transmissible pathogens that it is becoming increasingly plausible for a plant pathologist to gain expertise in diagnosing and identifying representatives of each of these groups of organisms. Hence using only one or two researchers to establish a wide diagnostic capability to detect all graft-transmissible pathogens (not just viruses or viroids or phytoplasmas) of citrus is completely feasible.

4) One general area of research receiving attention worldwide (eight such oral presentations on CTV specifically) but not being planned over the short term locally, is the use of transgenic plants for resistance to citrus viruses and other citrus graft-transmissible pathogens. Presentations at the IOCV however reinforced the delegate's contention that while negative aspects and risks of the technology are rapidly being addressed, and underlying mechanisms are being elucidated, the technology has not yet reached the level where countries or industries with limited resources can embark on such projects with some degree of guarantee of practically applicable outcomes. The delegate therefore is still of the opinion that expensive advances in the technology, done at well-resourced international laboratories overseas, must be monitored, and theoretical expertise be maintained, but that only once the majority of "teething" problems are sorted out must this technology be applied locally.

Direct outcomes to having attended the IOCV 2004 conference

Contact was made and promises of collaboration was made with Dr. Mark Hilf, who has subsequently sent unpublished modifications of primer sequences used to determine CTV genotypes. Has also promised to send appropriate strain specific positive controls of T3, expect shortly.

Contact was made with Prof. Pedro Moreno, with whom email correspondence had been conducted earlier. Positive controls to the planned CTV target region for PCR to differentiate severe and mild strains were obtained. From discussions it was determine that they use MeHg to denature dsRNA before PCR (this was not reported within their publication) and had held-up Me. Stewarts work for a short while.

Contact was made with Prof. J-M. Bove, and followed up with email correspondence. He has promised to send a non-infectious positive control of *Liberibacter asiaticus* for use in PCR systems already established to *L. africanus* at CRI@UP.

Contact was renewed with Prof. M. Bar-Joseph, and followed up by email correspondence in which DNA to specific target sequences of genotyping primers of CTV –VT, a unique Israeli strain, were requested. It is expected that these will arrive soon.

Contact was made with Dr. Nuredin Duran-Villa and Dr. Peggy Sieburth, with both promising help with viroid detection. Dr. Duran Villa sent an article with an excellent protocol on cloning and sequencing unknown viroids.

Contact was made with Dr. Carmen Vivez who has researched Citrus leaf blotch virus over the past few years. Plasmids containing the coat protein gene and the RNA-dependant DNA polymerase gene respectively were obtained from her to serve as positive controls in establishing PCR to the virus. The seed-transmitted nature of this virus accords it special status as a risk for introduction to South Africa, and if within South Africa to the CIP, as the protocols are based on the assumption that citrus seed is free of viruses.

Gaps in expertise at the UP@CRI program, as identified by the scope of research presented at IOCV 2004.

Expertise in research on plant virus and other pathogen resistance, both natural and transgenic, will have to be addressed within the foreseeable future, in order to equip the program best in studies on control of graft-transmissible diseases of citrus.

Acknowledgement

I wish to thank and compliment CRI management for their foresight in budgeting and providing funds to attend this important meeting even prior to the appointment of a new plant virologist. This is a clear sign of their commitment to the Plant Virology program at CRI, and will directly influence the success of the program.

9 KNOWLEDGE TRANSFER (H.F. le Roux)

9.1 KNOWLEDGE TRANSFER GROUPS (KTGs)

There are currently 23 citrus KTGs spread throughout the citrus producing regions of southern Africa. In the areas where a strong presence of technical personnel and citrus consultants exist, these groups are functioning well and the inputs from their technical committees add valuable inputs towards the research planning (see Figs. 9.1.1 and 9.1.2 for relative funding support of research programmes and projects). Although the research priorities did not show major differences from those determined during 2003 and 2004, peteca on lemons and FCM have been rated higher than in previous years. Recommendations for fertigation is also a problem and Dr. Hannes Coetzee is in the process of updating the Production Guideliness on fertilization and irrigation.

All the requests from the KTGs with regard to presentations on production inputs were met. It was especially Drs. Tony Ware (Fruit fly) and Sean Moore (FCM) who were in high demand as well as Keith Lesar (Post Harvest). After the 3rd Citrus Research Symposium, Dr. Hennie le Roux visited all the KTGs and presented a summary to each of these groups. This resulted in the number of growers who were exposed to the research feedback increasing more than five fold.

The highest demand on Extension inputs were, however, not from the current KTGs but from new citrus growers joining the industry as part of the transformation process. The current black citrus growers, with the exception of those in the Eastern Cape, do not attend grower meetings despite being invited. The reason for this needs to be identified. From 2006, citrus courses will be available on different levels of expertise and this should solve the problem. It may be that the technical level of the current grower meetings is on to high a level. If so Extension will address this problem.

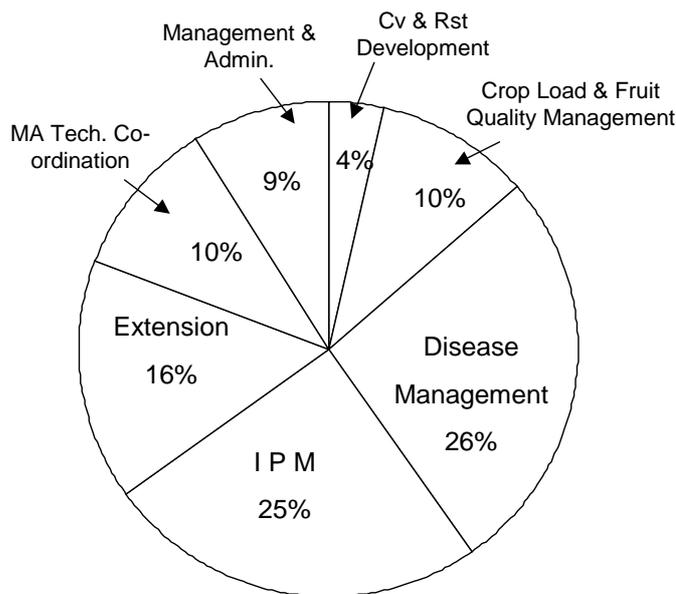


Fig. 9.1.1. Percentage funds in each CRI programme.

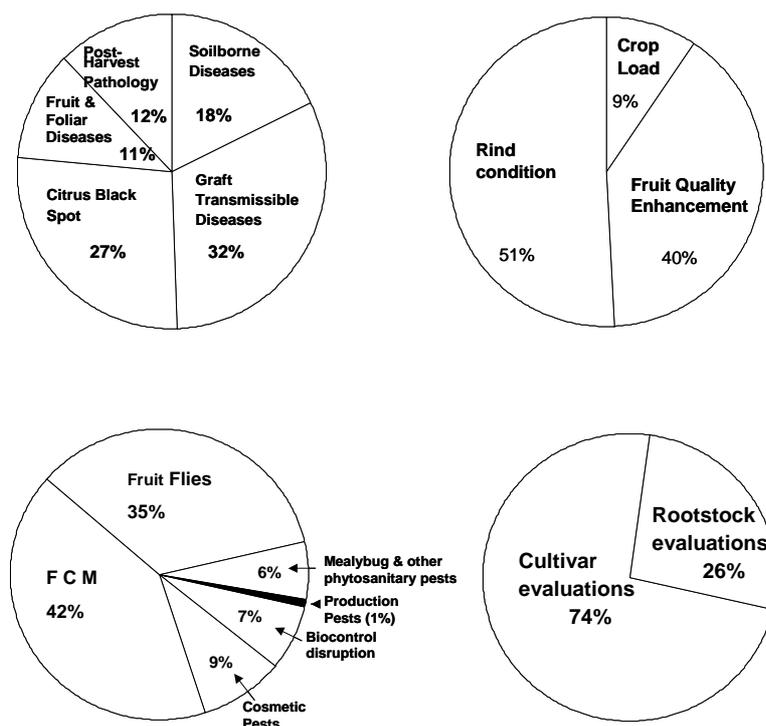


Fig. 9.1.2. Percentage funds in CRI Research Projects – Disease Management (Top left), Crop Load and Fruit Quality Management (Top right), Integrated Pest Management (Bottom left) and Cultivar and Rootstock Development (Bottom right).

EXTENSION PRESENTATIONS BY CRI GROUP RESEARCHERS IN 2004			
Name	Date	Area	Topic
Alexander, C. (Private)	24-27/05/2994	3 rd Citrus Research Symposium, Modimolle	Discussion on cultivars evaluated in the Cape areas
Barry, G.H. (CRI)	04/03/2004	Patensie	Fruit Quality Management: How can I optimise fruit quality?
	09/03/2004	USA Navel Alliance	Time-temperature protocol for optimal rind colour
	26/03/2004	USA Navel Alliance	Time-temperature protocol for optimal rind colour
	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Amelioration of sunburn on Miho Wase Satsuma mandarin using kaolin particle film technology Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate Post-harvest manipulation of rind colour
	11/08/2004	Citrus Exporters Forum	Fruit Quality Enhancement: Rind colour
Bijzet, Zelda (ARC)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	The ARC-ITSC citrus breeding programme: Current successes and future prospects
Cronjé, P. (CRI)	24/05/2004	3 rd Citrus Research Symposium, Modimolle	Rind disorders workshop: International citrus rind disorders research Die uitwerking wat ontgroening en die duurte voor verkoeling op skil afbraak van 'Nules Clementines' mandaryne het
	11/08/2004	Exporters Forum	Post-harvest research at CRI during 2003 season

	13/08/2004	SRCC	Rind pitting of citrus fruit
	15/04/2004	Stellenbosch	PECK, Hot stuffing of citrus
	25/11/2004	Stellenbosch	USA monthly alliance & USA technical committee meeting
Du Toit, M.N. (CRI)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	The South African Citrus Improvement Programme
Grout, T. (CRI)	11/02/2004	Nelspruit	An update on FCM management
	24-27/05/2004	3 rd Citrus Symposium, Modimolle	Augmenting natural enemies of citrus thrips Thrips control prospects with soft or systemic insecticides CRI Group research: Who does what and why
	28/07/2004	Nelspruit	Fruit Fly Strategic Planning meeting
Hattingh, V. (CRI)	03/03/2004	Tshipise (growers)	Systems approach to CBS for exports to USA
	03/05/2004	Pretoria	National Agricultural Research Forum: Intellectual Property Rights – an overview
	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	The role of research and technical support services in Market Access Modelling the potential distribution of CBS
	11/08/2004	Cape Town	Citrus Exporters Forum: Relevance of CRI Research portfolio to citrus exporters
	07/09/2004	Stellenbosch	Fruit Industry Plan, R&D Workshop: Research and technical support services in the citrus industry
	28/09/2004	Nelspruit	Fruit Industry Plan, R&D Workshop: Research and technical support services in the citrus industry
Hofmeyr, H. (CRI)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Navorsing op die bestralingsbiologie en F1-steriliteit van valskodlingmot
	20/09/2004	Clanwilliam	Valskodlingmot SIT
	21/09/2004	Citrusdal	Valskodlingmot SIT
Kaizer, C. (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	A review of citrus creasing in South Africa
Kirkman, W. (CRI)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Pest status and control of Oleander mealybug, <i>Paracoccus burnerae</i>
Korsten, L. (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Overview of citrus black spot research at Plant Pathology Laboratories
F.J. Kruger (ARC)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Investigation into the pre-harvest causes of rind pitting, stem end rind breakdown and chilling injury in export citrus from the Mpumalanga Lowveld
Labuschagne, (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Citrus resistance in the context of citrus decline Resistance of citrus rootstocks against root pathogens: Screening and identification of compounds with antifungal activity
Le Roux, H.F. (CRI)	14-17/04/04	Lower Orange River KTG	Fruit set workshop with Drs. G. Barry, H. Coetzee & J. Kruger
	20/04/2004	Barberton BEE	Moodies BEE group discussions on citrus production
	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Voorligting / Extension
	28/-6/2004	Winterveld BEE	Visit BEE group with Dr. Sam Moganjane
	12-15/07/2004	Free State	Visit growers re CBS freedom

	16/07/2004	Vaalharts Knowledge Transfer Group (KTG)	Feedback on research results presented at the 3 rd Citrus Research Symposium and determining of Research Priorities of the different citrus producing areas (3 rd CRS & RP)
	01/08/2004		Feedback on 3 rd CRS & determine RPs
	02/08/2004	Hoedspruit KTG	Feedback on 3 rd CRS & determine RPs
	03/08/2004	Constantia KTG	Feedback on 3 rd CRS & determine RPs
	04/08/2004	Tshipise KTG	Feedback on 3 rd CRS & determine RPs
	04/08/2004	Komatipoort KTG	Feedback on 3 rd CRS & determine RPs
	05/08/2004	Malelane KTG	Feedback on 3 rd CRS & determine RPs
	10/08/2004	Nelspruit KTG	Feedback on 3 rd CRS & determine RPs
	12/08/2004	Pongola KTG	Feedback on 3 rd CRS & determine RPs
	13/08/2004	Richmond KTG	Feedback on 3 rd CRS & determine RPs
	16/08/2004	Rustenburg KTG	Feedback on 3 rd CRS & determine RPs
	17/08/2004	Citrusdal KTG	Feedback on 3 rd CRS & determine RPs
	17/08/2004	Paarl KTG	Feedback on 3 rd CRS & determine RPs
	18/08/2004	Swellendam KTG	Feedback on 3 rd CRS & determine RPs
	18/08/2004	Knysna KTG	Feedback on 3 rd CRS & determine RPs
	19/08/2004	Patensie KTG	Feedback on 3 rd CRS & determine RPs
	19/08/2004	Sundays River KTG	Feedback on 3 rd CRS & determine RPs
	23/08/2004	Kat River Valley KTG	Feedback on 3 rd CRS & determine RPs
	23/08/2004	Burgersfort KTG	Feedback on 3 rd CRS & determine RPs
	24/08/2004	Marble Hall KTG	Feedback on 3 rd CRS & determine RPs
	26/08/2004	Lower Orange River	Feedback on 3 rd CRS & determine RPs
	03/09/2004	Swaziland KTG	Feedback on 3 rd CRS & determine RPs
	16/09/2004	Nelspruit	Meeting with Brazilian visitors re Greening
	23/09/2004	Nkwaleni KTG	Feedback on 3 rd CRS & determine RPs
	24/09/2004	Chequtu KTG	Feedback on 3 rd CRS & determine RPs
	27/09/2004	Harare KTG	Feedback on 3 rd CRS & determine RPs
	07/10/2004	Beitbridge KTG Gillemburg BEE	Meet and have discussions with the Bakone Development Trust
	08/10/2004	Rust de Winter BEE	Discussions with Obed Lekala's community on citrus production
	20/08/2004	Uppington BEE	Represent CGA at an Agri BEE meeting
	01/11/2004	Swellendam	Citrus Greening
	02/12/2004	Cairn Trust BEE	Lemon oil production.
	09/12/2004	Araraquara (Brazil)	Status of Citrus Greening in South Africa
	17/12/1004	Van der Kloof	CBS Area freedom
	20/12/2004	Kat River Valley	CBS Area freedom
Lesar, K.H. (CRI)	18/02/2004	Malelane - FMC launch attended by Onderberg & Swaziland P/houses, chemical companies and FMC Wax Int. Manager	Presentation on current progress of wax regulatory issues in the Industry
	25/02/2004	Nelspruit	Meeting with Agricultural Protection Systems at Broham packhouse re Terminator packhouse trials
	15/03/2004	Marble Hall	Packhouse meeting and visits
	22-26/03/2004	E. Cape – Patensie Co-op, Katco, all SRCC producers and packhouses	Packhouse meetings and advisory visits
	29/03/2004	Nelspruit	Crocodile Valley Citrus Co. – 2,4-D (Alternatives) trials
	15/04/2004	Malelane Hectorspruit	Waste and rind condition workshop
	18-23/04/2004	W. Cape	Packhouse meetings and advisory visits

	24-28/05/2004	3 rd Citrus Research Symposium, Modimolle	Evaluation of Phosphonates for the post-harvest control of <i>Phytophthora</i> brown rot The supporting role of the sanitizing agent Sporekill in the integrated post-harvest decay control of citrus
	03/06/2004	Burgersfort	Orchards and packhouse consultation
	25/06/2004	Nelspruit	Broham Packhouse advisory visit
	11/08/2004	Hectorspruit	Waste Workshop - Tecklenburg
	30/10 – 02/11/2004	Swellendam	Study group waste meeting
Meyer, L. (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Sensitivity of the one-day PCR-based technique to detect the citrus black spot pathogen, <i>Guignardia citricarpa</i>
Moore, S.D. (CRI)	26/02/2004	Addo	General Pest Management: Workshop: E. Cape Citrus Technical Association
	29/04/2004	Patensie	FCM Management: Grower Technical meeting
	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Cryptogran FCM and navel oranges: answering some unasked questions Lemon borer moth complex: who is the real culprit?
	11/08/2004	Marble Hall	FCM management: Grower technical meeting
	17/08/2004	Kirkwood	Cryptogran: Grower Technical & Business meeting
	21/09/2004	Addo	FCM management: Grower technical meeting
	22/09/2004	Kirkwood	FCM management: Grower technical meeting
	06/10/2004	Hartswater	Red scale, mealybug & FCM: Grower technical meeting
	11/10/2004	Kirkwood	Management of the spring pest complex; FCM: Sun Citrus growers technical meeting
	23/11/2004	Patensie	FCM: Grower technical meeting
	07/12/2004	Nkwaleni	FCM & mealybug management: Grower Technical meeting
Paul, Ida (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Climate change and the potential distribution of <i>Guignardia citricarpa</i> Kiely, the causal agent of CBS in South Africa
Pretorius, M.C. (CRI)	24-27/05/2004	3 rd Citrus Symposium, Modimolle	Maintaining a healthy root system on citrus trees by managing root pathogens and soil conditions
	14/09/2004	Nelspruit	SAMAC meeting: <i>Phytophthora</i>
Schutte, G.C. (CRI)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Investigation into a new fruit and leaf 'disease' of unknown origin on navel and Valencia oranges at Naboomspruit Control of <i>Alternaria</i> brown spot on Mandarins with strobilurin fungicides Control options for Citrus Black Spot
	08/09/2004	Pretoria	Agchem, Swartvlek
	02/11/2004	Nkwaleni Studiegroep	Swartvlek

Swart, S.H.	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Reducing inoculum, an important aspect in Citrus Black spot management for a zero tolerance market Correlation between ascospore inoculum and infection in an orchard
Timm, Alicia (Stell. Univ.)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Molecular investigation of the population structure of false codling moth, Molecular investigation of the population structure of false codling moth, <i>Cryptophlebia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae), in South Africa: application to pest management
Truter, M. (Univ. Pretoria)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Inoculation of citrus leaves at different leaf ages with <i>Guignardia citricarpa</i> <i>In vitro</i> germination conditions of <i>Guignardia</i> species
Van Vuuren, S.P. (CRI)	24-27/05/2004	3 rd Citrus Research Symposium, Modimolle	Citrus Tristeza Virus (CTV) Cross-Protection of Grapefruit Citrus Tristeza Virus (CTV) Cross-Protection of Sweet Orange and Mandarin
Ware, A.B. (CRI)	14/01/2004	Malelane Growers	Fruit fly
	20/01/2004	Hoedspruit Growers	Fruit fly
		Letsitele Growers	Fruit fly
		Letsele Growers	Fruit fly
	21/01/2004	Limpopo Growers	Fruit fly
	22/01/2004	Swaziland Growers	Fruit fly
	03/02/2004	Richmond Growers	Fruit fly
	03/02/2004	Nkwaleni Growers	Fruit fly
	04/02/2004	Pongola Growers	Fruit fly
	11/02/2004	SAAGA, Tzaneen	Disinfestation
	18/02/2004	Early Warning Workshop	Fruit fly
	12/05/2004	SAAGA, CRI	Disinfestation
	24/05/2004	3 rd Citrus Research Symposium, Modimolle	Fruit fly
	07/06/04	Market Access, China	Market Access
08/06/2004	Brits, Quest Dev.	Fruit fly	
11/06/2004	SAAGA, CRI	Market Access	
22/06/2004	ITSC, Litchi	Disinfestation	
07/10/2004	ITSC, SAAGA	Fruit fly	

The listing of the different KTGs below will assist growers who would like to become involved with the KTG in his/her area. To ensure maximum research feedback growers must belong to a KTG.

CHAIRMEN OF KNOWLEDGE TRANSFER GROUPS				
AREA	NAME	TEL. NO.	FAX NO.	EMAIL
Benede-Oranjerivier	Francois Reyneke	082 771 6758	054-4310780	sabeth@mweb.co.za
Burgersfort	Elbert de Kock	013-2317757	013-2318334	moronesitrus@intekom.co.za
Citrusdal	Dirk Visser	082 550 0158	022-9212962	dirkvisser@kingsley.co.za
Groblersdal	Chris van Ginkel	082 662 8426	013-2611671	valleiadvies@lantic.net
Hoedspruit	Org Boshoff	015-7955048 / 082 560 6309	015-7955971	grotro@worldonline.co.za
Katrivier	Lawrie Pringle	083 232 7943	046-6452345	technical@katco.co.za
Komatipoort	Dirk Horn	013-7937536 / 083 259 3359	013-7937536	sommerreg@soft.co.za

Knysna	John Stanwix	082 789 5051	044-3884611	knycit@mweb.co.za
Letsitele	Christo Viljoen	083 310 3868	015-3458506	cgviljoen@wol.co.za
Limpopo	B.J. Nicholson	015-5390763 / 083 306 0552	015-5390718	alicedal@lantic.net
Malelane	Leon Esselen	013-7900160	013-7900492	esselenk@mweb.co.za
Marble Hall	John Howard (Midnight Study Grp)	013-2611203 / 082 562 1203	013-2611203	
Nelspruit	Graham Piner	013-7522141 / 072 804 6495	013-7522560	crocval@mweb.co.za
Nkwaleni	Bester Snyman	035-4600634 / 082 896 2856	035-4600634	bsnyman@netactive.co.za
Paarl	Corrie Muller	083 631 7727	021-8621129	corriem@worldonline.co.za
Patensie	Ian Grieb	082 823 3960	042-2830893	iangrieb@gamnet.co.za
Pongola	André Barnard	083 229 8539	034-4351083	mhlali@idhweb.com
Richmond	Trevor Jukes	082 945 7317	033212-3342	katnatal@futurenet.co.za
Rustenburg	Johan-Chris Grobler	082 922 1579	014-5733036	witkrans1@mweb.co.za
Sondagsrivier vallei (Kirkwood & Addo)	Dave Gerber	072 292 2151	042-2331037	summersby@telkomsa.co.za
Swaziland	Gerd Höppner	09268-3232311	09268-3232317	ghoppner@iysiscitrus.co.sz
Swellendam	Sarel Neethling	028-5123606 / 082 551 2357	028-5123659	sarel@thornlands.net
Vaalharts	Tom Fouché	053-4710277 / 082 783 4842	053-4710277	marithaminnie@mweb.co.za
Zimbabwe	Graham Crutchley			gjcrutchley@mailblocks.com

Extension visit to Zimbabwe

By Hennie le Roux

Zimbabwe was visited during the period 21-27 September 2004 to determine the research priorities of the citrus growers in that area and to give feedback on the research results presented at the 3rd Citrus Research Symposium. Three meetings were held at Chegutu, Harare and Beitbridge. The current situation in Zimbabwe is as follows:

1. All citrus producers north of Mazoe were evicted from their farms. Yields in this area have dropped and very little fruit will be exported from these farms in future years.
2. The citrus farms south of Harare (Chegutu and Beitbridge) are still intact and the total exports from Zimbabwe for this past season will be about 3 000 000 cartons. This indicates a loss of 2 000 000 cartons from the northern areas. In the Chegutu and Beitbridge area in the south there are expansions.
3. *Pseudocercospora angolensis* is not controlled on the farms taken over by the war veterans. Unlike a few years ago when the disease was reduced to only two districts, the disease can again be found in the Karoi, Lions Den, Mvurwi, Mutepatepa and Bindura areas. It is also not limited to leaf lesions only. In Karoi fruit infested with the disease was for sale on the A8 main road going south. *Pseudocercospora* has also been seen on fruit from Mutepatepa. However, the incidence of *P. angolensis* has been very low because of the past dry seasons. In a few cases the neglected orchards have also been destroyed by fire, reducing the risk of the disease spreading.

Once the situation has normalised in Zimbabwe, CRI must ensure that every effort is made to eradicate *P. angolensis* in Zimbabwe. This was attempted once in the 1990s and although the disease was not eradicated it was reduced to undetectable levels in all but two districts.

Although the chances are slight that the disease will find its way into South Africa, it is important that the NDA are aware of the current situation and that increased awareness at the Messina border post be implemented so that untreated citrus fruit is not allowed through the border. Waxed fruit in transit to the ports will be safe.

9.2 OTHER MEANS OF TECHNOLOGY TRANSFER

By Hennie le Roux and Tim Grout (CRI)

SA Fruit Journal

The SA Fruit Journal is probably the most important means of transferring knowledge to the growers because all growers receive it and it is attractive and easy to read. The Extension Briefs are used to remind growers of specific management requirements that are important at certain times of the year.

CRInet

The number of members in the CRInet discussion group reached 200 during 2004 and many more people made use of this service to enquire about various technical problems. A total of 187 emails were sent in 2004 compared with 85 emails during 2003 (Table 9.2.1). Residents in southern Africa can join CRInet by filling in the registration form on CRI's website www.cri.co.za.

Table 9.2.1. Numbers of messages circulated per month on CRInet during 2004 and 2003

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004	7	26	13	28	27	26	12	9	15	12	12	0
2003	1	4	6	14	22	4	3	6	5	6	11	3

Cutting Edge

In contrast to expectations, the Cutting Edge has not lost its relevance and still plays a very important role in quickly getting certain technical information to the producer. Cutting Edge is only circulated via email so it is important that growers let CRI know if their email address changes.

CRI website

Usage of the website (www.cri.co.za or www.citrusres.com) during 2004 leveled off to between 2000 and 5000 page requests per month. Most requests were made from dot net or dot com domains followed by co.za. Only members can access the download area of the website and most of the material available is first circulated via email so this may explain the lack of growth in visitors. Individual staff email addresses were removed from the website during 2004 to reduce spam sent to staff. A general enquiry email address is being monitored. The public part of the website will be updated in 2005.

3rd Citrus Research Symposium / 3de Sitrusnavorsingsposium 2004

Die tweejaarlikse Sitrusnavorsingsposium, wat in 2000 in Nelspruit en in 2002 in Stellenbosch aangebied is, is in 2004 in Modimolle (Nylstroom) aangebied. Dit was dus die 3de Sitrusnavorsingsposium en is gehou vanaf 24-27 Mei 2004. Die groot trekpleisters was die afgelope twee jaar se vordering met betrekking tot valskodlingmotbeheer en sitruswartvlek (CBS). Daar is 51 referate gelewer en daar was 22 plakkaataanbiedings. Tabel 9.2.2 toon die program terwyl Tabel 9.2.3 die plakkaataanbiedings se temas gee.

Die simposium is deur 210 afgevaardigdes bygewoon. Hierdie afgevaardigdes het sowat 90% van die sitruskonsultante wat tans in die Suid Afrikaanse sitrusbedryf werksaam is ingesluit. Die lesings was van hoogstaande gehalte, die akkomodasie en kos was goed maar daar was een teleurstelling. Weinig van die produsente in die Marble Hall en Groblersdal area het die moeite gedoen om die simposium by te woon. Dit was in kontras met die vorige twee simposiums wat goed bygewoon is deur produsente van die onderskeie gebiede waar die simposiums aangebied is.

Bayer Crop Science het as die hoof borg opgetree terwyl Ag-Chem Africa, BP, CAL Laboratories, Capespan, Colors, Dole, FMC Technologies, Hygrotech, Horticura, ICA International Chemicals, Intertrading, Philagro, Quest Developments, Standard Bank, Syngenta, Ukulima, en Unisun ook as borge opgetree het. 'n Werkswinkel wat oor skilprobleme gehou is het die talle onbeantwoorde vrae wat daar nog op hierdie gebied is na vore laat kom. 'n Werkswinkel is ook na afloop van die simposium gehou om te verseker dat die Gedurende Augustus en September 2004 is 'n besoek aan die verskillende sitrusstudiegroepe gebring om die onderskeie areas se navorsingsprioriteite te bepaal. Tydens hierdie besoek is die navorsingsresultate wat tydens die Sitrusnavorsingsposium gelewer is by elk van die studiegroepe aangebied. Dit het daartoe gelei dat 'n aansienlik groter persentasie sitrusprodusente op hoogte gebring is van die jongste navorsingsdeurbrake.

Die 4de Sitrusnavorsingsimposium sal gedurende 2006 in die Oos Kaap aangebied word. Daar word beoog om tydens hierdie simposium ook besoeke te bring aan die hawe, die Sitrusgrondvesblok en van die Oos - Kaapse spogboorde. 'n Beroep word veral op die Oos Kaapse produsente gedoen om die simposium by te woon.

Table / Tabel 9.2.2.

PROGRAMME / PROGRAM	
VENUE / PLEK: WEESGERUS, NYLSTROOM/MODIMOLLE	
MONDAY – 24 MAY 2004	
17:00 – 19:00	REGISTRATION
19:00 – 21:00	WELCOME by PROF. VAUGHAN HATTINGH COCKTAILS & FINGER SUPPER
TUESDAY – 25 MAY 2004	
07:30 – 08:45	REGISTRATION
08:45 – 09:15	SESSION 1: INTRODUCTORY SESSION CHAIRPERSON : Prof. V. Hattingh Verwelkoming: Die belangrikheid van navorsing soos gesien uit 'n produsent se oogpunt <i>D. Eksteen (CGA Verteenwoordiger)</i>
09:00 – 09:15	CRI Group Research: Who does what and why? <i>T.G. Grout (Research & Technical Manager)</i>
09:15 – 10:45	SESSION 2: FALSE CODLING MOTH CHAIRPERSON: Dr. T.G. Grout
09:15 – 09:40	CRYPTOGRAN: a virus for the biological control of false codling moth <i>Sean Moore, Garth Richards, Wayne Kirkman, Peter Stephen, Hendrik Hofmeyr</i>
09:40 – 10:05	Navorsing op die bestralingsbiologie en F1-steriliteit van Valskodlingmot <i>Hendrik Hofmeyr, Marsheille Hofmeyr, Stephanie Bloem, Jim Carpenter</i>
10:05 – 10:25	Molecular investigation of the population structure of false codling moth, <i>Cryptophlebia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae), in South Africa: application to pest management <i>A.E. Timm, H. Geertsema, L. Warnich</i>
10:25 – 10:45	False codling moth and navel oranges: answering some unasked questions <i>Sean Moore, Garth Richards, Wayne Kirkman, Peter Stephen</i>
10:45 – 11:15	TEA
11:15 – 12:15	SESSION 3: INTEGRATED PEST MANAGEMENT CHAIRPERSON: Dr. S.D. Moore
11:15 – 11:35	Augmenting natural enemies of citrus thrips <i>Tim G. Grout, Peter R. Stephen</i>
11:35 – 11:55	Pest status and control of oleander mealybug, <i>Paracoccus burnerae</i> <i>Wayne Kirkman, Sean Moore</i>
11:55 – 12:15	Thrips control prospects with soft or systemic insecticides <i>Tim G. Grout, Sean D. Moore, Peter R. Stephen, Garth I. Richards</i>
12:15 – 14:20	SESSION 4: CROP LOAD & FRUIT QUALITY ENHANCEMENT CHAIRPERSON: Dr. H.F. le Roux
12:15 – 12:35	Amelioration of sunburn on Miho Wase Satsuma mandarin using kaolin particle film technology <i>Graham H. Barry</i>
12:35 – 12:55	Post-harvest manipulation of rind colour <i>Angelique van Wyk, Graham H. Barry</i>
12:55 – 13:15	Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate <i>Graham H. Barry</i>
13:15 – 13:35	The effect of synthetic auxins on the fruit size and post-harvest reaction of citrus fruit <i>P.J.J. van Rensburg</i>
13:35 – 14:20	LUNCH

14:20 – 16:00	SESSION 5: RIND CONDITION CHAIRPERSON: Dr. F. Kruger
14:20 – 14:40	Investigation into the pre-harvest causes of rind pitting, stem end rind breakdown and chilling injury in export citrus from the Mpumalanga Lowveld <i>F.J. Kruger, P. Chibi</i>
14:40 – 15:00	Die uitwerking wat ontgroening en die duurte voor verkoeling op skil afbraak van “Nules Clementines” mandaryne het <i>P.J.R. Cronjé, P.J.J. van Rensburg</i>
15:00 – 15:20	Factors influencing Rind Breakdown in citrus fruit <i>P.J.J. van Rensburg, J. Joubert, P. Cronjé, G. Gambetta, M. Bruwer</i>
15:20 – 15:40	Factors affecting the occurrence of creasing in citrus fruit <i>S.F. du Plessis, J.G.J. Maritz</i>
15:40 – 16:00	A review of citrus creasing in South Africa <i>S. le Roux & C. Kaiser</i>
16:00 – 16:30	TEA
16:30 – 17:50	RIND CONDITION WORKSHOP CHAIRPERSON: Dr. T.G. Grout
16:30 – 16:50	Historical background <i>P.J.J. van Rensburg, P.J.R. Cronjé</i>
16:50 – 17:00	Current research <i>T. G. Grout</i>
17:00 – 17:20	Area Feedback: <i>D. Gerber</i> <i>P. Wahl</i> <i>J. Warrington</i> <i>B. Offer</i>
17:20 – 17:50	Discussion
18:30 – 22:00	BRAAI
	WEDNESDAY – 26 MAY 2004
08:00 – 08:30	SESSION 6: INTRODUCTORY SESSION The future of the Southern African citrus industry <i>J. Chadwick (CEO: CGA)</i>
08:30 – 10:10	SESSION 7: DISEASE MANAGEMENT (PRE-HARVEST) CHAIRPERSON: R. ANELICH
08:30 – 08:50	Control of Alternaria brown spot on Mandarins with strobilurin fungicides <i>Tian Schutte, Hennie Korf</i>
08:50 – 09:10	Maintaining a healthy root system on citrus trees by managing root pathogens and soil conditions <i>M.C. Pretorius</i>
09:10 – 09:30	Integrated control of <i>Tylenchulus semipenetrans</i> with <i>Paecilomyces lilacinus</i> active ingredient of PL Plus <i>H.J.G. Korf, N. Neethling</i>
09:30 – 09:50	Citrus rootstock resistance in the context of citrus decline <i>N. Labuschagne, A. Fourie</i>
09:50 – 10:10	Investigation of unusual fruit and leaf symptoms of unknown origin on navel and Valencia oranges at Naboomspruit <i>Tian Schutte, Tim G. Grout, M.C. Pretorius, T. Goszczynska</i>
10:10 – 10:40	TEA
10:40 – 11:20	SESSION 8: DISEASE MANAGEMENT (POST-HARVEST) CHAIRPERSON: Dr. B. MANICOM
10:40 – 11:00	Evaluation of Phosphonates for the post-harvest control of <i>Phytophthora</i> brown rot <i>K.H. Lesar</i>
11:00 – 11:20	The supporting role of the sanitizing agent Sporekill in the integrated post-harvest decay control of citrus <i>K.H. Lesar</i>
11:20 – 12:00	SESSION 9: GRAFT TRANSMISSIBLE DISEASES CHAIRPERSON: Dr. J. MOLL
11:20 – 11:40	Citrus Tristeza Virus (CTV) cross-protection of grapefruit <i>S.P. van Vuuren, B.Q. Manicom</i>
11:40 – 12:00	Citrus Tristeza Virus (CTV) cross-protection of sweet orange and mandarin <i>S.P. van Vuuren, B.Q. Manicom</i>
12:00 – 13:00	LUNCH

13:00 – 14:10	SESSION 10: INTEGRATED PEST MANAGEMENT CHAIRPERSON: K. DE KOCK
13:00 – 13:20	The lemon borer moth complex: who is the real culprit? <i>Sean Moore, Wayne Kirkman, G. Richards</i>
13:20 – 14:10	Natal Fruit Fly <i>Tony Ware</i>
14:10 – 15:00	SESSION 11: FERTILIZATION & IRRIGATION CHAIRPERSON: Dr. J.G.K. COETZEE
14:10 – 14:45	Besproeiing en Bemesting: Kommersiële resultate en beginsels <i>J.A. Kruger</i>
14:45 – 15:45	TEA + POSTER SESSION
15:15 – 18:00	SACNA (Break away session) (Nyl Hall) South African Citrus Nursery Association (SACNA) Annual General Meeting <i>Peter Kingston</i>
19:30 – 22:00	DINNER
	THURSDAY – 27 MAY 2004
08:00 – 08:30	SESSION 12: INTRODUCTORY SESSION CHAIRPERSON: Dr. H.F. LE ROUX
08:00 – 08:15	Research needs as perceived by the exporter community <i>S. Symington (CEO: Fresh Produce Exporters Forum)</i>
08:15 – 08:30	The role of research and technical support services in Market Access <i>Prof. V. Hattingh (CEO: CRI)</i>
08:30 – 12:40	SESSION 13: CITRUS BLACK SPOT CHAIRPERSON: Prof. J.M. KOTZÉ
08:30 – 08:50	Overview of Citrus Black Spot research at Plant Pathology Laboratories <i>L. Korsten, M. Truter, L. Meyer, R. Jacobs</i>
08:50 – 09:10	Inoculation of citrus leaves at different leaf ages with <i>Guignardia citricarpa</i> <i>M. Truter, P.M. Labuschagne, L. Korsten</i>
09:10 – 09:30	Sensitivity of the one-day PCR-based technique to detect the citrus black spot pathogen, <i>Guignardia citricarpa</i> <i>Linda Meyer, René Jacobs, Lise Korsten</i>
09:30 – 09:50	<i>In vitro</i> germination conditions of <i>Phyllosticta citricarpa</i> pycnidiospores <i>M. Truter, B. Greyvenstein, P.M. Labuschagne, L. Korsten</i>
09:50 – 10:10	Modelling the potential distribution of Citrus Black Spot <i>I. Paul, V. Hattingh, L. Korsten, A.S. van Jaarsveld</i>
10:10 – 10:30	Climate change and the potential distribution of <i>Guignardia citricarpa</i> Kiely, the causal agent of Citrus Black Spot in South Africa <i>I. Paul, L. Korsten, A.S. van Jaarsveld</i>
10:30 – 11:00	TEA
11:00 – 11:20	Correlation between ascospore inoculum and infection in an orchard <i>W. van Broekhuizen, S.H. Swart, J.M. Kotzé</i>
11:20 – 11:40	Control options for Citrus Black Spot <i>G.C. Schutte</i>
11:40 – 12:00	Efficacy of pyraclostrobin under South African commercial farming conditions <i>H.J.G. Korf, O. van Eysen, T. Richards</i>
12:00 – 12:20	Reducing inoculum, an important aspect in Citrus Black Spot management for a zero tolerance market <i>S.H. Swart, W. van Broekhuizen, J.M. Kotzé</i>
12:20 – 12:40	Survey of Citrus Black Spot in the Limpopo, Northern, Eastern and western Cape Provinces <i>V. Hattingh, H. le Roux, G.C. Schutte, J.M. Kotzé</i>
12:40 – 13:40	LUNCH
13:40 – 15:00	SESSION 14: CITRUS IMPROVEMENT PROGRAMME CHAIRPERSON: THYS DU TOIT
13:40 – 14:00	The South African Citrus Improvement Programme <i>Thys du Toit</i>
14:00 – 14:20	Discussion on cultivars evaluated in the Cape areas <i>C.J. Alexander</i>

14:20 – 14:40	The ARC-ITSC citrus breeding programme: Current successes and future prospects <i>Zelda Bijzet, Nikki Combrink</i>
14:40 – 15:00	Grower Clubs – a brief summary of the development within TopFruit (Pty) Ltd. <i>Peter Allderman</i>
15:00 – 15:30	SESSION 15: EXTENSION & CLOSING DISCUSSION CHAIRPERSON: Dr. H.F. LE ROUX
15:30 – 16:00	TEA
16:00 – 17:00	SESSION 16: BREAK AWAY GROUP (Waterberg Hall) KNOWLEDGE TRANSFER / EXTENSION BRIEFS CHAIRPERSON: Dr. H.F. LE ROUX
18:30	CURRY AND RICE

Table / Tabel 9.2.3

PROGRAMME / PROGRAM	
24-27 MAY 2004	
POSTERS / PLAKKATE	
1	Screening of rootstocks for Citrus Blight tolerance <i>J.H.J. Breytenbach, L.J. Marais, S.P. van Vuuren</i>
2	Comparison of promising field and single aphid <i>Citrus Tristeza Virus</i> isolates in the glasshouse <i>J.H.J. Breytenbach, S.P. van Vuuren, L.J. Marais</i>
3	The monitoring and control of fruit fly in South Africa <i>Carel Buitendag, Wena Naudé, Tony Ware</i>
4	A promising new hybrid from the citrus breeding program <i>Nikki Combrink, J.G.J. Maritz</i>
5	Post-harvest calyx retention of citrus fruit <i>P.J.R. Cronjé, E.M. Crouch, M. Huysamer</i>
6	An "Organic" M3 Bait Station <i>John-Henry Daneel, Tony Ware</i>
7	Resistance of citrus rootstocks against root pathogens: Screening and identification of phenolic compounds with antifungal activity <i>A. Fourie, T.J.C. Regnier, N. Labuschagne</i>
8	Die LNR-ITSG Sitrus mutasie-teelprogram – 2003 / The ARC-ITSC Citrus mutation breeding programme – 2003 <i>I.J. Froneman, J.H. Husselman, Zelda Bijzet, J.G.J. Maritz, Nikki Combrink</i>
9	<i>In vitro</i> embryo rescue for the development of triploid citrus cultivars <i>Karin Hannweg, Zelda Bijzet, Gerrit Visser</i>
10	Use of sectoral chimeras for the improvement of citrus cultivars <i>Karin Hannweg, Johan Maritz, Nikki Combrink</i>
11	<i>In vitro</i> development of tetraploid breeding parents for use in controlled crosses <i>Karin Hannweg, Zelda Bijzet, Gerrit Visser</i>
12	Radiation Biology and Inherited Sterility in False Codling Moth (Lepidoptera: Tortricidae) <i>Stephanie Bloem (IAEA), James E. Carpenter (USDA-ARS) and Hendrik Hofmeyr</i>
13	Acceptability and suitability of eggs of false codling from irradiated parents to parasitism by <i>Trichogrammatoidea cryptophlebiae</i> <i>James E. Carpenter (USDA-ARS), Stephanie Bloem (IAEA) and Hendrik Hofmeyr</i>
14	Steriele Insekloslatings: Boordhoknavorsing oor oorgeërfde steriliteit in valskodlingmot <i>Cryptophlebia Leucotreta</i> (Lepidoptera: Tortricidae) <i>J. Hendrik en Marsheille Hofmeyr</i>
15	Evaluation of citrus rootstocks for lemons and Valencias in replant conditions in the Eastern Cape <i>P.J.J. Koekemoer, J.G.J. Maritz, Nikki Combrink</i>
16	A comparison of accelerated degradation rates of nematicides applied through drip and micro irrigation <i>H.F. le Roux, M.C. Pretorius, L. Huisman</i>
17	The potential geographic distribution of <i>Guignardia citricarpa</i> (Kiely) under climate change <i>I. Paul, L. Korsten, A.S. van Jaarsveld</i>
18	Detection of <i>Hemicyclophora</i> sp on citrus in the Gamtoos River Valley <i>M.C. Pretorius, L. Huisman, H.F. le Roux, E. van den Berg, L.R. Tiedt</i>

19	Chinch Bug – a real tough bugger <i>Tony Ware, Bruce Tate</i>
20	Reducing citrus black spot inoculum in the orchard <i>M. Truter, L. Korsten</i>
21	The effect of trap colour on Questlure-baited female fruit fly catches <i>Tony Ware</i>
22	Influence of diet on fruit fly response to Questlure <i>Tony Ware</i>

10 PUBLICATIONS IN 2004

BARRY, G.H.

- Barry, G.H., Castle W.S. & Davies, F.S. Rootstocks and plant water relations affect sugar accumulation of *Citrus* via osmotic adjustment. *Journal of the American Society for Horticultural Science*; 129(4): 881-889.
- Barry, G.H., Castle, W.S. & Davies, F.S. Soluble solids accumulation in 'Valencia' sweet orange as related to rootstock selection and fruit size. *Journal of the American Society for Horticultural Science*; 129(4):594-598.
- Barry, G.H., Castle, W.S. & Davies, F.S. Rootstocks and plant water relations affect sugar accumulation of 'Valencia' sweet orange via osmotic adjustment. *Acta Horticulturae*; No. 632: 159-165.
- Barry, G.H., Castle, W.S. & Davies, F.S. Juice quality of 'Valencia' sweet oranges borne on different inflorescence types. *Hortscience*; 39(1):33-35.
- Barry, G.H. Citrus colour intensity chart. Citrus Research International publication.

CRONJÉ, P.J.R.

- Cronjé, P.J.R., Crouch, E.M., Huysamer, M. 2004. Postharvest calyx retention of citrus fruit. 5th International Postharvest Conference, Verona, Italy.

GROUT, T.G.

- Grout, T.G. 2004. Citrus research scope in 2004-5. *SA Fruit J.* 3(5): 52-53, 55.
- Grout, T.G. and G.C. Schutte. 2004. Citrus grey mite causing necrotic spot. *SA Fruit J.* 3(2): 32-34.
- Ware, AB & Grout, TG. 2004. Vrugtevliegbestryding in sitrus. *SA Vrugte J.* 3(2): 30-31.

HOFMEYR, J.H.

- Hofmeyr, J.H., Hofmeyr, M., Bloem, S. & Carpenter, J.E. 2004. Bestryding van Valskodlingmot met behulp van Steriele Insekloslatings: Laboratoriumproewe. *S.A. Vrugtejoernaal* 3 (4): 55-59.
- Hofmeyr, J.H., Hofmeyr, M., Bloem S. & Carpenter, J.E. 2004. Bestryding van Valskodlingmot met behulp van Steriele Insekloslatings: Hokproef. *S.A. Vrugtejoernaal* 3 (5): 61-66.
- Carpenter, J.E., Bloem, S. & Hofmeyr, J.H. 2004. Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae). *Biological Control* 30: 351-359.

LESAR, K.H.

- Lesar, K.H. 2004. Evaluation of Phosphonates for the post-harvest control of *Phytophthora* brown rot on citrus fruit. *S.A. Fruit Journal* 3 (6): 43-47.

MOORE, S.D.

- Moore, S.D. & Hattingh, V. 2004. Augmentation of natural enemies for control of citrus pests in South Africa: A guide for growers. *SA Fruit Journal* 3(4): 45-47, 51, 53.
- Moore, S.D., Kirkman, W. & Stephen, P. 2004. CRYPTOGRAN: A virus for the biological control of false codling moth. *SA Fruit Journal* 3(6): 35-39.
- Moore, S.D., Pittaway, T., Bouwer, G. & Fourie, J.G. 2004. Evaluation of *Helicoverpa armigera* nucleopolyhedrovirus, HaNPV, for control of *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera) on citrus in South Africa. *Biocontrol Science and Technology* 14(3); 239-250.

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- Da Graca, J.V. & Korsten, L. 2004. Citrus huanglongbing: review, present status and future strategies. In: Diseases of Fruits and Vegetables: Diagnosis and Management. (ed. S.A.M.H. Naqvil). Kluwer Academic Publishers. Vol 1, pp 229-245.
- Da Graca, J.V. & Korsten, L. Huanglongbing: History, symptoms and control. Brazil. In press.
- Truter, M., Kotzé, J.M., Janse van Rensburg, T.N. & Korsten, L. 2004. A sampler to determine available *Guignardia citricarpa* inoculum on citrus leaf litter. *Biosystems Engineering* 89(4): 515-519.

WARE, A.B.

- Ware, A.B., Tate, B.A.W., Stephen, P.R., Daneel, J-H. & Beck, R.R. 2004. Cold disinfestations of Mediterranean *Ceratitis capitata* [Wiedemann] and Natal (*Ceratitis rosa* Karsh) fruit fly-infested litchis (*Litchi chinensis* Scnn.). S.A. Litchi Growers' Association Yearbook 16:47-51.
- Ware, A.B. & Grout, T.G. 2004. Vrugtevliegbestryding in sitrus. SA Vrugte J. 3(2): 30-31.

11 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

- Barry, G.H., Van Wyk, A.A. Novel approaches to rind colour enhancement of citrus. International Society of Citriculture Congress, Agadir, Morocco, February 2004.
- Barry, G.H. The quest for seedless citrus fruit. International Society of Citriculture Congress, Agadir, Morocco, February 2004.
- Barry, G.H., Rabe, E. Clementine Mandarin production in South Africa. International Society of Citriculture Congress, Agadir, Morocco, February 2004.
- Hattingh, V. Changes in the driving forces behind pest control strategies in export fruit industries such as the South African citrus industry. XXII Int. Congr. Entomol., Brisbane, Australia. 12-26 August 2004.
- Paul, I, Barry, G.H., Collingham, Y.C., Huntley, B., Van Jaarsveld, A.S. A review of global citrus production with reference to potential areas for citrus production and potential spread of pathogens and pests. International Society of Citriculture Congress, Agadir, Morocco, February 2004.
- Grout, T.G. Indigenous Vectors Of Citrus Pathogens In Southern Africa Now More Challenging. 10th International Society of Citriculture Congress, Morocco. 15-20 February 2004.
- Grout, T.G. and Hattingh, V. Multi-Institutional Task Teams: The CRI Group Approach To Citrus Research. 10th International Society of Citriculture Congress, Morocco. 15-20 February 2004.
- Van Vuuren, S.P. Strain prevalence of *Citrus tristeza virus* (CTV) cross-protecting isolates altered by red grapefruit hosts. 16th Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 2004.
- Van Vuuren, S.P. The effect of the rootstock and *Citrus tristeza virus* isolates on Huanglongbing infection of Palmer navel and Delta Valencia. 16th Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 2004.
- Van Vuuren, S.P. The response of Star Ruby grapefruit to different *Citrus tristeza virus* isolates. 16th Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 2004.
- Van Vuuren, S.P. The Association of Group III citrus viroids with gum pocket disease in South Africa. 16th Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 2004.
- Van Vuuren, S.P. The effect of pruning and graft transmissible isolates on Huanglongbing infection. 16th Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, November 2004.
- Ware, A.B. South African experience in cold disinfestations research. XXII Int. Congr. Entomol., Brisbane, Australia. 15-21 August 2004.
- Ware, A.B. Field results using the M₃ bait station. XXII Int. Congr. Entomol., Brisbane, Australia. 15-21 August 2004.